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Variable Neuroendocrine-Immune Dysfunction in Individuals with Unfavorable Outcome after Severe Traumatic Brain Injury

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Abstract

Bidirectional communication between the immune and neuroendocrine systems is not well understood in the context of traumatic brain injury (TBI). The purpose of this study was to characterize relationships between cerebrospinal fluid (CSF) cortisol and inflammation after TBI, and to determine how these relationships differ by outcome. CSF samples were collected from 91 subjects with severe TBI during days 0–6 post-injury, analyzed for cortisol and inflammatory markers, and compared to healthy controls (n=13 cortisol, n=11 inflammatory markers). Group-based trajectory analysis (TRAJ) delineated subpopulations with similar longitudinal CSF cortisol profiles (high vs. low cortisol). Glasgow Outcome Scale (GOS) scores at 6 months served as the primary outcome measure reflecting global outcome. Inflammatory markers that displayed significant bivariate associations with both GOS and cortisol TRAJ (interleukin [IL]-6, IL-10, soluble Fas [sFas], soluble intracellular adhesion molecule [sICAM]-1, and tumor necrosis factor alpha [TNF]-α) were used to generate a cumulative inflammatory load score (ILS). Subsequent analysis revealed that cortisol TRAJ group membership mediated ILS effects on outcome (indirect effect estimate= −0.253, 95% CI (−0.481, −0.025), $p=0.03$). Correlational analysis between mean

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Conflicts of Interest

The authors report no actual or potential conflicts of interest.

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cortisol levels and ILS were examined separately within each cortisol TRAJ group and by outcome. Within the low cortisol TRAJ group, subjects with unfavorable 6-month outcome displayed a negative correlation between ILS and mean cortisol ($r=-0.562$, $p=0.045$). Conversely, subjects with unfavorable outcome in the high cortisol TRAJ group displayed a positive correlation between ILS and mean cortisol ($r=0.391$, $p=0.006$). Our results suggest that unfavorable outcome after TBI may result from dysfunctional neuroendocrine-immune communication wherein an adequate immune response is not mounted or, alternatively, neuroinflammation is prolonged. Importantly, the nature of neuroendocrine-immune dysfunction differs between cortisol TRAJ groups. These results present a novel biomarker-based index from which to discriminate outcome and emphasize the need for evaluating tailored treatments targeting inflammation early after injury.

Keywords

traumatic brain injury; cerebrospinal fluid; cortisol; hypothalamic-pituitary-axis; inflammation; cytokines; cell adhesion molecule; GOS; trajectory analysis; Rehabilomics

1. Introduction

The pathogenesis of traumatic brain injury (TBI) is a highly heterogeneous process. The wide variability associated with acute secondary injury mechanisms has led to significant challenges in discerning effective diagnostic and treatment modalities (Lowenstein, 2009; Faden, 2001). Therefore, recent research has begun to examine biomarker profiles to characterize unique injury responses (Jain, 2008). At present, research suggests that both inflammatory markers and hormones may serve as reliable indicators of acute injury pathology and outcome, findings that may eventually aid in the development of individualized treatment plans (Dash et al., 2010; Kochanek et al., 2008; Wagner et al., 2011; Santarsieri et al., 2014; Juengst et al., 2014).

A growing body of evidence suggests that neuroinflammation contributes to injury and repair after TBI, and thus, has both beneficial and detrimental effects (Morganti-Kossmann et al., 2002; Amor et al., 2009; Woodcock and Morganti-Kossmann, 2013). Although the brain has long been considered immunologically privileged, new evidence suggests that the intrinsic immuno-regulatory properties of central nervous system (CNS) cells, as well as peripheral immune cells and molecules crossing the blood-brain barrier (BBB), influence the brain's response to injury (Becher et al., 2000; Dardiotis et al., 2012). Additionally, cytokine trafficking may occur via receptor-mediated transcytosis or activation of afferent vagal fibers (Banks, 2005; Maier et al., 1998). The central component of the brain's innate immune system is microglia. Activated microglia is largely indistinguishable from peripheral macrophages and can release numerous inflammatory mediators, including cytokines and adhesion molecules (Rivest, 2009; Gehrmann et al., 1995). Additionally, bone marrow-derived microglial precursors migrate into the CNS after injury (Hickey, 1999; Ransohoff et al., 2003). Leukocyte migration into brain parenchyma is facilitated by binding to endothelial selectins and adhesion molecules (Rahman and Fazal, 2009). Depending on the extent, timing or location, the inflammatory response may be characterized as either neuroprotective or neurodegenerative (Morganti-Kossmann et al., 2002). For example,

microglial activation is necessary for the clearance of apoptotic cells after injury and may facilitate injury-induced neural plasticity through various mechanisms, including BDNF signaling (Neumann et al., 2009; Parkhurst et al., 2013). However, chronic inflammation and sustained microglial activation have been linked to cytotoxicity and neurodegeneration (Bal-Price and Brown, 2001; Gentleman et al., 2004). Importantly, microglial activation, and associated inflammatory responses, can persist for years after TBI (Johnson et al., 2013; Ramlackhansingh et al., 2011).

Hypothalamic-pituitary-adrenal (HPA) axis activation after trauma and illness has been extensively studied (Munck et al., 1984; Desborough, 2000). Although increased cortisol secretion is a critical component of the body's response to injury, continued activation or dysfunctional regulation of this pathway can lead to elevated CNS cortisol levels, which can have adverse effects on mood, cognition, and neuron survival (Sapolsky, 1992; Traskman et al., 1980; Pearson et al., 2010). Our prior research showed that elevated CSF, but not serum, cortisol during the first week post-TBI is associated with unfavorable outcomes, and BBB disruption and impaired efflux transporter mechanisms may contribute to harmful levels of cortisol in the CNS (Santarsieri et al., 2014). Additionally, diminished sensitivity of pituitary corticotropin releasing hormone (CRH) to glucocorticoids and/or diminished glucocorticoid receptor affinity may alter HPA negative feedback mechanisms that affect physiological cortisol production (Reincke et al., 1993; Van den Berghe, 1998). Conversely, relatively low cortisol levels in the face of critical illness, often labeled acute adrenal insufficiency (AI), has been linked to unfavorable outcomes as well (Van den Berghe, 1998; Marik and Zaloga, 2002). Various factors including genetic variation, acute care treatments with drugs that impact the HPA axis, and the environment may contribute to an individual's unique systemic cortisol response (Jabbi et al., 2007; Kirschbaum et al., 1995; Mahon et al., 2013; Hildreth et al., 2008; Llompарт-Pou et al., 2007), and its impact on CNS cortisol levels, a fact that highlights the need for research addressing individual variability in hormonal and immune responses after TBI.

Until recently, the immune system and neuroendocrine system were believed to function independently. However, a bi-directional relationship between the two systems is now well established (Reichlin, 1993; Maier, 2002). The HPA axis can be activated by endogenous and exogenous cytokines via multiple humoral and neural mechanisms (Besedovsky and del Rey, 2001; Turnbull and Rivier, 1999). For example, the pro-inflammatory cytokines like IL-1, IL-6 and TNF α are considered potent activators of the HPA axis (Sapolsky et al., 1987; Mastorakos et al., 1993; Besedovsky et al., 1991; Turnbull and Rivier, 1995). Further, the traditional view that glucocorticoids have mainly anti-inflammatory properties has been challenged; cortisol regulation of inflammation is now recognized as both dualistic and dynamic (Yeager et al., 2011; Sapolsky et al., 2000; Munck and Naray-Fejes-Toth, 1994; Yeager et al., 2004). That is, cortisol can both promote and suppress inflammation, and these properties are dose-dependent and temporally dynamic. In the *periphery*, low or basal cortisol levels permissively allow up-regulation of the immune system and increased pro-inflammatory cytokine production (Munck et al., 1984; Yeager et al., 2011). When elevated, however, glucocorticoids have negative-feedback regulatory control that can shut down inflammatory cascades, thereby preventing accumulation of harmful levels of inflammatory

markers (Sapolsky et al., 2000; Besedovsky and del Rey, 2000). Thus, it is high or chronic plasma cortisol levels, such as those associated with stress or pharmacological glucocorticoid administration, which are responsible for the hormone's well-established anti-inflammatory or immunosuppressive properties. The balance of this intricate negative feedback mechanism is an integral part of a healthy immune response, as evidence suggests that dysfunction in this communication pathway may facilitate a chronic inflammatory state, as observed with rheumatic diseases (Silverman and Sternberg, 2012; Sternberg et al., 1989).

