Estradiol to Androstenedione Ratios Moderate the Relationship between Neurological Injury Severity and Mortality Risk after Severe Traumatic Brain Injury

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Abstract

Early declines in gonadotropin production, despite elevated serum estradiol, among some individuals with severe traumatic brain injury (TBI) suggests amplified systemic aromatization occurs post-injury. Our previous work identifies estradiol (E2) as a potent mortality marker. Androstenedione (A), a metabolic precursor to E2, estrone (E1), and testosterone (T), is a steroid hormone substrate for aromatization that has not been explored previously as a biomarker in TBI. Here, we evaluated serum A, E1, T, and E2 values for 82 subjects with severe TBI. Daily hormone values were calculated, and E2:A and E1:T ratios were generated and then averaged for days 0–3 post-injury. After data inspection, mean E2:A values were categorized as above (high aromatization) and below (low aromatization) the 50th percentile for 30-day mortality assessment using Kaplan-Meier survival analysis and a multivariable Cox proportional hazard model adjusting for age, and Glasgow Coma Scale (GCS) to predict 30-day mortality status. Daily serum T, E1, and E2 were graphed by E2:A category. Serum E1 and E2 significantly differed over time ($p < 0.05$); the high aromatization group had elevated levels and a significantly lower probability of survival within the first 30 days ($p = 0.0274$). Multivariable Cox regression showed a significant E2:A*GCS interaction ($p = 0.0129$), wherein GCS predicted mortality only among those in the low aromatization group. E2:A may be a useful mortality biomarker representing enhanced aromatization after TBI. E2:A ratios may represent non-neurological organ dysfunction after TBI and may be useful in defining injury subgroups in which GCS has variable capacity to serve as an accurate early prognostic marker.

Keywords: androstenedione; aromatization; estradiol; estrone; mortality; steroidogenesis; traumatic brain injury

Introduction

APPROXIMATELY 1.7 MILLION PERSONS in the United States sustain a traumatic brain injury (TBI) annually.¹ TBI is considered a critical public health problem because of its high prevalence, with 275,000 hospitalizations and 52,000 fatalities reported in 2010.² The role of biomarkers to characterize the secondary injury response after TBI is increasingly important because of the quantitative nature of these assays and their potential capacity to inform clinical decisions and prognosis beyond clinical and demographic characteristics alone. While most TBI biomarker discovery and clinical development efforts to date have focused on central nervous system (CNS) specific markers, biomarkers reflective of the systemic response to TBI may also provide unique, yet relevant, data informing prognosis and management.

A high percentage of TBI associated mortality due to non-neurological organ dysfunction (NNOD),^{3,4} much of which arises from systemic nervous system (SNS) overactivity and dysfunction.^{3–5} Aseptic peripheral inflammation occurs, in part, from the acute hepatic response after TBI,⁶ a phenomenon resulting from the autonomic SNS in response to TBI.^{6,7} Also, overactivity of SNS-innervated peripheral lymphoid tissue stimulates the systemic inflammatory response to TBI, including ongoing tumor necrosis factor (TNF) α and other cytokine production.^{8,9} TNF α , in addition to contributing to neurological injury,¹⁰ indirectly targets aromatase gene transcription by increasing prostaglandin and estradiol (E2) mediated transcription factor of the aromatase gene.¹¹ This process fuels aromatization by converting androstenedione (A) to E2 in adipose tissue.¹¹ E2 production can further amplify the sympathetic response to injury and estrogen mediated vasodilatory effects^{12–20} by contributing to systemic hypotension and multi-organ dysfunction that leads to systemic compromise.^{21,22}

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In gonadal tissues, as well as extragonadal tissues such as adrenal and adipose tissue, progesterone can be used to generate intermediate hormones in the steroidogenesis pathway such as A (**Fig. 1**). In addition to being a substrate for further androgen metabolism, A is the synthetic precursor for E2 production.²³ Despite experimental evidence of the neuroprotective properties of E2,^{24,25} and previous evidence by our group that higher E2:T (testosterone) ratios in the cerebrospinal fluid (CSF) are associated with better outcome among individuals with severe TBI,²³ our previous serum biomarker work shows strong relationships between increased serum E2 levels and elevated mortality risk after TBI.²⁶ Also, our work²⁶ and work from others^{27–29} show that elevated T and estrone (E1) levels can adversely impact pathophysiology and outcome after CNS injury, suggesting intermediate products within the aromatization pathway may independently and/or indirectly influence TBI outcomes. Moreover, our previous work shows that genetic variability across several single nucleotide polymorphisms within the aromatase gene is associated with global outcomes after TBI.²³

To our knowledge, however, no studies have examined A specifically as a substrate for E2 and E1 production and its relationship to outcome after severe TBI. Understanding E2 synthesis pathways after injury may be relevant to determining if/how systemic E2 production may affect TBI physiology and outcome beyond its association as a biomarker reflective of mortality. Further, it may be clinically useful to assess if/how aromatization pathway biomarkers provide unique information beyond standard clinical metrics, like the Glasgow Coma Scale (GCS),³⁰ thought to reflect extent of neurological injury.

