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1 Neuroimmune activation is associated with neurological 2 outcome in anoxic and traumatic coma

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8 Abstract

9 The pathophysiological underpinnings of critically disrupted brain connectomes resulting in coma
10 are poorly understood, but inflammation is potentially an important but still undervalued factor.
11 Here we present a first-in-human prospective study using translocator protein 18 kDa (TSPO)
12 radioligand (F¹⁸-DPA714) for PET imaging, to allow in vivo neuroimmune activation
13 quantification on patients with coma (n = 17) following either anoxia or traumatic brain injuries
14 and compare with age and sex-matched controls. Our findings yield novel evidence that an early
15 inflammatory component that is predominantly located within key cortical and subcortical brain
16 structures which are putatively implicated in consciousness emergence and maintain after severe
17 brain injury (i.e. mesocircuit and frontoparietal networks). We observed that traumatic and anoxic
18 patients with coma have distinct neuroimmune activation profiles, both in terms of intensity and
19 spatial distribution. Finally, we demonstrated that both the total amount and the specifically
20 distributed PET-measurable neuroinflammation within the brain mesocircuit were associated with
21 patient's potential of recovery. We suggest that our results can be developed for use both as a new
22 neuroprognostication tool and as promising biometric to guide future clinical trials targeting glial
23 activity very early after severe brain injury.

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1 **Keywords:** disorders of consciousness, neuroimmune activation, TSPO PET scan, prognosis,
2 traumatic brain injury, brain anoxia, mesocircuit

3

4 **Introduction**

5 Coma, typically arising from traumatic or anoxic brain injury, is a major health issue worldwide;
6 prolonged coma often predicts long-term, typically lifelong, cognitive and behavioral
7 impairments^{1,2}. Despite recent developments in the characterization of structural and functional
8 brain damage underlying coma, prediction of neurological recovery for these patients remains a
9 conundrum, thereby necessitating a better understanding of brain injury mechanisms that are
10 responsible of consciousness abolition in this setting³⁻⁶ and those supporting its recovery⁴.
11 Actually, filling this knowledge gap holds promise for both the discovery of accurate and
12 actionable biomarkers of clinical outcomes and the development of highly needed new targeted
13 patient-tailored interventions.

14 Regardless of etiology, a growing body of evidence suggest that the common pathophysiological
15 mechanism underlying coma is a broad withdrawal of excitatory synaptic activity across key
16 neocortical and subcortical brain structures (i.e. mesocircuit and frontoparietal networks)^{7,8}.
17 However, the pathophysiological processes underpinning the disruption of these critical brain
18 connectomes is poorly understood, but acute neuroinflammation might be one important factor.
19 Indeed, it is well known that severe brain injury, induce significant inflammatory central and
20 systemic response^{4,7,9,10}. Increasing evidence suggests that the neuroinflammatory counterpart of
21 this adaptative mechanism could be located within brain regions that are the critical hubs of these
22 consciousness-related networks. Hence, a marked frontal, parietal and thalamic accumulation and
23 activation of glial cells have been reported in the post-mortem specimen from patients who died
24 from either acute traumatic¹¹ or anoxic¹² brain injury, and animal models studies have identified
25 early inflammatory process in both traumatic¹³ and anoxic¹⁴ conditions, particularly within the
26 associative cortices and sub-cortical grey matter.

27 At a cellular level, microglia are key players of the central immune response, in which they act as
28 brain-resident macrophages¹⁵. In response to brain insults, microglia are transformed from a sentry
29 state into an active state and increase the expression of a mitochondrial protein, the 18-kDa

1 translocator protein (TSPO). As a result, in response to microglial activation, TSPO is
2 overexpressed compared with its expression in normal tissues, making it a key marker of
3 neuroimmune activation¹⁶. In addition to their role in inflammation, microglia support neuronal
4 viability and regeneration¹⁷ and play a critical role in brain plasticity after brain injury¹⁸. However,
5 it should be noted that chronic activation of microglia can become deleterious for neuronal cells
6 and constitute an important factor in neurodegenerative processes¹⁹.

7 Because of its ability to provide in vivo measurements of selected proteins at low concentrations,
8 positron emission tomography (PET) seems exceptionally well suited to in vivo quantify
9 neuroinflammation in brain injured patients. For example, TSPO radioligands for PET imaging
10 are well-validated and widely used biomarkers of neuroinflammation to assess diverse central
11 nervous system (CNS) pathologies in preclinical and clinical studies²⁰. Although TSPO is
12 predominantly expressed in the brain by microglia, expression by other cell types should be
13 considered. TSPO was originally found in peripheral tissue but is also expressed in the brain by
14 astrocytes and in the vascular endothelium²⁰. Migration of peripheral myeloid cells into the brain
15 can also contribute to the TSPO signal²¹. Furthermore, despite the fact that second-generation
16 TSPO radioligands as ¹⁸F-DPA-714 have improved the ratios of specific to non-specific binding,
17 they are sensitive to a common polymorphism (rs6971) in the TSPO gene. Individuals with two
18 copies of the rare allele (ie, low-affinity binders) bind these radioligands with a lower affinity than
19 people with two copies of the major allele (ie, high-affinity binders), and people who are
20 heterozygous for this allele (ie, mixed-affinity binders) express both high-affinity and low-affinity
21 binding sites in similar proportions. This obstacle has been addressed by doing TSPO genotyping,
22 which allow to exclude low-affinity binders from further analysis. Despite these limitations, the
23 study of PET-measurable neuroimmune activation have allowed the detection of significant
24 neuroinflammation in a limited number of small in vivo brain imaging studies of individuals
25 months to years after moderate to severe traumatic brain injury^{19,22,23}. Hereafter, a report of
26 moderate to severe traumatic brain injured (TBI) patients who were scanned while awake at least
27 11 months after the primary brain insult, revealed that patient's TSPO binding potentials were
28 significantly higher than those of controls in the thalami, the putamen and the posterior limb of the
29 internal capsule²³. However, to the extent of our knowledge, there is no in vivo evidence about
30 neuroimmune activation from either anoxic nor in traumatic coma patients in the acute setting.