Interestingly, cortisol's effects on the immune response in the injured brain differ significantly from its effects in the periphery. Studies, both in vivo and in cell culture, examining inflammatory cell extravasation and migration, inflammatory messenger levels, and transcription factor activation after glucocorticoid exposure have concluded that chronic glucocorticoid exposure is not uniformly anti-inflammatory in the injured CNS (for a thorough review, see Sorrells and Sapolsky, 2007). Our current understanding is that, in the CNS, basal or acute-stress glucocorticoid levels suppress inflammation, while chronic, high-dose glucocorticoid levels exacerbate it (Sorrells and Sapolsky, 2007). The concept is relatively novel and complicated by a variety of factors influencing glucocorticoid effects (e.g., brain region, cell type, synthetic vs. endogenous glucocorticoid, timing of administration, and dose). Regardless, this literature underscores the need to revisit the classic model of glucocorticoids as having strictly anti-inflammatory effects, and to assess, in clinical populations, the effects of TBI on cortisol-inflammatory relationships.

We used a Rehabilomics based framework (Wagner and Zitelli, 2013) to guide our research design; that is, we identified biomarker relationships informative to secondary injury mechanisms and outcome prognostication with a focus on how this information may help shape individualized treatment plans. We hypothesized that the initial inflammatory insult of a TBI triggers an HPA response, and that cortisol regulates the ongoing inflammatory response. Further, we hypothesized that dysfunctional neuroendocrine-immune communication could be defined through unique CSF cortisol and immune marker relationships associated with unfavorable outcome. Our goals were to 1) identify cerebrospinal fluid (CSF) inflammatory markers associated with outcome and cortisol physiology, and based on these criteria, create an inflammatory load score (ILS), 2) characterize the *relationship* between the immune and neuroendocrine systems after TBI and 3) determine if/how the neuroendocrine-immune relationship differs by both outcome and cortisol trajectory (TRAJ) in the setting of TBI.

2. Materials and Methods

2.1. Subjects

This prospective observational cohort study was approved by the Institutional Review Board at the University of Pittsburgh. Informed consent was provided by next-of-kin. When possible, subjects provided assent and/or were later reconsented if their cognitive and informed decision making capacity improved satisfactorily. Samples and data obtained in acute care were obtained as a part of a year-long study. Thus, if a surviving subject became cognitively able, self-consent was obtained during that time. We evaluated 91 adults with

severe TBI at our level 1 trauma center. Subjects were enrolled if they were between the ages of 16 and 75, had a severe TBI based on an admission GCS ≥ 8 with positive findings on head CT, required an extraventricular drainage catheter (EVD) for intracranial pressure (ICP) monitoring and management, and had at least two CSF samples available for analysis of cortisol and inflammatory markers. Patients with penetrating head injury or with prolonged cardiac or respiratory arrest at injury were excluded from the study. Patients were also excluded if they had a previous history of pituitary or hypothalamic tumor, history of breast cancer requiring chemotherapy treatment/tamoxifen, history of prostate cancer requiring orchiectomy or LH suppression agents, or untreated thyroid disease. Women with TBI were not on hormone replacement or oral contraceptive therapy during the sample collection period. All 91 subjects had Glasgow Outcome Scale (GOS) data at 6 months post-injury, and of these 91 subjects, 65 had serum inflammatory markers.

Healthy adult control subjects were separately enrolled to establish reference levels for the biomarkers analyzed. Two independent control groups were used: thirteen subjects (n=6 men, n=7 women) had samples analyzed for CSF cortisol and 11 different subjects (n=6 men, n=5 women) had samples analyzed for CSF and serum inflammatory markers. Control subjects were 18–70 years old and self-reported that they had no current or past history of brain injury, neurological disease, bleeding disorder or endocrine disorder. Women were excluded if pregnant, taking oral contraceptives or hormone replacement therapy, or had any history of reproductive disorder.

2.2. Clinical Care

TBI subjects were admitted to the neurotrauma intensive care unit to receive treatment consistent with *The Guidelines for the Management of Severe Head Injury* (Brain Trauma Foundation, 2007). This care included initial EVD placement, central venous catheter, and arterial catheter. When clinically necessary, surgical intervention for decompression of mass lesions was provided. Elevated ICP was treated in a stepwise fashion to regain control and maintain the pressure within normal parameters (<20 mmHg), and cerebral perfusion pressure (CPP) was maintained at >60 mmHg. Temperature was monitored, and a small subset of subjects received moderate hypothermia (temperature $32.5\text{--}33.5^{\circ}\text{C}$ for 48 hours) if they were enrolled in a randomized controlled clinical trial evaluating hypothermia after severe TBI. All subjects not receiving hypothermia were treated to maintain a normothermic state. In total, n=7 patients received hypothermia, and n=84 remained normothermic. Seven subjects were also involved in the Citicoline Brain Injury Treatment (COBRIT) study, a randomized, double-blind, placebo-controlled, multi-center trial studying 90 days of citicoline treatment on functional outcome after TBI (Zafonte et al., 2009). Two subjects were randomly assigned to receive citicoline (1000 mg twice daily), and 5 subjects were assigned to receive placebo beginning 24 hours after injury. Although not examined for this study, in a previous cohort that includes individuals in this current cohort, glucocorticoids were administered to $\sim 20\%$ of subjects. However, mean serum cortisol levels did not differ based on glucocorticoid use. Also, adrenal suppression agents were commonly used in this previous cohort (Wagner et al., 2011).

2.3. Sample Collection and Processing

CSF samples (n=531 for cortisol, n=443 for inflammatory markers) were collected passively via EVD placed for clinical care, and samples were collected up to 2 times daily (~7:00 AM and ~7:00 PM) for up to 6 days after injury. After collection, CSF samples were stored at 4°C until processing. Blood samples (~10–20ml per sample) were collected as a part of study procedures, and a portion of each sample (n=244 samples) was for biomarker analysis. Blood samples were collected at ~7:00 AM daily for the first 6 days after injury. Clinical care issues, such as medical stability, minimal CSF output, or removal from the intensive care unit (ICU), affected the duration over which CSF sample collection occurred. Control subjects' CSF was obtained at ~7:00AM via a single lumbar puncture (up to 30 ml per sample) for research purposes only. Control blood samples (up to 15 ml per sample) were collected during the same study visit. All CSF and serum samples were centrifuged, aliquoted, and stored at –80°C until batch analysis.

2.4. Sample Analysis

CSF cortisol levels were measured using radioimmunoassay with the Coat-A-Count In-vitro Diagnostic Test Kit (Siemens Healthcare Diagnostics Inc., Los Angeles, CA). Each kit was a solid-phase ¹²⁵I radioimmunoassay designed for the direct, quantitative measurement of cortisol from CSF. The inter-assay and intra-assay coefficients of variation (CV) were less than 10% for this assay. Samples with out of range (low) or undetectable levels were assigned the detection limit (1 ng/ml) of the assay for analysis purposes.

Serum and CSF inflammatory markers, including the cytokines interleukin (IL)-1 β , IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, and tumor necrosis factor alpha (TNF α), as well as the inflammatory cell surface markers, soluble vascular adhesion molecule 1 (sVCAM-1), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble Fas (sFAS), were measured using a LuminexTM bead array assay (Millipore, Billerica, Massachusetts). Multiplex bead array assays use a microsphere tagged with multiple fluorescent-labelled markers. A fluorescence detection laser optic system was used to simultaneously analyse individual protein binding. Single samples were used for analysis for each Luminex assay.