In this study, we examined the relationship between A and its conversion to E1, T, and E2 as a predictor of outcome after TBI. We earlier showed that increased E2 was associated with decreased survival and asked whether aromatase conversion of A was responsible for the increase in E2. We also hypothesized that A paired with E2 as a ratio reflecting aromatization may be a useful biomarker for early mortality prediction after severe TBI that

contributes unique and systemic physiological information about mortality risk. Thus, our objectives are to: (1) describe temporal profiles for A and sex hormone intermediates within the aromatization pathway, (2) analyze A and E2 together as a ratio and its associations to 30-day mortality, (3) analyze if/how covariates representing injury severity can be paired with E2:A ratios to develop a highly predictive prognostic model of mortality after severe TBI, and (4) assess the predictive capacity of the E2:A ratio using model prognostication measures.

Methods

Study design and participants

This study was approved by the Institutional Review Board at the University of Pittsburgh. We assessed 82 adults with severe TBI ($n=65$ men and $n=17$ women) recruited through our level 1 trauma center. Participants had a GCS score of ≤ 8 and received standard care as outlined in *The Guidelines for the Management of Severe Brain Injury*. Participants were between 16 and 73 years of age. Additional inclusion criteria included positive findings on head computed tomography (CT) and extraventricular drainage catheter placement for intracranial pressure monitoring. Subjects with a penetrating brain injury, history of hypothalamic or pituitary tumors, history of breast cancer necessitating chemotherapy, history of prostate cancer necessitating orchiectomy or LH suppression agents, or untreated thyroid disease were excluded.

Demographic variables such as age, sex, and race were collected. Clinical variables included mechanism of injury, GCS (best in 24 h),³⁰ non-head Injury Severity Score (ISS),³¹ length of hospital stay, hospital complications, and admission CT findings assessing brain injury type. Hospital complications were grouped by system assessed and included pulmonary, infectious disease, cardiovascular, musculoskeletal, hematological (HEME), renal, wounds, gastrointestinal, neurological, and other complications. In addition, mortality status was determined at one month post-injury by extracting date of death from the Social Security Death Index.³² Control subjects ($n=11$ men and $n=21$ women), who did not have a history of endocrine dysfunction and were not taking hormone replacement therapy or contraceptives, were enrolled for a single serum sample collection. Demographic information collected from controls at the time of blood draw included age, sex, and race. We assessed mortality at 30 days as a measure of acute care hospital-based outcomes given previously reported mean acute hospital stays of three to five weeks after severe TBI^{33,34} and given that most deaths in this cohort occurred within this time frame.

Sample collection and hormone measurements

Blood samples were collected daily from enrolled TBI subjects during the first three days post-injury. On collection, samples were centrifuged, aliquoted, and stored at -80°C until the time of assay. Serum hormone assessments for E2 ($n=324$ samples) and T ($n=320$ samples) were measured using radioimmunoassay with the Coat-A-Count[®] In-Vitro Diagnostic Test Kit (Siemens Healthcare Diagnostic). Kits included a solid-phase ¹²⁵I radioimmunoassay and were designed for direct, quantitative measurement of each serum hormone. Serum hormone assessments for A ($n=228$ samples) and E1 ($n=205$ samples) were measured using competitive enzyme-linked immunosorbent assays (DRG International, Springfield, NJ). The interassay and intra-assay coefficients of variance were $<15\%$, and any samples that fell below the detection limit were assigned the value of the detection limit for the respective assay. Subjects who had at least one serum sample in the first 0–3 days were included in graphical and statistical analyses.

FIG. 1. Steroidogenesis concept figure. Relevant markers within the steroidogenesis pathway that contribute to the aromatization of E2 (estradiol) are depicted. A (Androstenedione) is a hormone derivative from progesterone. A and Testosterone (T) are steroid hormone precursors that via the aromatase gene produce E1 (estrone) and E2 respectively.