1 In this study we sought to investigate the relationship between in vivo detected acute
2 neuroinflammation and patients with coma neurological outcome 90 days after primary brain
3 injury. We hypothesized that brain injuries associated with coma triggers microglia within the
4 selective brain structures who are putatively responsible for both loss and recovery of
5 consciousness (e.g., mesocircuit and fronto-parietal networks)^{7,8}. We further anticipated that
6 traumatic and anoxic patients with coma demonstrate distinct in vivo microglial activation profiles,
7 both in terms of intensity and spatial distribution, and that neuroimmune activation during coma
8 would will show a significant relationship with the patient's further neurological outcome. Finally,
9 in traumatic patients with coma, we also investigated PET-measurable neuroinflammatory signals
10 within traumatic focal lesions, derived from structural magnetic resonance imaging.

11

12 **Materials and methods**

13 **Experimental design**

14 We undertook a cross-sectional study of traumatic and anoxic patients with coma and compared
15 with age matched controls. This prospective study was undertaken in three intensive care units
16 affiliated with the University Hospital of Toulouse (Toulouse, France) between February 2018 and
17 February 2022. TSPO PET (F¹⁸-DPA-714), MRI and clinical assessment (Glasgow Coma Scale,
18 GCS; Full Outline of Unresponsiveness, FOUR)²⁴ were performed at baseline. Patients were
19 followed up at 3 months after primary brain injury, including scoring with the revised version of
20 the Coma Recovery Scale (CRS-R)⁷. Our study was approved by the Ethics Committee of the
21 University Teaching Hospital of Toulouse, France. Informed and written consent to participate to
22 the study was obtained from the subjects themselves in the case of healthy subjects and from legal
23 surrogate of the patients. Clinical trials identifier: NCT03482115.

24 **Population**

25 Patients were included in the study after they had a behavioral assessment with GCS and had been
26 diagnosed as been in coma (GCS score at the admission to hospital ≤ 9 with motor responses < 6)
27 induced by either severe traumatic or anoxic brain injury. Patients needed to maintain a GCS score
28 ≤ 9 with M <6 without sedation at the time of brain imaging acquisition. Patients not meeting this
29 criterion at assessment were excluded (Figure 1). Additional exclusion criteria were: significant

1 neurological or psychiatric illness prior to coma; the use of steroidal or non-steroidal anti-
2 inflammatory drugs before imaging; and a low affinity genotype for TSPO (rs6971 gene)^{16,25}.
3 Indeed, patient's peripheral blood samples were drawn the day of TSPO PET scan to characterize
4 TSPO genotypes. Based on the rs6971 polymorphism within the TSPO gene²⁶ we classified all
5 subjects into three groups: high affinity binders (HAB), mixed affinity binders (MAB) or low
6 affinity binders (LAB). TSPO PET scan data from LAB patients were discarded from further
7 analysis^{19,26-29} (Figure 1).

8 All patients were managed according to standard of care recommendations^{30,31} by physicians
9 blinded to neuroimaging data. To avoid confounding factors, all patient's assessments were
10 conducted at least 2 days (4 +/- 2 days) after complete withdrawal of all sedative drug therapy and
11 were performed under normothermic conditions. The day of brain imaging, urine benzodiazepine
12 and barbiturates screening tests were used in patients to rule out residual sedation in case of
13 prolonged utilization of these drugs. Over the same recruitment period, 24 controls, matched by
14 age and sex, were recruited. Control participants denied history of substance dependence, current
15 use of anti-inflammatory treatment, including over-the-counter medications. Participants were
16 included if they had normal neurological examination results, no history of neurological or
17 psychiatric disorder and had either a TSPO (rs6971) genotype other than low-binding.

18 **Clinical assessment**

19 In patients, standardized clinical examination (GCS and FOUR)^{32,33} was performed by raters
20 blinded to neuroimaging data on the day of patient's admission to the hospital and the day of MRI
21 and PET scanning³⁴. Patient's neurological outcome was assessed 3 months after the primary brain
22 injury by using the CRS-R³⁵. The CRS-R is a 23-points scale measuring arousal level, auditory,
23 language, visuo-perception, communication abilities and motor function³⁶. This scale enables the
24 distinction between patients in a vegetative state (VS also coined Unresponsive Wakefulness
25 Syndrome - UWS) and patients in a minimally conscious state (MCS) or patients who emerged
26 from MCS (EMCS) and recovered consciousness as reflected by functional communication or
27 functional use of objects. According to this scale, patient's 90 days outcome was binarized as either
28 favorable (MCS or EMCS) or unfavorable (VS/UWS or deceased).

29

1 **MRI acquisitions**

2 In all participants, high-resolution anatomical image, using 3-dimensional T1-weighted sequence
3 were acquired on the same 3T magnetic resonance scanner (Philips Achieva, Dstream, The
4 Netherlands). Monitoring of vital measures was performed by a senior intensivist throughout the
5 experiment.

6 **PET image acquisition and preprocessing**

7 PET scanning was performed using standard protocols at the Cyclotron Building, Toulouse
8 University Hospital, Toulouse, France, using a hybrid PET/CT tomograph (Siemens, Biograph
9 TruePoint 6.0). Thirty seconds after the start of emission scanning, a mean dose of 3,5 MBq/kg of
10 ^{18}F -DPA -714 (maximal dose: 377MBq) was administered intravenously over 10 seconds into the
11 antecubital vein. This was followed by a 60-minutes mode list emission PET scan acquired as 32-
12 time frames.

13 Images were reconstructed into a total of 32-time frames, with the frame setting of 6 ten-second,
14 8 30-second, 5 one-minute, 5 two-minute, 8 five-minute frames. Attenuation correction, time-of-
15 flight information and point-spread-functions were incorporated in the PET reconstruction. The
16 image volume was 336 x 336 x 148 with a pixel size of 1.018 mm and a slice thickness of 1.5 mm.
17 All PET images were corrected for head movement with a frame-by-frame 3D image registration
18 to a composite frame.