The minimum detectable concentrations reported for each cytokine (measured as a 9-plex assay--catalog number: HSCYTO-60SK) were as follows: IL-1 β : 0.06 pg/ml, IL-4: 0.13 pg/ml, IL-5: 0.01pg/ml, IL-6: 0.1 pg/ml, IL-7: 0.12 pg/ml, IL-8: 0.11 pg/ml, IL-10: 0.15 pg/ml, IL-12: 0.11 pg/ml, and TNF α : 0.05 pg/ml. The minimum detectable concentration reported for cell surface markers sICAM and sVCAM (measured as a 2-plex assay--catalog number: HSEP-63K) were 30 pg/ml and 33 pg/ml respectively, and the minimum detectable concentration for sFAS (measured as a 1-plex--catalog number: HSEP-63K) was 7.0 pg/ml. The intra-plate % coefficient of variance (CV) ranged from 3.11%–5.86%, while the inter-plate %CV ranged from 2.16%–14.27% for these assays.

Inflammatory biomarker data generated for serum and CSF samples were scaled prior to analysis due to recognized variability between assay kits and across sample plates (Brand et al., 2011). The scaling procedure utilized both concentration standards (CS) and quality control (QC) samples duplicated across plates. Both CS and QC used were entirely on par

with a minimization function, Ω , set to minimize the sum of weighted differences between common QC samples as well as common CS on all plates being scaled.

$$\Omega = \sum_p \left(\alpha_{1p} A_{1p}^2 + \alpha_{2p} (A_{2p} - 1)^2 \right)$$

Offset, A_1 , and scaling factor, A_2 , were determined for each plate so as to minimize the sum of weighted differences between common QC samples as well as common CS on all plates being scaled. Weight α_{1p} , and α_{2p} were set to a minimum so as to only ensure that the set of data was not shifted and the scaling process did not change the scale as a whole.

2.5. Demographic and Clinical Injury Variables

Independent variables included gender, age, Glasgow Coma Scale (GCS) score, body mass index (BMI), injury severity score (ISS), hospital length of stay (LOS), mechanism of injury, radiological injury type, hypothermia treatment, citicoline treatment, and acute systemic adrenal insufficiency status. The best GCS score taken within 24 hours of injury was recorded from the electronic medical record. Injury type was abstracted from clinical head CT radiology reports obtained at the time of injury. Acute AI status during the sampling period was coded using criteria previously published by Cohan and associates (2005), which included two consecutive morning serum cortisol levels of <150 ng/ml or one morning serum cortisol level of 50 ng/ml.

2.6. Outcome Variables

GOS obtained at 6 months post-injury served as the primary outcome measure. Research-trained neuropsychometrists, blinded to biomarker analyses, collected the data used to generate GOS scores for this cohort. GOS is a frequently used measure developed by Jennett and Bond (1975). The scale reflects the degree of overall mental and physical disability and serves as an indicator of how an injury has affected functioning in major areas of daily life. The GOS categories are: dead (1), vegetative (2), severely disabled (3), moderately disabled (4), and good recovery (5). For this study, GOS categories were collapsed into 1/2/3 (unfavorable outcome) vs. 4/5 (favorable outcome) for analysis. The GOS is frequently dichotomized in this way to discriminate outcomes. Although dichotomization reduces sensitivity, some have claimed that using non-dichotomized outcome measures do not confer a significant advantage over the dichotomized GOS (Teasdale, 1999; Tolia, 2004). From a data analysis perspective, outcome dichotomization facilitated sub-group correlational analysis to assess relationships between biomarkers as a function of outcome (*see statistical analysis*).

2.7. CSF Inflammatory Load Score

Numerous inflammatory markers are co-elevated early after TBI, and these markers have dual (or multiple) roles that are highly context-dependent (Morganti-Kossmann, 2007). Thus, we hypothesized that individual inflammatory markers may not be as informative of TBI pathology or predictive of outcome as an aggregate inflammatory score. The skewness of inflammatory biomarker data requires either transformation or conversion into categorical

variables. For the purposes of this analysis, we used quartiles in order to simultaneously compare several markers without losing interpretability by log-transforming the data (Grund and Sabin, 2010). Several other clinical research studies have generated similar aggregate scores to evaluate inflammatory load by establishing 1) a cut-point above and below the 75th percentile (Andaluz-Ojeda et al., 2012; Correia et al., 2010; Juengst et al. 2014), 2) cut-points below the 25th, between 25th–75th percentile, and greater than 75th percentile (Wakelkamp et al., 2003; Boelen et al., 1995), or 3) quartiles (Kumar et al. 2014).

To generate a cumulative CSF inflammatory load score (ILS), a weekly mean for each inflammatory marker was calculated for each subject. Then, individuals were assigned a quartile (1–4), based on the relative ranking of that individual, for each ordered cytokine. An inflammatory marker value that was >75th percentile was assigned a 4, while those values <25th percentile were assigned a score of 1. Inflammatory markers that displayed significant bivariate associations with both 6-month GOS and cortisol trajectory (TRAJ) group membership were included in ILS formulation. A cumulative CSF ILS for each subject was calculated by summing the quartile rank for each of the five selected inflammatory markers. Therefore, the possible range for each subject's ILS was 5–20. Exploratory analyses using ILS scores derived from inflammatory marker levels on each day, or over successive 2–3 days intervals post injury were explored, and relationships to cortisol were consistent with relationships delineated using ILS scores derived from weekly mean inflammatory values (data not shown).

2.8. Statistical Analysis

Prior to data analysis, we averaged multiple hormone and inflammatory biomarker values collected from samples taken the same day for the same subject. One TNF α outlier value that was 8 SD outside of the mean was removed from the data set prior to analysis. Mean hormone and inflammatory marker values across the weekly sampling period were calculated for each subject. Statistical analyses were performed using SPSS Version 21.0 (Chicago, IL) and SAS Version 9.2 (Cary, NC).

Descriptive statistics, including mean, median, and standard error of the mean (SE) were computed for all continuous variables. Mann-Whitney U and Kruskal-Wallis tests were conducted to assess differences between groups for continuous variables. Frequencies and percentages were determined for categorical variables, and Chi-square, with Fisher's exact test, was used to determine associations for categorical variables. All tests were two tailed, with a significance level set at $\alpha=0.05$.

Group based trajectory analysis (TRAJ) was used to develop temporal CSF cortisol profiles that characterize distinct subpopulations similar to methods previously described (Wagner et al., 2011; Niyonkuru et al., 2013). TRAJ is a specialized application of finite mixture modeling that assesses patterns of change over time (Nagin, 2005), and models were estimated using the PROC TRAJ Macro (Jones et al., 2001) for SAS software. TRAJ analyses can identify clusters of individuals with similar CSF hormone profiles over time. This cohort had CSF cortisol TRAJ group membership assignments generated from a previous study (Santarsieri et al., 2014). Three distinct TRAJ group profiles were identified

for CSF cortisol, and the two highest TRAJ groups were combined for comparison to those in the lowest TRAJ group (Santarsieri et al., 2014).

To examine whether the association between CSF ILS score and GOS at 6 months was mediated by cortisol TRAJ group, models were fitted based on procedures outlined by Baron and Kenny (1986). Analyses were performed using the M-plus software (Version 7.11). Because the variables included in our models were ordinal or binary (e.g. GOS and cortisol TRAJ), we used a non-parametric bias-corrected bootstrap method to estimate both direct and indirect mediating effects of CSF ILS score on outcome (Efron, 1987). The bootstrapping method utilized random sampling with replacement from the original cohort. There were 5,000 bootstrap samples in this analysis. The bias-corrected bootstrapping method was used because of its rigorous approach for computing confidence intervals for mediating effects (MacKinnon et al., 2004). Mediation effects were evidenced by: (i) the direct path from CSF ILS to GOS no longer being statistically significant after the introduction of cortisol TRAJ group, and (ii) the statistical significance of the indirect path from CSF ILS through cortisol TRAJ group membership to GOS. In addition to mediation, multivariate logistic regression was utilized to test associations while controlling for relevant clinical and demographic variables. There was an a priori decision to control for age and GCS in the multivariate logistic regression models characterizing outcome. Additionally, variables that were significant in bivariate associations with cortisol TRAJ, ILS, and GOS were tested as covariates in the multivariate model to control for potential confounding effects. The initial multivariate model was reduced to obtain a final parsimonious model by stepwise backwards elimination that included only covariates with p-values<0.2. Models were constructed to assess odds of unfavorable versus favorable GOS at 6 months.