Statistical analysis

Statistical analyses were computed using SAS software (version 9.4. SAS Institute, Inc., Cary, NC). Means and standard error of the mean (SE) were calculated for all continuous variables. Categorical variables were summarized using frequencies and percentages. Associations between continuous variables and differences between categories were determined using the Mann-Whitney test. Associations between categorical variables were assessed using the chi-square analysis and Fisher exact test where appropriate.

In cases where multiple samples were obtained within a particular day for a given individual, daily mean levels were first calculated to generate a day 0, day 1, day 2, and day 3 mean value for each hormone. E2:A and E1:T ratios were then calculated by day to evaluate temporal trends with aromatization pathway intermediates/products, characterized by E2:A ratios, and also the production of aromatization intermediates. After inspecting these profiles, E2:A ratios were then averaged over 0–3 days to evaluate this ratio as an informative time-averaged marker reflecting mortality. Quartiles were initially assessed for E2:A levels; there was a higher percentage of non-survivors in quartiles three and four compared with quartiles one and two (20 vs. 11). Thus, E2:A was dichotomized at the median, with E2:A ratios above and below the 50th percentile representing *high aromatization* and *low aromatization*, respectively. E2:A ratios were used as an index from which to identify high versus low-aromatization risk groups and assess their mortality risk. Average daily hormone levels for A, T, E1, E2, and E1:T were compared by aromatization group. Average hormone levels for control subjects were also graphed to serve as a reference group.

A Kaplan-Meier survival curve was generated to test the potential association between E2:A ratio and time to 30-day mortality. The log-rank statistic was used to compare survival rates among those above and below the median E2:A ratio. A multivariable Cox proportional hazard model was then generated to identify relationships between E2:A ratio and mortality status while adjusting for covariates. All the independent variables that were included were tested for the assumption of proportional hazards before inclusion in the model. The independent variables and covariates were first analyzed in a bivariate analysis before inclusion in the multivariable model and were selected *a priori* based on previous literature.³⁵ All main effects were tested for interactions with E2:A ratios, and significant interaction terms were included in the multivariable model. Hazard ratios and 95% confidence intervals are reported for all main effects and interactions in the multivariable model. Logistic regression models to 30-day mortality were generated to assess the area under the curve (AUC) statistic to describe model prognostication.

Results

Demographic and clinical information for cohort

A detailed description of the cohort by E2:A group (above/below 50th) is presented in Table 1. The mean age for the high-aromatization group was 38.78 (SE, 2.85) years, and the mean age for the low-aromatization group was 39.02 (SE, 2.62). Both groups included more men than women, with most participants self-reporting being White. The median GCS was 7 for the low-aromatization group and 6 for the high-aromatization group. The most common mechanisms of injury for both groups were motor vehicle accidents and motorcycle accidents. There were significant difference in hospital length of stay between the aromatization groups ($p=0.041$), wherein the high aromatization group had a shorter length of stay. Extracerebral trauma scores, as captured by non-head ISS, did not differ by E2:A group.

Complications significantly associated with the aromatization group were HEME ($p=0.032$) and neurological ($p=0.015$). More individuals in the low aromatization group had HEME complications, which include coagulopathy and post-operative hemorrhage. Interestingly, more individuals in the high aromatization group had neurological complications, which include CNS infection, diabetes insipidus, neurosequelae, progression of neurologic insult, and seizures (see Supplementary Table 1; see online supplementary material at <http://www.liebertpub.com>).

Similarly, a description of the cohort stratified by mortality status is shown in Table 2. Non-survivors had a significantly higher mean age compared with survivors (45.23 [SE, 3.23] years vs. 32.25 [SE, 2.28] years, $p=0.010$). Both survivors and non-survivors groups included mostly men with the most prevalent race being White. The median GCS was 7 for the survivors and 6 for the non-survivors. Motor vehicle and motorcycles collisions were the most common mechanisms of injuries for both groups. Extracerebral trauma scores, as captured by non-head ISS, did not differ by mortality group. As expected, non-survivors had a significantly shorter hospital length of stay compared with survivors ($p<0.0001$). Injury types that significantly differed by mortality status were subarachnoid hemorrhage ($p=0.032$) and contusion ($p=0.005$), with non-survivors experiencing more of these injuries; survivors had higher frequencies of intraventricular hemorrhage ($p=0.042$). There were no complications associated with mortality status.