19 **PET image quantification**

20 The quantification of PET images was performed with a simplified reference tissue model
21 (SRTM2) approach, which avoids arterial blood sampling. Considering the widespread
22 distribution of TSPO in the brain, the reference time activity curve was estimated with a
23 SuperVised Cluster Analysis (SVCA) as no anatomical region can be a priori supposed to be
24 devoid of specific binding. Based on previously reported procedure²⁶ our pipeline for PET images
25 quantification was composed of four main steps : (i) segmentation of the cortical and subcortical
26 structures, (ii) creation of two sets of classes from controls participants for both HAB and MAB,
27 (iii) linear unmixing of the PET voxels all subjects and creation of the reference curves and (iv)
28 reference based quantification of individual PET images. Segmentation of the brain were obtained
29 using the *recon-all* pipeline of Freesurfer on each subject's T1-weighted MRI, which includes the

1 segmentation of the subcortical structures, the extraction of subcortical surfaces, the spatial
2 normalization onto the FreeSurfer surface template and a parcellation of the cortical regions for
3 two atlases: Destrieux and Desikan-Kiliany. The PET image of each subject was then registered
4 to its own T1-weighted MR image with a rigid transformation using PMOD v4.2 (PMOD
5 Technologies Ltd, Switzerland). All the frames of the PET images were normalized by subtracting
6 the mean activity of the brain in the frame and by dividing by the standard deviation of the frame.
7 The SVCA classes were created independently for the HAB and MAB subjects. 10 HAB control
8 subjects and 10 MAB control subjects were used for the creation of SVCA classes. Normalized
9 curves of the typical blood, non-specific binding grey matter, white matter and specific binding
10 grey matter were generated. The blood class was created using the 40 voxels having the most
11 activity within the first 3 minutes of acquisition. Gray and white matter class was derived from the
12 supratentorial regions obtained from FreeSurfer previously eroded to limit the partial volume
13 effect. Similarly non-specific binding grey matter class was obtained from the cerebellum grey
14 matter after erosion. Lastly, specific binding grey matter class was obtained using the eroded
15 thalamus region obtained from FreeSurfer. One HAB control participants and one MAB controls
16 participant were excluded from the class creation as their typical kinetic curves presented a
17 significantly different non-specific binding grey matter profile compared to the other controls. All
18 retained controls subject normalized curves were averaged separately among HAB and MAB to
19 create the two sets of SVCA kinetic classes. Dynamic PET images of all controls, anoxic and
20 traumatic subjects were normalized using each subject brain mask obtained from FreeSurfer and
21 decomposed voxel-wise as weighted linear combinations of the kinetic classes using nonnegative
22 least squares. For each subject four 3D weight maps were generated, corresponding to each of the
23 four classes of their corresponding affinity genotype. A low-binding grey matter weight ratio was
24 calculated and used to identify the voxels over 0.9 that were used to generate the ad-hoc reference
25 curve of each subject. Parametric non-displaceable binding potential (referred as TSPO level in
26 the rest of the manuscript) images were generated using SRTM2, where the efflux rate constant is
27 first estimated and subsequently fixed globally. All methods were implemented and performed on
28 Matlab 2017b.

29

1 **Statistical 3D parametric mapping**

2 All T1-weighted MR images were registered onto the Hammer's template from PMOD v4.2 in the
3 MNI space with nonrigid transformations. The estimated transformations were then applied to the
4 TSPO parametric maps to register them in the same reference space. Brain differences in TSPO
5 levels between the three groups (controls, anoxic coma, traumatic coma) were measured via a
6 voxel-based analysis using unpaired Student's two-tailed t-test with p-values corrected for multiple
7 comparisons using the Benjamini-Hochberg control of false discovery rate. 3D Z-score maps with
8 a threshold of $p = 0.05$ were generated, along with d-value maps to evaluate the effect size
9 (moderate, large or very large effect size for d-values inferior to 0.80, between 0.80 and 1.20, or
10 superior to 1.20, respectively). The Hammer's template from PMOD v4.2 software (PMOD
11 Technologies Ltd, Switzerland) was applied to z-score and d-value maps to obtain cerebral z and
12 d values.

13 Partial least squares (PLS-R) regression was used to investigate the association between patient's
14 outcome and whole-brain and voxel-wise TSPO levels. Considering the very large number of brain
15 voxels, that each act as an independent factor, and the fact that many of these voxels can be
16 redundant (collinear in homogeneous regions), classic multiple linear regression approaches are
17 not suited for constructing a predictive model of coma CRS-r outcome. Conversely, PLS-R is well-
18 suited for problems that involve a large set of collinear factors and does not require strict
19 assumptions with regard to the variable and residual distributions. In addition, it is well-suited
20 when the matrix of factors has more variables than observations. All the voxels of the brain were
21 considered as input factors to estimate patient's outcome. To avoid overfitting, a few underlying
22 latent factors (voxels) that account for most of the variation in the outcome are extracted.
23 Conversely to principal component analysis, the loading vectors and the latent variables not only
24 account for variations in the factors, but also model the outcome as well as possible, and remain
25 directly interpretable. To enable better interpretation of the PLS-R models, the "variable
26 importance in projection" (VIP) coefficients were calculated. They estimate the importance of each
27 factor in the outcome prediction of the PLS model, with a VIP Score greater than 1 indicating an
28 important contribution. As each factor corresponds to a voxel in the brain, the VIP values were
29 reshaped as 3D VIP maps of the brain to visualize the regions where the TSPO levels contributed

1 the most to the patient's outcome classification model. Cross-validation was used to predict the
2 CRS-R outcome of each patient, with a leave one out strategy.

3 **Volume of interest definition**

4 To test our hypothesis, ¹⁸F-DPA-714 binding potentials were sampled from an ensemble of
5 anatomically defined gray and white matter volume of interest (VOIs), defined by the mesocircuit
6 and frontoparietal network model⁴: brainstem, thalamus, caudate, globus pallidum, putamen,
7 corpus callosum, anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), mesial
8 prefrontal cortex (mPFC) and precuneus. Because they have been reported as likely to be affected
9 by anoxic brain injuries, the superior parietal, the cuneus and the hippocampus were also
10 considered as VOIs (Supplementary Material, Fig S1). In addition, bilateral precentral gyrus was
11 used as control brain region not typically related to conscious processing^{4,7}. VOIs containing focal
12 brain injury were removed to conduct this analysis. However, in the four traumatic patients with
13 focal brain injury, we investigated TSPO level within the lesion, the lesion penumbra, the thalamus
14 and the normal ipsilateral and contralateral gray and white matter (Supplementary Material, Fig
15 S2). We defined spheric sample VOIs with a 5 mm radius in and around the most prominent lesion
16 (Supplementary Material, Fig S2). One VOI was placed centrally within the lesion, and a second
17 within tissue adjacent to the lesion that returned abnormal T1 signal. Based on the severity of our
18 cohort and previous reports of increased neuroimmune activation remote from focal damage in
19 moderate to severe TBI patients²³, spheric VOIs were placed in gray and white matter returning
20 normal magnetic resonance signal both in ipsilateral and contralateral brain hemispheres and
21 thalami.