Associations between continuous variables were assessed using Spearman rank order correlations due to the non-normal distribution of the biomarker data. Within each cortisol TRAJ, subjects were stratified by GOS group, and correlations between ILS and mean weekly cortisol levels were assessed. Correlations between serum and CSF mean inflammatory marker levels were also assessed.

Exploratory analyses were conducted to examine the frequency of acute systemic AI among cortisol TRAJ and outcome groups. Further, associations between AI status and ILS were explored in the overall cohort as well as in each cortisol TRAJ group.

3. Results

3.1. Description of the Cohort

Table 1a/b outlines the population characteristics by CSF cortisol TRAJ group membership, 6 month GOS and ILS. The mean age of the cohort was 35.80 ± 1.73 years, with an age range of 16–73 years. Women represented 20% of the population. The primary mechanism of injury among both men and women was motor vehicle collisions (45%) followed by motorcycle collisions (21%). The median GCS score for the cohort was 7, and the mean $\pm$ SEM ISS score was 33.69 ± 0.88 . The most common injury types observed on CT scan were subarachnoid hemorrhage (SAH) (74%) and subdural hematoma (SDH) (66%). This population had a 26% acute care mortality rate and a mean acute care hospital LOS of

20.96±1.17 days. There were a total of 30 individuals (36%) in the cohort that met clinical criteria for adrenal insufficiency. At 6 months post-injury, 67% of the population had a GOS score of 1, 2 or 3, and 33% had a GOS of 4 or 5. Age, diffuse axonal injury (DAI), and SDH were significantly associated with the predictor variables (cortisol TRAJ and ILS) as well as the outcome variable (GOS) and were tested as covariates, along with GCS, in the subsequent multivariate logistic regression model. Acute systemic AI was more common among those in the low cortisol TRAJ (46.4%), although roughly one-third of individuals in the high cortisol TRAJ still had AI. Overall, those with AI had significantly lower ILS scores compared to those without AI ($p=0.026$).

Among those controls with CSF cortisol values, the mean age was 34.88±4.03, 53.8% of the population was comprised of women, and the mean CSF cortisol was 4.50±0.41 ng/mL with no differences in CSF cortisol levels based on sex. Among those with inflammatory marker values in the control population, the mean age was 25.048±3.66 and 45.5% of the population included women.

3.2. CSF Cortisol Trajectory Groups

Figure 1 shows that cortisol levels were significantly different between TRAJ groups on days 0–5 post-TBI ($p<0.01$), but not day 6. Cortisol levels for all TBI subjects, regardless of cortisol trajectory group membership, were significantly higher than control levels on day 0–5 ($p<0.01$, all comparisons). On day 6, only TBI subjects in the high cortisol TRAJ had cortisol levels higher than controls ($p<0.01$).

3.3. CSF Inflammatory Markers: Associations with CSF Cortisol TRAJ Group Membership

To begin to characterize how cortisol regulates inflammatory biomarker profiles in the CSF, Table 2 summarizes individual bivariate inflammatory marker associations with each cortisol TRAJ group. Subjects in the *high* cortisol TRAJ group were more likely to have higher levels of IL-10, IL-1 β , IL-6, sFas, sICAM-1, sVCAM-1 and TNF α ($p \leq 0.01$ all comparisons, except IL-1 β , $p = 0.05$) compared to subjects in the *low* cortisol TRAJ group. Levels for each inflammatory marker included in the ILS are also graphed as a function of CSF cortisol TRAJ, compared to controls (see Figure 2). Regardless of cortisol TRAJ group membership, levels of the inflammatory marker levels included in the ILS were higher for TBI subjects compared to controls ($p \leq 0.01$ all comparisons).

3.4. CSF Cortisol and CSF Inflammatory Markers: Associations with Outcome

Table 2 summarizes the individual associations between cortisol and outcome, as well as inflammatory marker associations at 6 months post-injury. Mean CSF cortisol was significantly associated with GOS ($p<0.01$), with lower levels of cortisol associated with more favorable outcome (i.e., membership in the GOS 4/5 group). Additionally, there were significant associations between GOS and mean levels of IL-10, IL-6, IL-8, sFas, sICAM-1 ($p<0.01$) and TNF α ($p<0.05$), with lower levels associated with favorable outcome.

3.5. ILS and Multivariate Regression

IL-10, IL-6, sFas, sICAM-1, and TNF α were included in the ILS due to their significant associations with both GOS and cortisol TRAJ (see sections 3.3 and 3.4 above). To assess

whether ILS or cortisol was the primary variable influencing outcome, a multivariate regression model was generated using both biomarkers in the model (Table 3). Age, GCS, DAI, and SDH were entered as covariates in the initial multivariate regression model. Upon stepwise backwards elimination, SDH was removed from the model ($p=0.296$). No other covariates were removed from the model. Multivariate logistic regression models showed that cortisol TRAJ significantly predicted GOS after controlling for covariates and ILS (Table 4). Membership in the high vs. low cortisol TRAJ group conferred an approximately 3.5-fold increased odds of unfavorable versus favorable outcome at 6 months (adjusted OR=3.495, 95% CI (1.123, 10.884), $p=0.031$). The best GCS in the first 24 hours was also a significant predictor of outcome at 6 months ($p=0.020$). Age, DAI, and ILS did not significantly predict GOS in the multivariate model.

3.6 ILS Mediation Analysis

The multivariate model generated above identified cortisol TRAJ as the primary biomarker variable driving outcome. However, given our hypothesis of unique regulatory effects of cortisol on inflammation, we then used mediation analyses to assess indirect relationships between ILS and 6 month GOS via cortisol TRAJ. The standardized coefficients for the mediation analyses are provided for direct comparison in Figure 3. The model shows that higher ILS is associated with increased likelihood of high cortisol TRAJ group membership ($p<0.001$). Further, high cortisol TRAJ group membership was significantly associated with unfavorable GOS scores ($p=0.006$). The test for mediation effects shows that the relationship between ILS and GOS is indirectly mediated through cortisol TRAJ group membership (indirect effect estimate= -0.253 , 95% CI (-0.481 , -0.025), $p=0.030$).

3.7. Nonparametric Correlations between Inflammation and Cortisol

Correlations between cortisol and ILS for subjects in each cortisol TRAJ are depicted in Figure 4 to show the unique interactions between CSF cortisol and ILS for subjects within each TRAJ group. Among those in the low cortisol TRAJ, mean CSF cortisol levels and ILS were not significantly correlated for individuals in the *low* cortisol TRAJ ($r=0.023$, $p=0.902$), but were highly correlated in the *high* cortisol TRAJ ($r=0.336$, $p=0.009$) (Figure 4A). However, to better understand ILS relationships with CSF cortisol in the context of outcome, ILS and mean cortisol values for favorable vs. unfavorable outcome groups were compared within each cortisol TRAJ group (Figure 4B). For subjects with unfavorable outcome in the low CSF cortisol TRAJ group, mean cortisol levels and ILS were negatively correlated ($r=-0.562$, $p=0.045$). However, mean cortisol levels and ILS were positively correlated for subjects with unfavorable outcome in the high CSF cortisol TRAJ group ($r=0.391$, $p=0.006$).