Aromatization hormone profiles

All hormone levels are depicted graphically by day post-injury for T, E2, A, E1, and E1:T ratios, and stratified by aromatization group (Fig. 2). All control hormone levels displayed in each figure are provided in Supplementary Table 2; see online supplementary material at <http://www.liebertpub.com>. Figure 2a shows serum E2 levels were significantly higher days 0–3 in the high aromatization group compared with the low group ($p<0.03$ all comparisons). E2 levels in the high aromatization group were on average three-fold greater than male controls, but were only 1.5-fold greater than male controls in the low aromatization group.

A levels were initially higher than controls, but decreased over time, returning to control levels over the first three days post-injury (Fig. 2b). On average, A levels for the low aromatization group were two-fold greater than male control, whereas A levels for the high aromatization group were only 1.3-fold greater than male control on average. Similarly, serum T levels declined from 0–3 days post-injury with no significant differences by day between aromatization groups; however, T values were approximately one-third lower than male control values (Fig. 2c).

In contrast, E1 levels were elevated compared with male controls by at least 15-fold across both aromatization groups (Fig. 2d). Also, E1 levels were significantly higher over the first three days post-injury in the high aromatization group compared with the low group ($p<0.05$ for all comparisons). E1:T levels gradually increased over the first three days, and increased to four-fold greater than female control (male control values not produced) in the high aromatization group (Fig. 2e). E1:T were significantly larger in the high aromatization group for days 2–3 versus the low aromatization group ($p<0.02$ for both days).

Aromatization risk association with 30-day mortality status

The Kaplan Meier survival curve, shown in Figure 3, depicts a significant difference between aromatization group and time to 30-day mortality (log rank $p=0.027$). Individuals in the high

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BY ESTRADIOL/ANDROSTENEDIONE RATIO GROUP

Variable	Below 50th percentile E2/A (n=41)	Above 50th percentile E2/A (n=41)	p
Age, mean (SE)	39.02 (2.62)	38.78 (2.85)	<i>p</i> = 0.755
Sex, Men (%)	31 (75.61)	34 (82.93)	<i>p</i> = 0.414
Race, n (%)			<i>p</i> = 0.384
Caucasian	39 (95.12)	39 (95.12)	
African-American	2 (4.88)	2 (4.88)	
GCS best in 24, median (IQR)	7 (2)	6 (2)	<i>p</i> = 0.098
Non-head ISS, mean (SE)	12.95 (1.65)	12.91 (1.59)	<i>p</i> = 0.735
Length of Stay, mean (SE)	22.07 (1.80)	17.25 (2.01)	<i>p</i> = 0.041
MOI, n (%)			
MVA	19 (46.34)	14 (34.15)	
Motorcycle	5 (12.20)	10 (24.39)	
Truck	2 (4.88)	2 (4.88)	
Train	1 (2.44)	0 (0.00)	
Off-road vehicle	1 (2.44)	2 (4.88)	
Bicycle	2 (4.88)	1 (2.44)	
Fall/jump	7 (17.07)	9 (21.95)	
Assault/fight	2 (4.88)	2 (4.88)	
Construction, industrial, or farm machine	0 (0.00)	2 (4.88)	
Other	1 (2.44)	0 (0.00)	
Complications, n (%)			
Pulmonary	23 (67.65)	25 (68.44)	<i>p</i> = 0.871
Infectious disease	5 (14.71)	8 (22.22)	<i>p</i> = 0.419
Cardio	3 (8.82)	5 (13.89)	<i>p</i> = 0.239
MSK	0 (0.00)	2 (5.56)	<i>p</i> = 0.261
HEME	8 (23.53)	2 (5.56)	<i>p</i> = 0.031
Renal	6 (17.65)	6 (16.67)	<i>p</i> = 0.913
Wounds	3 (8.82)	8 (8.33)	<i>p</i> = 0.942
GI	3 (8.82)	8 (8.33)	<i>p</i> = 0.942
Neurological	2 (5.88)	10 (27.78)	<i>p</i> = 0.015
Other	3 (8.82)	2 (5.56)	<i>p</i> = 0.669
Injury type, n (%)			
SDH	27 (65.85)	29 (70.73)	<i>p</i> = 0.635
SAH	31 (75.61)	32 (78.05)	<i>p</i> = 0.794
DAI	(17, 41.46)	10 (24.39)	<i>p</i> = 0.100
EDH	6 (14.63)	10 (24.39)	<i>p</i> = 0.265
Contusion	19 (46.34)	21 (51.22)	<i>p</i> = 0.659
IVH	17 (41.46)	11 (26.83)	<i>p</i> = 0.162
ICH	15 (36.59)	11 (26.83)	<i>p</i> = 0.343
Other	1 (2.44)	0 (0.00)	<i>p</i> = 0.500

E2/A, estradiol/androstenedione; SE, standard error; IQR, interquartile range; ISS, Injury Severity Score; MOI, mechanism of injury; MVA, motor vehicle accident; MSK, musculoskeletal; HEME, hematological; GI, gastrointestinal; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; DAI, diffuse axonal injury; EDH, epidural hematoma; IVH, intraventricular hemorrhage; ICH, intracerebral hemorrhage.