22 **Statistical analysis**

23 In accordance with recent reports^{19,23,37}, we pooled the mixed and high affinity binders together
24 and used the TSPO genotype as a covariate in all statistical analyses. Data were analyzed using R,
25 a software environment for statistical computing and graphics (<http://www.R-project.org/>).
26 Normality distribution was tested using Shapiro-Wilk test. Differences between groups were
27 assessed using X²-test, Kruskal Wallis test, or two-ways ANOVA when appropriate. Mandatory
28 normal assumptions for the use of parametric ANOVA were assessed and ANOVA post-hoc
29 testing was performed with the Tukey's Honestly-Significant-Difference (Tukey-HSD) test.

1 **Results**

2 **Population**

3 Between February, 2018, and February, 2022, 53 patients were enrolled. Among excluded patients,
4 five were low affinity binders, two needed sedation during PET scan and one was excluded due to
5 failure of MRI brain normalization process (Figure 1). Therefore, we eventually compared 17
6 severely brain injured patients who met the clinical definition of coma (GCS score at the hospital
7 admission < 9 with motor responses < 6; 11 patients with traumatic and 6 with anoxic brain
8 injuries; age range, 18-65 and 21-79 years, respectively) with 24 age and sex matched controls
9 (age range, 19-69 years). The mean interval between coma onset and PET scan was 13 +/- 8 days.
10 Detailed demographic and clinical data are summarized in Table 1 and Supplementary Material
11 (Tab. S1 and Tab. S2).

12 **Neuroimmune activation profiles according to brain injury mechanisms**

13 Whole brain PET-measurable neuroinflammation was significantly greater in anoxic compared to
14 traumatic patients with coma (Supplementary Material, Fig. S3). Regions of abnormal high TSPO
15 levels, were found in most of patients with coma compared to controls (Figure 2). However, the
16 intensity and spatial distribution of these binding significantly differed between anoxic and
17 traumatic patient's groups. Indeed, a voxel-wise comparison of anoxic patients with coma versus
18 controls showed a significant bilateral increase in ^{18}F -DPA-714 binding in the thalamus, pallidum,
19 putamen, PCC, mPFC, precuneus and the cuneus (Figure 3A-B). VOIs analysis confirmed these
20 observations (Figure 3C). Indeed, we found a statistically-significant difference in average ^{18}F -
21 DPA-714 binding by both coma etiology [$F(2) = 100.29$, $p\text{-value} < 10^{-16}$] and VOIs [$F(12) = 14.03$,
22 $p\text{-value} = 10^{-16}$], though the interaction between these factors was not significant. A Tukey-HSD
23 test revealed significant pairwise differences between anoxic coma and control groups in all the
24 studied VOIs: brainstem ($p\text{-value} = 0.043$), thalamus ($p\text{-value} < 0.0001$), caudate ($p\text{-value} <$
25 0.0001), globus pallidum ($p\text{-value} < 0.0001$), putamen ($p\text{-value} < 0.0001$), PPC ($p\text{-value} < 0.0001$),
26 mPFC ($p\text{-value} = 0.009$), ACC ($p\text{-value} = 0.043$), precuneus ($p\text{-value} = 0.005$), cuneus ($p\text{-value} <$
27 0.0001), corpus callosum ($p\text{-value} = 0.049$) and hippocampus ($p\text{-value} = 0.003$). The post-hoc
28 pairwise comparison between traumatic patients with coma and controls only identified a
29 significant TSPO levels difference in the mPFC ($p\text{-value} = 0.02$, Figure 3). There was no

1 significant difference of ^{18}F -DPA-714 binding between the core and penumbra of traumatic focal
2 lesions²³ (Supplementary Material, Fig. S2).

3 **Relationship between neuroimmune activation and neurological outcome**

4 In patients, we investigated the relationship between baseline neuroinflammatory signal and
5 subsequent neurological outcome at 6 months after primary brain injury. Significant higher TSPO
6 levels were observed in coma patients with unfavorable outcome (Figure 4A). Specifically, VOI
7 analysis revealed significant higher TSPO levels in patients with unfavorable outcome compared
8 to those with favorable outcome in the brainstem, thalamus, pallidum, putamen, caudate, PCC,
9 mPFC, ACC and the precuneus (Figure 4B).

10 In addition, ANOVA analysis allowed the identification of statistically-significant differences of
11 ^{18}F -DPA-714 binding by both patients outcome [$F(1) = 55.99$, p -value $< 10^{-12}$] and ROIs [$F(12) =$
12 3.84 , p -value $= 10^{-5}$], however the interaction between these factors was not significant. A post
13 hoc testing revealed significant pairwise differences between patient's outcomes groups at the
14 level of the following VOIs: brainstem (p -value $= 0.0032$), thalamus (p -value $= 0.032$), globus
15 pallidum (p -value < 0.0001), putamen (p -value $= 0.007$), PPC (p -value $= 0.041$), mPFC (p -value
16 $= 0.009$), ACC (p -value $= 0.018$). There was not interaction between patient's outcome and coma
17 etiology. Furthermore, a significant correlation was observed between the ^{18}F -DPA-714 binding
18 and further patient's behavioral level assessed by the CRS-R three months after the primary brain
19 injury in the pallidum (Spearman's $\rho = -0.73$, p -value $= 0.001$), the putamen (Spearman's $\rho = -$
20 0.58 , p -value $= 0.02$) and the PCC (Spearman's $\rho = -0.49$, p -value $= 0.04$).

21 These findings were corroborated and expanded using a whole-brain and voxel-wise cross-
22 validated predictive model (Figure 4C). While in the model all the voxels are independently
23 considered, the VIP maps (Figure 4D) contained topological features with average value above 1
24 that can be interpreted as regions where the TSPO level best explain the patient outcome.