3.8. CSF and Serum Inflammatory Marker Comparisons

Figure 5 depicts the median CSF and serum levels of inflammatory markers included in the ILS. Serum IL-10, sFAS, sICAM and TNF α were significantly higher than CSF levels ($p < 0.001$ all comparisons). Conversely, CSF IL-6 levels were significantly higher than serum levels ($p<0.001$). Out of the 12 inflammatory markers measured, the following cytokines had CSF and serum levels that were significantly and positively correlated with

each other: IL-10 ($r=0.329$, $p=0.004$), IL-6 ($r=0.226$, $p=0.050$), IL-8 ($r=0.365$, $p=0.001$), and TNF α ($r=0.431$, $p<0.001$). Notably, CSF values for each of these cytokines was significantly associated with CSF cortisol TRAJ group membership, GOS score, or both.

3.9 Acute Systemic AI Status and Neuroendocrine-Immune Dysfunction

The relationship between AI status and ILS within each cortisol TRAJ group is provided in Table 5. Among those in the low CSF cortisol TRAJ, there were no significant differences in median ILS by AI status; however, among individuals in the high cortisol TRAJ, those with a clinical designation of AI had significantly lower median ILS compared to those with a sufficient systemic adrenal response ($p=0.030$).

4. Discussion

There exists compelling data regarding the dual role of neuroinflammation in the injured brain (Morganti-Kossmann et al., 2002; Woodcock and Morganti-Kossmann, 2013) and also for the bi-directional communication between the immune and neuroendocrine systems (Reichlin, 1993; Maier, 2002; Chrousos, 1995). Thus, the aims of the present study were to 1) examine the interrelationships between cortisol and inflammatory markers after TBI and 2) determine if/how the nature of this relationship varied by cortisol TRAJ and could help inform long-term outcome. Our data supports previous reports that CSF inflammatory markers are elevated after TBI (Bell et al., 1997; McClain et al., 1991), and higher levels of cytokines and adhesion molecules are associated with unfavorable outcome (Kirchhoff et al., 2008). Unique to this study, we demonstrated that the association between these inflammatory markers and outcome is mediated by cortisol TRAJ group membership. These results suggest that while it is the traumatic event that elicits initial neuroinflammation, the activated stress response and ongoing presence of high cortisol modulate the subsequent inflammatory response and effects on outcome. Because of this mediating effect, the nature of the neuroendocrine-immune relationship was independently examined within each TRAJ group. We report that individuals with unfavorable outcome after TBI seem to display dysfunctional neuroendocrine-immune regulation and, strikingly, the nature of this dysfunction differs between cortisol TRAJ groups. Specifically, our data suggest that those with low CSF cortisol and unfavorable outcome have a subphysiologic inflammatory response that may be insufficient to facilitate early reparative processes in the injured brain. Conversely, in the setting of high cortisol, those with unfavorable outcome have a dysfunctionally permissive immune response that may exacerbate CNS damage (Figure 6).

Surprisingly, cortisol's effects on the CNS immune response differ from its effects on peripheral inflammation, at least in the injured brain (Sorrells and Sapolsky, 2007). In this case, basal levels of glucocorticoids and the physiological response to acute stress are anti-inflammatory while chronic or high stress levels exacerbate inflammation (Sorrells and Sapolsky, 2007; Sorrells et al., 2009). This concept is based, in part, on findings by Dinkel and colleagues (2003) that hippocampal brain tissue from corticosterone-treated adrenalectomized animals displayed higher levels of pro-inflammatory cytokines (including IL-1 β and TNF- α) after excitotoxic insult compared to adrenalectomized animals. Follow-up in vitro work by this group evaluated hippocampal cultures exposed to a range of physiological glucocorticoids and found that low- to mid-range glucocorticoid exposure was anti-

inflammatory, while high doses were not (MacPherson et al., 2005). Glucocorticoids' dualistic effects on inflammation may be due to simultaneous upregulation of inflammatory marker receptor expression and suppression of inflammatory marker release (Wiegers et al., 1995; Wiegers and Reul, 1998). Evidence suggests that differential binding to two different intracellular receptors, the glucocorticoid (GR) and mineralocorticoid (MR) receptors, may partially explain cortisol's opposing, yet complementary, effects on inflammation (Reul and De Kloet, 1985). MR are stimulated to help initiate the inflammation response at low/moderate concentrations of glucocorticoids, which is followed by GR-mediated suppression of inflammation at higher concentrations of glucocorticoids (Chantong et al., 2012).

In our overall cohort (i.e. not stratified by outcome) relatively low acute cortisol levels (i.e., low cortisol TRAJ group membership) displayed no correlation with ILS, while elevated cortisol levels (i.e. high cortisol TRAJ) were positively correlated with ILS (Figure 4a). These results support previous findings that, in the injured brain, high cortisol levels may exacerbate inflammation (Sorrells and Sapolsky, 2007). While membership in the high cortisol TRAJ was associated with increased inflammation and unfavorable outcome for the majority of subjects, we noted that some individuals within the high cortisol TRAJ had a favorable outcome. Likewise, some individuals in the low cortisol TRAJ had an unfavorable outcome. To explore this heterogeneity, we further stratified individuals in each cortisol TRAJ category by GOS group.

Within the high cortisol TRAJ group, subjects who experience an *unfavorable* outcome display a positive correlation between cortisol and ILS, consistent with current literature stating that high stress levels of cortisol promote inflammation in the *injured* brain (Sorrells and Sapolsky, 2007). Conversely, cortisol and ILS were not correlated in individuals with favorable outcome. Therefore, we speculate that in a subset of subjects with elevated CSF cortisol levels after TBI, unfavorable outcome is associated with dysfunctionally permissive neuroendocrine-immune regulation. Conversely, subjects with high cortisol who experience a favorable outcome may have a "healthier," self-limited neuroinflammatory response to brain injury, supporting the hypothesis that inhibition of excess inflammation is, when necessary, beneficial.

In the low cortisol TRAJ group, correlational directions were reversed; that is, cortisol and ILS were negatively correlated for those with *unfavorable* outcome, supporting findings that low glucocorticoid levels in the *injured* brain display immunosuppressive actions (Sorrells and Sapolsky, 2007). Conversely, cortisol levels and ILS were positively, though not significantly, correlated for individuals with favorable outcome. When physiologically regulated, the inflammatory response is essential for preparing the CNS for reparative and regenerative processes such as debris phagocytosis, astrocytosis, cell proliferation and remyelination (Morganti-Kossmann et al., 2002; Correale and Villa, 2004; Lenzlinger et al., 2001), and acute hypercortisolism can support appropriate regulation of this inflammatory response (Van den Berghe et al., 1998; Marik and Zaloga, 2002). In the setting of low cortisol, it seems that individuals with unfavorable outcome have a sub-physiologic inflammatory response, whereas the maintenance of what is likely an adequate inflammatory response is associated with favorable outcome.

Our results suggest that outcome prediction based solely on cortisol levels or solely on inflammation is incomplete and reductive. Alternatively, we suggest a novel outcome discrimination method based on the relationship between cortisol and ILS such that individuals with favorable outcome after TBI retain what may be considered a functional neuroendocrine-immune relationship, whereas those with unfavorable outcome display evidence of impaired neuroendocrine-immune communication (Figure 6). Our definitions of “functional” and “impaired” are cortisol dependent, in that cortisol-ILS correlations in the *high* cortisol TRAJ group were opposite to those in the *low* cortisol TRAJ group. Interestingly, mean CSF cortisol levels of ~18–20 ng/ml seem to be a natural point of dichotomization for cortisol TRAJ (Figure 4a), suggesting a potential clinically relevant point of population stratification from which to further assess and validate these divergent neuroendocrine-immune relationships.