25

26 **Discussion**

27 Recent research supports the idea that microglia, the resident immune cells of the CNS, play a
28 major role over synaptic remodeling across lifespan and after brain injury¹⁷. Here, for the first time

1 we in vivo demonstrated that traumatic and anoxic brain injuries responsible of coma trigger
2 specific neuroimmune activation across the brain networks that are putatively implicated in
3 conscious processing^{7,8}. Interestingly, we observed that distinct neuroimmune activation profiles
4 were elicited by these two primary brain injury mechanisms. Last but not least, based on
5 converging data from both VOI-based methods and robust data-driven whole-brain and voxel-wise
6 statistical parametric mapping comparisons, we found a significant relationship between
7 neuroimmune activation intensity and spatial distribution and patient's neurological outcome.
8 Overall, we consider that our results provide new insights into the characterization of
9 neuroinflammation induced by severe brain injuries. Notwithstanding that we observed that both
10 the total amount and the specifically distributed TSPO levels within patient's brain mesocircuit
11 were associated with patient's potential of neurological recovery, it is worth noting that our results
12 do not provide any causal evidence between neuroimmune activation and patient's outcome and
13 we acknowledge that these findings, mostly reflects ongoing reparative processes³⁸ and may
14 simply be a reactive reflection of brain injury severity and not the driving force for further patient's
15 neurological status. Hence, our findings emphasize the need for future longitudinal studies tracking
16 of brain serial TSPO levels from coma onset in relation to neurological performance over time,
17 aiming to understand whether these neuroimmune signatures persist, progress and/or warrant
18 neuroimmune-modulating interventions.

19 To the extent of our knowledge, for the first time we have in vivo identified significant differences
20 between the acute neuroimmune profiles induced by either traumatic of anoxic severe brain
21 injuries. Notwithstanding that all patients were in coma at PET scan time, patient's characteristics
22 according to primary brain injury, showed significant difference that might contribute to explain
23 the observed TSPO levels differences at this group level. First, only clinically stable patients were
24 enrolled because particular attention was paid to patient's safety across brain imaging acquisitions.
25 Consequently, the delays between coma onset and patient's neuroinflammatory state assessment
26 were shorter and narrower in traumatic patients compared to cardiac arrest survivors. Second, due
27 to the intrinsic diversity of primary brain injuries induced by severe brain trauma, the reported
28 structural brain anomalies were more heterogenous both in terms of type and spatial distribution
29 in the traumatic compared to the anoxic group. Third, our cross-sectional findings may reflect
30 ongoing systemic inflammatory reactions to concomitant infections or extracranial traumatic
31 injuries. Besides, from a pathophysiological standpoint, we consider that in agreement with human

1 post-mortem evidence¹², anoxic coma was associated with a significant expression of TSPO which
2 was mainly situated in brain regions belonging to the default mode brain network (DMN). Actually
3 the DMN is known for being tonically active at rest³⁹, having the highest brain blood flow, oxygen
4 consumption and oxygen extraction fraction at baseline⁴⁰, and therefore being particularly
5 susceptible to energy delivery failure³⁹. Interestingly, in traumatic patients, we identified a
6 different profile of neuroimmune activation, which was characterized by predominant innate
7 immune cells activation within the frontal lobes that are known for their vulnerability regarding
8 traumatic brain injuries^{13,28}. It is worth noting that alternative mechanisms might also be
9 responsible of the neuroimmune responses that we have observed. Secondary neurodegeneration²⁷,
10 diaschisis^{41,42}, intracranial propagation of mechanical forces⁴³, and widespread endothelial cell
11 activation and blood-brain barrier leakage, might also contribute to boost both local and remote
12 brain inflammation⁴⁴. Altogether, these observations and putative mechanisms underscore the need
13 for a specific characterization of TSPO expression in larger groups of patients with coma according
14 to primary brain injury mechanisms.

15 Neurological impairment related to acute coma is now theorized as being contingent upon the
16 structural⁴⁵⁻⁴⁷ or functional⁴⁸⁻⁵⁰ disruption of dynamic interaction between anterior forebrain
17 mesocircuit and frontoparietal networks^{4,7}. Here we provided novel and converging evidence from
18 both VOI-based and whole-brain voxel-wise analysis, about the role of neuroimmune activation
19 as a potential major but still underrated mechanism of disturbance of key conscious processing
20 brain regions⁵¹. Indeed, the association between patient's outcome and their neuroinflammatory
21 profiles that we have identified is consistent with the view that neuroinflammation can
22 significantly contribute to the dysfunction of critical brain connectomes (i.e. inflammatory
23 penumbra)^{15,18}. Hence, it could be hypothesized that patient-tailored treatment approaches
24 specifically designed to early harness inflammation after severe brain injury might reduce
25 microglia-driven tau phosphorylation and neurofibrillary tangle formation¹⁵, enhance repair and
26 ultimately improve the neurological outcomes of patients with coma.

27 Our findings should be interpreted in light of the following potential limitations. First, we
28 acknowledge that patient's concomitant sedation is a potential confounder for TSPO PET
29 analysis^{20,52} and therefore might hinder the clinical applicability of this evaluation method in brain
30 injured patients with disorders of consciousness. During the current study we have controlled this
31 factor by scanning patients at least two days after total withdrawal of sedation and by testing the

1 day of brain imaging urine benzodiazepine and barbiturates screening levels, in case of previous
2 utilization of these drugs. Moreover, two patients were later excluded because they required
3 sedation during brain scanning. Second, despite the fact that both VOIs and whole- brain and
4 voxel-wise analyses suggest a relationship between acute neuroimmune activation and patient's
5 unfavorable neurological outcome, we should keep in mind recent data which emphasise that
6 TSPO expression in human myeloid cells is related to different phenomena than in mice, and that
7 TSPO-PET signals in humans reflect the density of inflammatory cells rather than activation state⁵³
8 and do not provide information about the dynamic proinflammatory/anti-inflammatory profiles
9 changes across the time. Future studies should investigate in larger cohorts of severe brain injured
10 patients, with and without disorders of consciousness, the beneficial or deleterious nature of the in
11 vivo neuroinflammation that we have identified, probably by leveraging on combined analysis of
12 fluid-derived biomarkers and in vivo and ex vivo brain imaging. Another limitation, is related to
13 the global spatial distribution of neuroimmune activation and therefore the absence of true
14 reference region for inter-subject analysis. Here, we have circumvented the need for kinetic model
15 based on arterial sampling by the use of robust cluster sampling techniques²⁶. This approach has
16 been successfully validated against arterial sampling methods and have been efficaciously used to
17 in vivo identify microglial activation several years after moderate and mild traumatic brain
18 injuries^{19,23,28}.