Further, our results support the idea that since outcome discrimination is a highly context-dependent process, steroid supplementation is not clinically indicated for all patients. Our findings suggest one possible mechanism by which results from glucocorticoid trials have been mixed and the Corticosteroid Randomization after Significant Head Injury (CRASH) Trial failed (Alderson and Roberts, 1997; Roberts et al., 2004; Annane et al., 2002). We suggest that part of the failure may be attributed to the lack of subject stratification based on baseline characteristics as well as cortisol and inflammation status. Although future studies would be required to assess the specifics of this concept, our data indicate that administration of exogenous glucocorticoid in individuals with high acute cortisol profiles and dysfunctional neuroendocrine-immune function would likely be deleterious, as it would contribute to an already exaggerated immune response. On the other hand, tailored glucocorticoid supplementation may be beneficial for some individuals with impaired neuroendocrine-immune response in the low cortisol TRAJ as it might facilitate an adequate acute inflammatory response that would otherwise be lacking. Similarly, the complex interplay between the neuroendocrine and immune responses is an essential consideration when evaluating the potential effectiveness of COX-inhibitor treatments (Hurley et al., 2002). Therapeutic agents targeting inflammation cannot follow a “one size fits all” paradigm, but must instead be carefully tailored to individual physiological characteristics. However, because synthetic glucocorticoids and prostaglandins differ from their endogenous counterparts in many respects, including binding properties, kinetics, metabolism, and BBB permeability, (Sorrells and Sapolsky, 2007; Jozefowski et al., 2003) it is critical for future research to examine differences in the immunomodulatory effects of exogenous vs. endogenous molecules.

Although we primarily investigated CSF cortisol levels and inflammatory mediators, a peripheral inflammatory response can contribute significantly to CNS inflammation. Following secondary injury, centrally derived cytokines, particularly IL-1 β , IL-6 and TNF α , can contribute to increased permeability of the BBB (Stamatovic et al., 2008), allowing blood-borne mononuclear cells and cytokines to enter the brain via paracellular routes. Additionally, cytokine trafficking may occur via receptor-mediated transcytosis or activation of afferent vagal fibers (Banks, 2005; Maier et al., 1998). Brain-to-blood transport for some cytokines may also occur (Phelps and Korneva, 2008). Although the distinct roles of

resident vs. systemic immune cells are still unclear, some have suggested that recruitment of systemic immune cells is actually necessary to terminate cytotoxic neuroinflammation (Schwartz and Shechter, 2010).

Our data show that, aside from IL-6, inflammatory marker concentrations were significantly higher in serum compared to CSF. In addition, serum and CSF levels of IL-10, IL-6, IL-8 and TNF- α were significantly correlated after TBI, a finding that has been used by others as a measure of BBB dysfunction (Kossmann et al., 1995). While purely observational, our results may support the idea that a blood-to-brain gradient contributes to CSF elevations of inflammatory markers. Others have also found that IL-6, a major regulator of the acute phase response, is elevated to a larger degree in CSF than serum, suggesting parenchymal CNS production as driving the CSF (and perhaps also the peripheral) levels observed (Kossmann et al., 1995). Therefore, both resident and systemic molecules may contribute to secondary injury progression, and both compartments should likely be evaluated to determine what constitutes an adequate CSF neuroendocrine-immune milieu to facilitate a therapeutic response to brain injury.

Previous work also shows that CSF cortisol is generated largely from peripheral synthesis and blood-to-brain transit (Santarsieri et al., 2014), and that AI status reflects serum cortisol production (Wagner et al., 2011). While AI tended to be more common for those in the low CSF cortisol TRAJ group, several individuals with AI also were in the high cortisol TRAJ group, suggesting that peripheral cortisol synthesis is not the sole factor contributing to CSF cortisol levels after severe TBI. It is likely that multiple factors, such as BBB permeability and active cortisol efflux capacity (Schinkel, 1999; Loscher and Potschka, 2005; Karssen et al., 2001), as well as the possibility of CNS cortisol synthesis (Yu et al., 2002; Stoffel-Wagner, 2003) and/or impaired CNS sterol metabolism (Holub et al., 2007) may contribute to CSF cortisol levels. Acute systemic adrenal insufficiency was associated with ILS, but only among those in the high cortisol TRAJ group, wherein (paradoxically), an adequate systemic adrenal response to major trauma was associated with significantly higher CSF ILS and, ultimately, unfavorable outcome. This finding further supports the concept of *variable* neuroendocrine-immune dysfunction in the context of severe TBI and may have significant clinical implications for pharmacological management of AI in this population.

While some inflammatory markers were associated with cortisol and outcome, other inflammatory markers were not included in the ILS. Delineating reasons for these markers being less relevant to outcome and cortisol are varied, and beyond the scope of this study. However, the literature suggests that glucocorticoids affect the balance between Th-1 and Th-2 immunity (Elenkov 2004), including some of these cytokines measured on our panel that were not sensitive to cortisol or outcome. Among the candidate inflammatory markers not included in the ILS, IL-12 is modulated by neurotransmitters like epinephrine and norepinephrine to a much greater degree compared to cortisol (Elenkov, 2008). Further, our previously published work suggests that CSF IL-4, IL-7, and IL-12 are not elevated above controls (Juengst, et al. 2014). IL-7 has some capacity for involvement with autoimmunity (Dooms, 2013; Wang et al., 2013) without much yet known about its potential function in TBI. IL-5 is predominantly synthesized by eosinophils and mast cells (Yu et al., 2014), with no known specific functioning in the context of clinical TBI (Kumar et al., 2014; Juengst et

al., 2014; Helmy et al., 2011). While sVCAM was associated with cortisol TRAJ membership, there was no association with outcome. Other markers known to be elevated after TBI, like IL-1 β and IL-8, were not included in the ILS because they were not associated with outcome or cortisol TRAJ. We explored scores that included various combinations of these inflammatory markers in our ILS without substantial improvement in statistical relationships to cortisol TRAJ or outcome.

Chronic stress and depression have also been implicated as predisposing factors to neuroendocrine-immune dysfunction (Cohen et al., 2012; Pace et al., 2007; Carroll et al., 1976). Thus, individual differences in stress-related premorbid factors (e.g. stress due to anxiety, depression, poor social supports) (Kumar et al., 2014; McCauley et al., 2001; Ruff et al., 1996), injury characteristics, and pharmacological treatment may influence interactions between the premorbid stress and immune responses after TBI that adversely impact outcome, though the injury itself may be the primary cause of dysfunctional immune communication (Meisel et al., 2005). Additionally, innate factors such as age and genetic variation may affect inflammation and CNS cortisol efflux across the BBB (Roubenoff et al., 1998; Cousar et al., 2013). Future work may capture these potential contributors for their role in neuroendocrine-immune dysfunction with TBI.

Finally, other hormones besides cortisol play a role in regulating the immune response. In particular, estradiol and testosterone can, both positively and negatively, modulate the immune response via regulation of inflammatory gene expression, (Bird et al., 2008) and some studies suggest sex differences in immune responses to injury/illness, including TBI (Ansar Ahmed et al., 1985) that might warrant future study.

Several limitations of the study exist. Because this study is naturalistic and reflects a true clinical sample, we could not control for, or effectively rule out, the impact of medications on subjects' neuroendocrine-immune responses. However, it is notable that most individuals in a similar cohort were exposed to one or more adrenal suppression agents (Wagner, et al., 2011). This study also cannot draw any conclusions about causation. As such, findings should be regarded as descriptive. Furthermore, sample sizes for our control groups and TBI sub-cohorts (based on TRAJ and GOS) were small, underlining the need to validate results in independent populations. With larger sample sizes, individual GOS categories may be able to be used, instead of dichotomous stratification, when evaluating outcome specific cortisol-inflammatory interactions. Finally, cortisol and inflammatory data was generated from different samples of control participants, precluding the evaluation of cortisol-ILS relationships among healthy controls.