19 Overall, our findings provide the first evidence that coma should be viewed as a condition with an
20 early inflammatory component which seems predominantly located in critical brain networks that
21 are implicated in consciousness abolition and recovery after severe brain injuries. A significant
22 spatial heterogeneity was observed between the neuroimmune activation profiles that were
23 triggered by either traumatic or anoxic brain injury. We demonstrated that TSPO PET brain scan
24 can be useful for exploring the association between in vivo neuroinflammation and severely brain
25 injured patients outcome and think that this approach can pave the way for development of
26 innovative personalized immunomodulatory therapeutics that ultimately permit to breach the wall
27 of decades of inconclusive poorly stratified clinical trials in coma^{29,54}.

28

29

1 **Data availability**

2 Anonymized data may be shared on request to the corresponding author from a qualified
3 investigator for non-commercial use, subject to restrictions according to participant consent and
4 data protection legislation.

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15 or writing of the report.

17 **Competing interests**

18 The authors report no disclosures relevant to the manuscript

20 **Supplementary material**

21 Supplementary material is available at *Brain* online.

22

23

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1 **Figure Legends**

2

3 **Figure 1 Study flow chart.** TSPO: third generation translocator protein 18 kDa radioligand for
4 PET imaging (^{18}F -DPA714).

5

6 **Figure 2 Individual neuroimmune activation.** TSPO PET scan data is depicted as co-registration
7 with subject's native T1-weighted MRI. Six anoxic and eleven traumatic coma patients are shown.
8 Brain images from one control subject are represented. Controls subjects and anoxic patient's
9 individual scans are depicted using canonical alignment to anterior and posterior commissure
10 (ACPC). In few cases, traumatic patients brain levels were displaced (\pm 20 mm from ACPC) to
11 better illustrate brain concussions and focal TSPO binding

12

13 **Figure 3 In vivo neuroimmune activation profiles according to primary brain injury**
14 **mechanisms.** (A). ^{18}F -DPA-714 binding potential group analysis. (B) Whole brain comparisons
15 between comatose patients' groups according to primary brain injury mechanism (Z scores). (C)
16 Hypothesis-driven comparisons between comatose patients' groups according to primary brain
17 injury mechanism (volume of interest, VOIs). * Corrected p value < 0.05. Abbreviations: ACC =
18 anterior cingulate cortex; mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex.

19

20 **Figure 4 Relationship between neuroimmune activation in vivo and coma patient's**
21 **neurological outcome.** (A). Whole-brain ^{18}F -DPA-714 binding potential difference between
22 patients with a favorable vs. unfavorable neurological outcome at 3-months after coma onset. (B).
23 Volume of interest ^{18}F -DPA-714 binding potential difference between patients with a favorable
24 vs. unfavorable neurological outcome at 3-months after coma onset. (C) Estimation of patients
25 CRS-R outcome after 90 days using whole-brain voxel-wise cross-validated PLS model (VIP
26 map). X-axis corresponds to observed outcomes while y-axis to estimated outcomes. (D).
27 Parametric map of VIP scores from the PLS-R. High values indicate important contribution of the
28 voxels to the model. Abbreviations: ACC = anterior cingulate cortex; CRS-R = coma recovery

1 scale revised; FO = favorable outcome; mPFC = medial prefrontal cortex; PCC = posterior
2 cingulate cortex; UO = unfavorable outcome.
3

ACCEPTED MANUSCRIPT

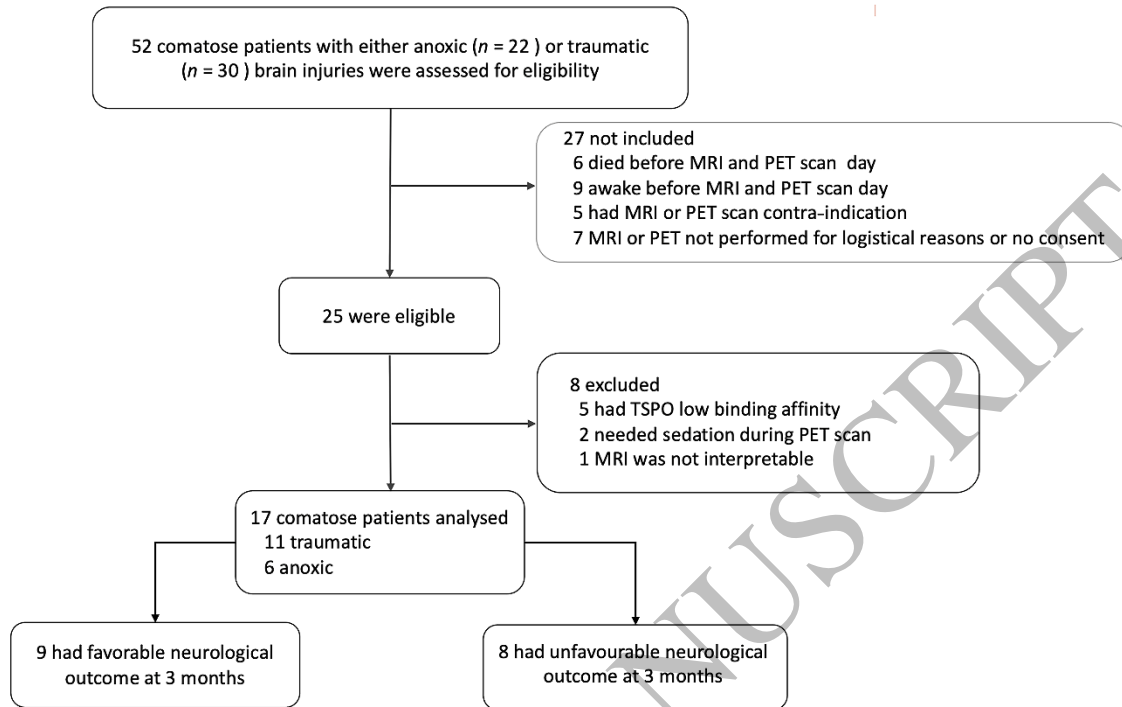


Figure 1
159x98 mm (x DPI)