The inflammatory response is essential in preparing the CNS for reparative and regenerative processes, but it can also facilitate secondary damage and neurological dysfunction when exaggerated or prolonged. Our results suggest that cortisol trajectories might serve as a useful lens through which to discern whether inflammation leads to favorable or unfavorable outcome. In the setting of low cortisol, subjects with unfavorable 6-month outcome displayed a negative correlation between inflammation and cortisol. Conversely, in the setting of high cortisol, subjects with unfavorable outcome displayed a positive correlation between inflammation and cortisol. We speculate that unfavorable outcome after TBI may

result, in part, from neuroendocrine-immune dysfunction such that, in the high cortisol TRAJ group, neuroinflammation is exaggerated or, alternatively, in the low cortisol TRAJ group, an adequate immune response is not mounted. Further, we emphasize that because the nature of this dysfunction appears to be cortisol-dependent, an individual's distinctive cortisol response must be evaluated before considering inflammatory treatment. While validation of these findings is needed, our results highlight the heterogeneity of the TBI population, and together with existing data, emphasize the need for evaluating targeted, personalized inflammatory treatment strategies early after TBI. Additional research is needed to explore the individual factors that can influence this complex and highly variable interaction of the stress and immune responses in the injured brain.

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Fig. 1.

Daily Mean CSF Cortisol by Cortisol Trajectory Group. Cortisol levels were significantly different between TRAJ groups on days 0–5 post-TBI ($p < 0.01$), but not day 6. Cortisol levels for all TBI subjects, regardless of cortisol trajectory group membership, were significantly higher than control levels on day 0–5 ($p < 0.01$, all comparisons). On day 6, only TBI subjects in the high cortisol TRAJ had cortisol levels higher than controls ($p < 0.01$). CSF: cerebrospinal fluid; TRAJ: trajectory; TBI: traumatic brain injury.

Fig. 2.

Median Inflammatory Marker Levels (pg/mL) by CSF Cortisol Trajectory Group, compared to controls. Both TBI and control levels were scaled by a multiple of 10 to fit a 0 to 100 range. Regardless of cortisol TRAJ group membership, levels of inflammatory marker levels included in the ILS were higher for TBI subjects compared to controls ($p < 0.01$ all comparisons). CSF: cerebrospinal fluid; TBI: Traumatic Brain injury; TRAJ: trajectory; ILS: Inflammatory Load Score; IL: interleukin; sFAS: soluble Fas; sICAM: soluble intracellular adhesion molecule; TNFα: tumor necrosis factor α.

Fig. 3.

Indirect Effect of Cortisol TRAJ on the Relationship between ILS and GOS. The following are the standardized coefficients for direct comparison: $A=0.517$, $B=-0.489$, $C=-0.173$. CSF ILS has a direct effect on cortisol TRAJ ($p<0.001$). Cortisol TRAJ has a direct effect on GOS ($p=0.006$). The relationship between ILS and GOS is indirectly mediated through cortisol TRAJ group membership (IE= -0.253 , 95% CI $(-0.481, -0.025)$, $p=0.030$). TRAJ: trajectory; ILS: Inflammatory Load Score; GOS: Glasgow Outcome Scale; CSF: cerebrospinal fluid; IE: indirect effect; CI: confidence interval.

Fig. 4.

Fig. 4A. Correlations between ILS and Mean Cortisol by Cortisol TRAJECTORY. Mean CSF cortisol levels and ILS were not significantly correlated for individuals in the low cortisol TRAJECTORY ($r=0.023$, $p=0.902$), but were significantly correlated in the high cortisol TRAJECTORY ($r=0.336$, $p=0.009$). ILS: inflammatory load score; TRAJECTORY: trajectory; CSF: cerebrospinal fluid.

Fig. 4B. Correlations between ILS and Mean Cortisol by Cortisol TRAJECTORY and GOS at 6 Months. Subjects with unfavorable outcome in the low CSF cortisol TRAJECTORY group had mean cortisol levels and ILS that were negatively correlated ($r=-0.562$, $p=0.045$). However, subjects with unfavorable outcome in the high CSF cortisol TRAJECTORY group displayed a positive correlation ($r=0.391$, $p=0.006$) between mean cortisol levels and ILS. ILS: Inflammatory Load Score; GOS: Glasgow Outcome Scale; CSF: cerebrospinal fluid; TRAJECTORY: trajectory group membership.

Fig. 5. Median Inflammatory Marker Levels (pg/mL) in CSF vs. Serum. Inflammatory marker levels were scaled by a multiple of 10 to fit a 0 to 100 range. Serum levels of IL-10, sFAS, sICAM and TNF α were significantly higher than CSF levels ($p < 0.01$ all comparisons). Conversely, CSF levels of IL-6 were significantly higher than serum levels ($p < 0.01$). CSF: cerebrospinal fluid; IL: interleukin; sFAS: soluble Fas; sICAM: soluble intracellular adhesion molecule; TNF α : tumor necrosis factor alpha.

Fig. 6.

Theoretical Model of Variable Neuroendocrine-Immune Dysfunction after Severe TBI.

Cortisol trajectories might serve as a useful lens through which to discern whether inflammation leads to favorable or unfavorable outcome. Individuals with low CSF cortisol and unfavorable outcome have a sub-therapeutic inflammatory response that may be insufficient to facilitate early reparative processes in the injured brain. Among individuals with high cortisol, those with unfavorable outcome have a dysfunctionally permissive immune response that may exacerbate CNS damage. TBI: traumatic brain injury; CSF: cerebrospinal fluid; CNS: central nervous system.

Table 1a
Population Description by Cortisol Trajectory Group Membership and Glasgow Outcome Scale Score at 6 Months

	Cortisol TRAJ			6 Month GOS		
	Low Cort TRAJ	High Cort TRAJ	p-value	GOS 1/2/3	GOS 4/5	p-value
Gender, # male (% male)	25 (80.6)	48 (80.0)	1.000	47 (77.0)	26 (86.7)	0.403
GCS, Median	7.00	7.00	0.844	6.00	7.00	0.057
BMI, Median	25.12	25.74	0.539	25.14	25.56	0.555
Age, Median	27.00	36.00	0.034*	37.00	27.00	0.026*
ISS, Median	34.00	31.00	0.650	34.00	30.00	0.462
LOS, Median	19.00	20.00	0.429	20.00	19.00	0.799
Mechanism of Injury, # (%)						
Motor Vehicle	18 (58.1)	23 (38.3)		25 (41.0)	16 (53.3)	
Motorcycle	6 (19.4)	13 (21.7)	0.199	12 (19.7)	7 (23.3)	0.510
Fall	2 (6.5)	13 (21.7)		12 (19.7)	3 (10.0)	
Other	5 (16.1)	11 (18.3)		12 (19.7)	4 (13.3)	
Radiological Injury Type, # (%)						
SDH	15 (48.4)	45 (75.0)	0.019*	45 (73.8)	15 (50.0)	0.034*
DAI	16 (51.6)	14 (23.3)	0.010**	14 (23.0)	16 (53.3)	0.005**
EDH	5 (16.1)	7 (11.7)	0.534	8 (13.1)	4 (13.3)	1.000
SAH	16 (51.6)	51 (85.0)	0.001**	47 (77.0)	20 (66.7)	0.319
Contusion	12 (38.7)	31 (51.7)	0.274	31 (50.8)	12 (40.0)	0.377
IVH	8 (25.8)	16 (26.7)	1.000	15 (24.6)	9 (30.0)	0.618
ICH	7 (22.6)	23 (38.3)	0.161	22 (36.1)	8 (26.7)	0.478
COBRIT Study, # (%)						
Citicoline	0 (0)	2 (40)	1.000	2 (33.3)	0 (0)	1.000
Placebo	2 (100)	3 (60)		4 (66.7)	1 (100)	

	Cortisol		6 Month GOS		
	Low Cort	High Cort	TRAJ	p-value	
Hypothermia Study, # (%)					
Hypothermia	5 (71.4)	2 (66.7)		1.000	5 (83.3)
Control	2 (28.6)	1 (33.3)			1 (16.7)
Acute Adrenal Insufficiency Status, # (%)					
AI	13 (46.4)	17 (30.9)		0.227	10(38.5)
					0.809

TRAJ, trajectory; GOS, Glasgow outcome scale; GCS, Glasgow coma scale; BMI, body mass index; ISS, injury severity score; LOS, length of stay; SDH, subdural hematoma; DAI, diffuse axonal injury; EDH, epidural hematoma; SAH, subarachnoid hemorrhage; IVH, intraventricular hemorrhage; COBRIT, Citicoline Brain Injury Treatment Trial; AI, adrenal insufficiency.