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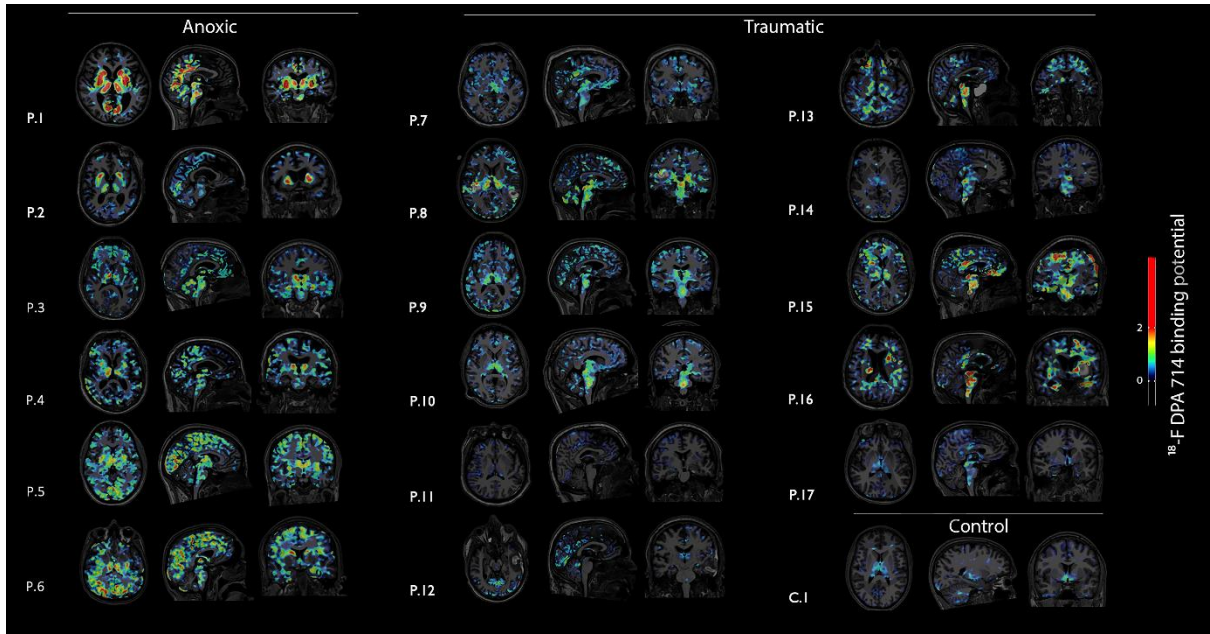


Figure 2
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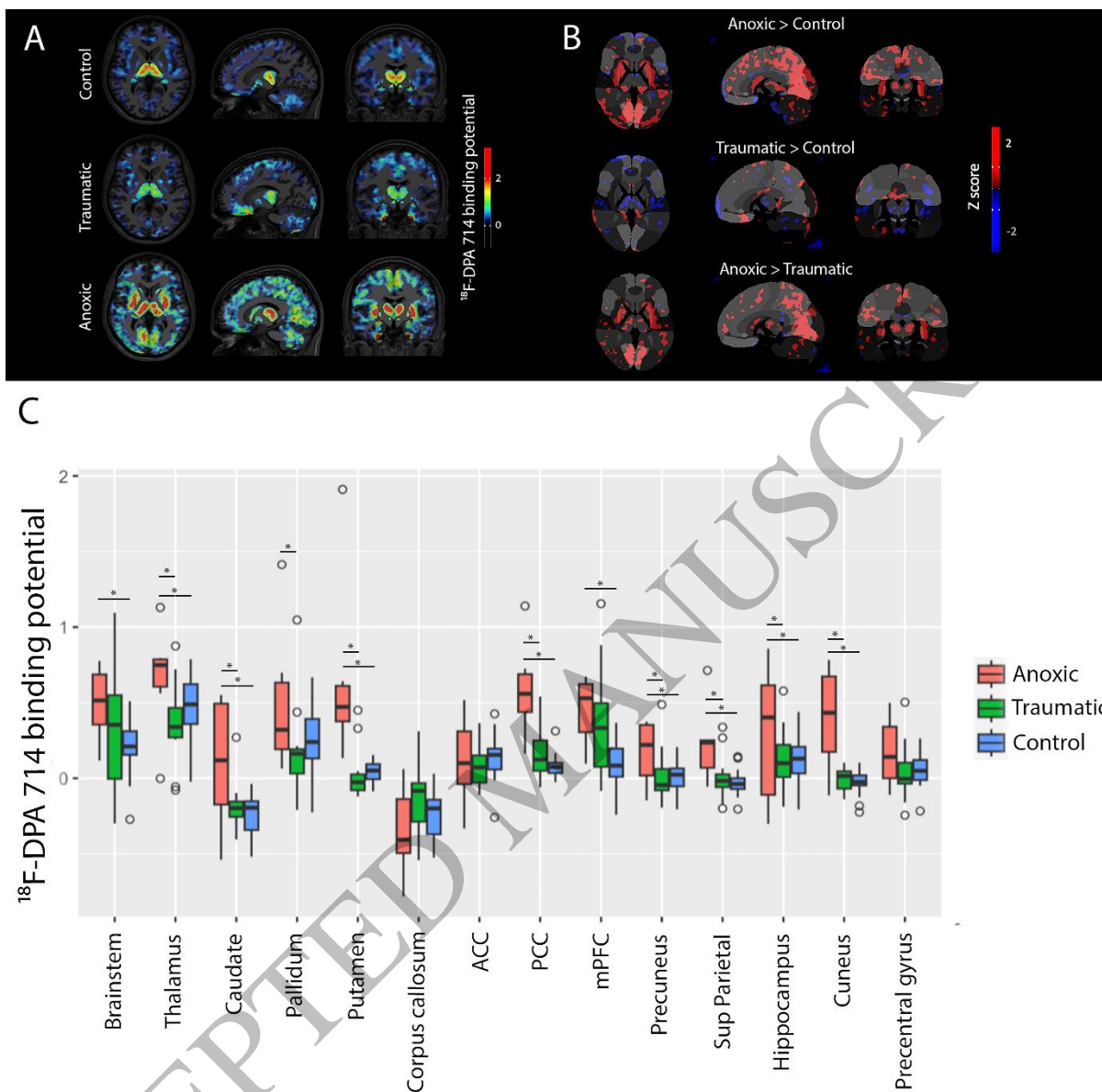