* indicates statistical significance at the 0.05 level (2-tailed)

** indicates statistical significance at the 0.01 level (2-tailed)

Table 1b**Population Description by Inflammatory Load Score**

	Inflammatory Load Score	p-value
Male, Median	16.00	0.963
Female, Median	15.00	
GCS, Spearman's r	−0.116	0.281
BMI, Spearman's r	−0.038	0.718
Age, Spearman's r	0.328	0.002**
ISS, Spearman's r	−0.080	0.452
LOS, Spearman's r	−0.088	0.406
Mechanism of Injury, Median		
Motor Vehicle	13.00	0.098
Motorcycle	16.00	
Fall	20.00	
Other	17.00	
Radiological Injury Type, Median		
SDH	17.00	0.019*
No SDH	13.00	
DAI	12.50	0.008**
No DAI	18.00	
EDH	11.50	0.264
No EDH	16.00	
SAH	17.00	0.007**
No SAH	11.00	
Contusion	15.00	0.973
No Contusion	16.00	
IVH	16.50	0.688
No IVH	15.00	
ICH	18.00	0.005**
No ICH	12.00	
COBRIT Study, Median		
Citicoline	20.50	0.476
Placebo	20.00	
Hypothermia Study, Median		
Hypothermia	12.00	0.967
Control	17.00	
Acute Adrenal Insufficiency Status, Median		

	Inflammatory Load Score	p-value
AI	10.00	0.026*
No AI	15.00	

GCS, Glasgow coma scale; BMI, body mass index; ISS, injury severity score; LOS, length of stay; SDH, subdural hematoma; DAI, diffuse axonal injury; EDH, epidural hematoma; SAH, subarachnoid hemorrhage; IVH, intraventricular hemorrhage; ICH, intracerebral hemorrhage; COBRIT, Citicoline Brain Injury Treatment Trial; AI, adrenal insufficiency.

* indicates statistical significance at the 0.05 level (2-tailed)

** indicates statistical significance at the 0.01 level (2-tailed)

Table 2

Mean CSF Inflammatory Marker Levels (pg/mL) by Cortisol Trajectory Group Membership

	Low Cort TRAJ	High Cort TRAJ	p-value
IL-10, Mean $\pm$ SE	20.02 $\pm$ 7.874	45.85 $\pm$ 11.95	0.002**
IL-12, Mean $\pm$ SE	0.142 $\pm$ 0.032	0.109 $\pm$ 0.007	0.312
IL-1 β , Mean $\pm$ SE	0.111 $\pm$ 0.036	0.305 $\pm$ 0.106	0.015*
IL-4, Mean $\pm$ SE	0.549 $\pm$ 0.070	0.491 $\pm$ 0.071	0.111
IL-5, Mean $\pm$ SE	0.213 $\pm$ 0.080	0.230 $\pm$ 0.047	0.621
IL-6, Mean $\pm$ SE	527.7 $\pm$ 90.42	990.6 $\pm$ 101.6	0.004**
IL-7, Mean $\pm$ SE	0.964 $\pm$ 0.162	0.703 $\pm$ 0.057	0.053
IL-8, Mean $\pm$ SE	466.9 $\pm$ 111.9	619.4 $\pm$ 83.04	0.075
sFas, Mean $\pm$ SE	183.7 $\pm$ 21.64	305.1 $\pm$ 28.91	0.006**
sICAM-1, Mean $\pm$ SE	3285 $\pm$ 464.5	6273 $\pm$ 627.8	0.002**
sVCAM-1, Mean $\pm$ SE	13912 $\pm$ 2240	86266 $\pm$ 25598	0.010**
TNF α , Mean $\pm$ SE	0.821 $\pm$ 0.360	2.080 $\pm$ 0.812	<0.001**

CSF, cerebrospinal fluid; TRAJ, trajectory; IL, interleukin; sFAS, soluble Fas; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TNF, tumor necrosis factor.

* indicates statistical significance at the 0.05 level (2-tailed)

** indicates statistical significance at the 0.01 level (2-tailed)

Table 3

Mean CSF Levels of Inflammatory Markers (pg/mL) and Cortisol (ng/mL) by Glasgow Outcome Scale Score at 6 Months

	GOS 1/2/3	GOS 4/5	p-value
IL-10, Mean $\pm$ SE	45.04 $\pm$ 11.79	20.79 $\pm$ 8.02	0.009**
IL-12, Mean $\pm$ SE	0.109 $\pm$ 0.007	0.142 $\pm$ 0.033	0.184
IL-1 β , Mean $\pm$ SE	0.303 $\pm$ 0.105	0.107 $\pm$ 0.028	0.388
IL-4, Mean $\pm$ SE	0.505 $\pm$ 0.068	0.523 $\pm$ 0.080	0.613
IL-5, Mean $\pm$ SE	0.231 $\pm$ 0.046	0.210 $\pm$ 0.083	0.363
IL-6, Mean $\pm$ SE	970.47 $\pm$ 100.20	553.17 $\pm$ 97.64	0.007**
IL-7, Mean $\pm$ SE	0.713 $\pm$ 0.055	0.953 $\pm$ 0.169	0.156
IL-8, Mean $\pm$ SE	675.32 $\pm$ 87.47	348.20 $\pm$ 85.56	0.008**
sFas, Mean $\pm$ SE	299.84 $\pm$ 28.17	190.36 $\pm$ 25.17	0.006**
sICAM-1, Mean $\pm$ SE	6279.94 $\pm$ 619.13	3171.72 $\pm$ 456.42	0.001**
sVCAM-1, Mean $\pm$ SE	84185.61 $\pm$ 25240.41	15729.96 $\pm$ 2489.06	0.061
TNF α , Mean $\pm$ SE	2.05 $\pm$ 0.82	0.848 $\pm$ 0.171	0.031*
Cortisol, Mean $\pm$ SE	29.39 $\pm$ 2.63	18.54 $\pm$ 2.12	<0.001**

CSF, cerebrospinal fluid; SEM, standard error of the mean; IL, interleukin; sFAS, soluble Fas; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TNF, tumor necrosis factor; GOS, Glasgow outcome scale.

* indicates statistical significance at the 0.05 level (2-tailed)

** indicates statistical significance at the 0.01 level (2-tailed)

Table 4

Multivariate Logistic Regression Model

6 month GOS (n=88)			
Predictor Variable	Odds Ratio [€]	95% CI	p-value
Age	1.034	(0.989, 1.080)	0.140
GCS	0.743	(0.579, 0.953)	0.020*
DAI	0.457	(0.140, 1.494)	0.195
Inflammatory Load Score [†]	1.070	(0.933, 1.226)	0.333
Cortisol Trajectory Group (2 vs. 1)	3.495	(1.123, 10.884)	0.031*

GOS, Glasgow outcome scale; GCS, Glasgow coma scale; DAI, Diffuse axonal injury; SDH, Subdural hematoma; CI, confidence interval.

* indicates statistical significance at the 0.05 level (2-tailed)

[€] Odds ratios represent GOS 1/2/3 vs. GOS 4/5

[†] the cytokines used for score were: IL-10, IL-6, sFAS, sICAM, and TNFα

Table 5
Relationship between AI Status and ILS by Cortisol Trajectory Group Membership

	Low Cort TRAJ			High Cort TRAJ		
	AI	No AI	p-value	AI	No AI	p-value
ILS, Median	9	9	0.918	11	16	0.030*

AI, adrenal insufficiency; ILS, inflammatory load score; Cort, cortisol; TRAJ, trajectory
* indicates statistical significance at the 0.05 level (2-tailed)