Figure 3
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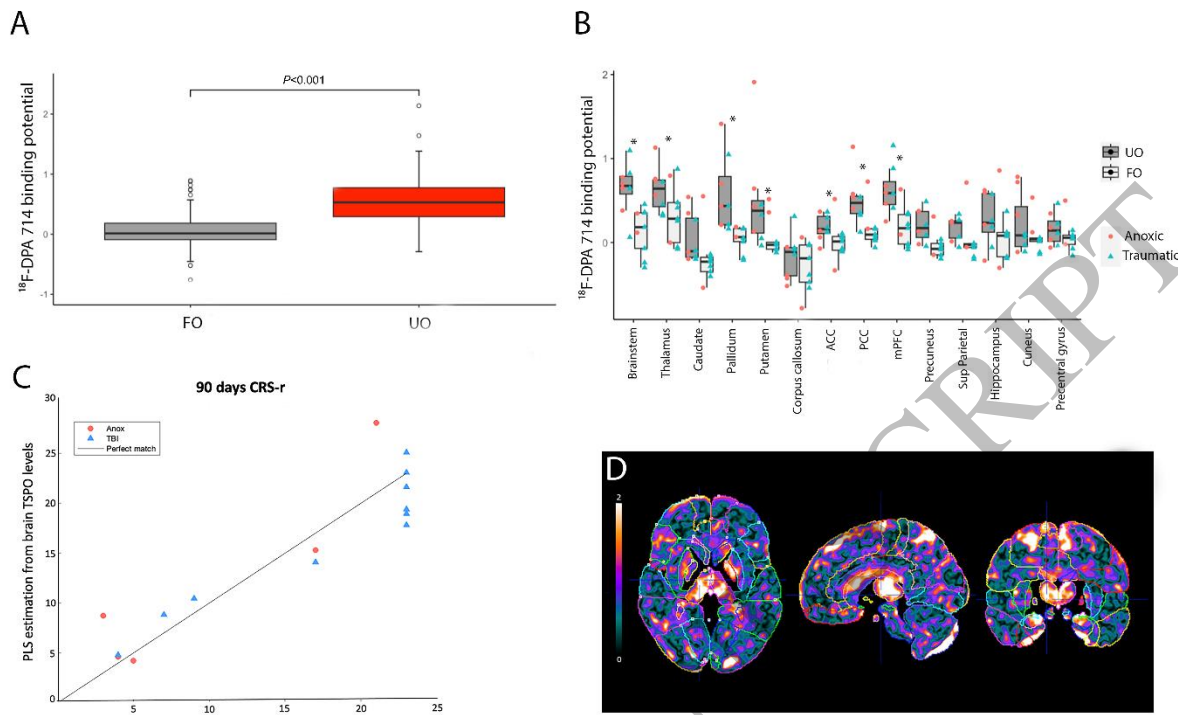


Figure 4
159x99 mm (x DPI)

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1 **Table I Clinical and demographic data**

| Patient n°. | Age | BI | GCS | TSPO | CRS-R | Standard MRI findings |
|-------------|-----|--------|------------|------|--------------------|---|
| P.1 | 18 | Anoxic | 6 (E1V1M4) | HAB | UWS / 3 | Corpus callosum FLAIR hyperintensity |
| P.2 | 65 | Anoxic | 9 (E4V1M4) | HAB | MCS /17 | Striatal and cuneus FLAIR hyperintensity |
| P.3 | 59 | Anoxic | 5 (E1V1M3) | HAB | UWS /5 | Striatal, occipital cortex FLAIR hyperintensity |
| P.4 | 59 | Anoxic | 3 (E1V1M1) | MAB | Death ^a | Striatal lesions |
| P.5 | 30 | Anoxic | 4 (E1V1M2) | MAB | UWS /4 | Striatal parieto-occipital FLAIR hyperintensity |
| P.6 | 23 | Anoxic | 6 (E1V1M4) | MAB | EMCS /21 | Normal |
| P.7 | 39 | TBI | 4 (E1V1M2) | HAB | UWS /7 | Thalamic petechiae; DAI; SAH |
| P.8 | 23 | TBI | 7 (E1V1M5) | HAB | EMCS /23 | Bi-hemispheric contusions; bi-frontal petechiae |
| P.9 | 21 | TBI | 5 (E1V1M3) | HAB | EMCS /23 | Corpus callosum contusion; DAI; SAH |
| P.10 | 36 | TBI | 8 (E2V1M5) | HAB | EMCS /23 | Bifrontal and corpus callosum contusion; SAH |
| P.11 | 40 | TBI | 3 (E1V1M1) | HAB | EMCS /23 | Frontal petechiae; SDH; SAH |
| P.12 | 70 | TBI | 5 (E1V1M3) | HAB | MCS /17 | Left temporal contusion; SDH; DAI; SAH |
| P.13 | 79 | TBI | 8 (E3V1M4) | MAB | Death ^b | Frontal petechiae; DAI; SDH; SAH |
| P.14 | 22 | TBI | 8 (E3V1M4) | MAB | EMCS /23 | DAI; SAH |
| P.15 | 32 | TBI | 8 (E3V1M4) | MAB | UWS /9 | DAI; SAH |
| P.16 | 69 | TBI | 6 (E1V1M4) | MAB | UWS /4 | DAI |
| P.17 | 21 | TBI | 7 (E2V1M4) | MAB | EMCS /23 | DAI, SDH |

2 Patient's CRS-R subscales are available in Supplementary Material. BI = Brain injury; CRS-R = Coma recovery scale revised; DA = Diffuse axonal injury; FLAIR = Fluid Attenuated Inversion Recovery; GCS = Glasgow coma scale (E = Eye; V = Verbal; M = Motor); MCS = minimally conscious state; SAH = Subarachnoid hematoma from traumatic origin; SDH = Subdural hematoma; TBI = traumatic brain injury; TSPO = 18-kDa translocator protein polymorphism encompassing high, mid and low radioligand affinity binders (HAB, MAB and LAB, respectively); UWS = unresponsive wakefulness syndrome.

3 ^aP4: best neurological status before dying: coma (GCS = 3 with E1V1M1); cause of death: multiple organ failure.

4 ^bP13: best neurological status before dying: UWS (CRS-R = 5); cause of death: septic shock.

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