Bold indicates statistically significant finding comparison (*p* < 0.05).

aromatization group had a lower survival probability (i.e., higher mortality rates), compared with those in the low aromatization group. Aromatization group, and its association with injury severity, was further explored to examine the risk of 30-day mortality in a multivariable Cox regression model (Table 3). The covariates included in this model were age, GCS, E2:A ratio, and a GCS*E2:A interaction term. The hazard ratios for age (HR: 1.041; 95% CI [1.017, 1.063], *p* < 0.001) and GCS*E2:A group interaction (HR: 1.784; 95% CI [1.130, 2.816], *p* = 0.013) were significant. The relationship between GCS and 30-day mortality varies by E2:A group, such that individuals in the low E2:A group have a lower HR for each unit increase in GCS, as observed below; those in the high E2:A group do not.

The GCS and E2:A relationship is descriptively characterized in Table 4. The mean GCS for survivors in the low aromatization group was 7.5, while for non-survivors in the same aromatization group, the mean GCS was 6.1, where the difference between the two groups tended to be significant (*p* = 0.0523). Both non-survivor groups, however, had a similar GCS of 5.7 and 6.2, respectively, for each aromatization group. Although GCS discriminated mortality status in the low aromatization group (GCS 7.5 vs. 5.7; *p* = 0.022), the ability to differentiate survival status based on neurological injury severity is limited (GCS 6.1 vs. 6.2; *p* = 0.997) in the high aromatization group.

A logistic regression for a baseline model that included the covariates age and GCS resulted in an AUC of 74.5%. The

TABLE 2. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BY 30-DAY MORTALITY STATUS

Variable	Survivors (n=52)	Non-survivors (n=30)	p
Age, mean (SE)	32.25 (2.28)	45.23 (3.23)	p = 0.010
Sex, men (%)	42 (80.77)	23 (76.67)	p=0.659
Race, n (%)			p=0.621
Caucasian	48 (96.15)	30 (93.33)	
African-American	2 (3.84)	2 (6.25)	
GCS best in 24, median (IQR)	7 (2)	6 (2)	p=0.052
Non-head ISS, mean (SE)	12.04 (1.35)	14.64 (2.10)	p=0.332
Length of stay, mean (SE)	24.38 (1.57)	11.41 (1.73)	p = <0.0001
MOI, n (%)			
MVA	24 (46.15)	9 (30.00)	
Motorcycle	10 (19.23)	5 (16.67)	
Truck	2 (3.85)	1 (3.33)	
Train	1 (1.92)	0 (0.00)	
Off-road vehicle	1 (19.2)	2 (6.67)	
Bicycle	2 (3.85)	1 (3.33)	
Fall/jump	6 (11.54)	10 (33.33)	
Assault/fight	3 (5.77)	1 (3.33)	
Construction, industrial, or farm machine	1 (1.92)	1 (3.33)	
Other	1 (1.92)	0 (0.00)	
Complications, n (%)			
Pulmonary	33 (75.00)	15 (57.69)	p=0.132
Infectious disease	10 (22.73)	3 (11.54)	p=0.136
Cardio	4 (9.09)	4 (15.38)	p=0.458
MSK	1 (2.27)	1 (3.85)	p=0.474
HEME	6 (13.64)	4 (15.38)	p=0.266
Renal	7 (15.91)	5 (19.23)	p=0.756
Wounds	2 (4.55)	4 (15.38)	p=0.108
GI	4 (9.09)	2 (7.69)	p=0.840
Neurological	5 (11.36)	7 (26.92)	p=0.095
Other	4 (9.09)	1 (3.85)	p=0.410
Injury type, n (%)			
SDH	31 (62.00)	25 (39.02)	p=0.126
SAH	36 (69.23)	27 (90.00)	p = 0.032
DAI	20 (38.46)	7 (23.33)	p=0.160
EDH	10 (19.23)	6 (20.00)	p=0.933
Contusion	21 (40.38)	19 (63.33)	p = 0.045
IVH	22 (42.31)	6 (20.00)	p = 0.040
ICH	17 (32.69)	9 (30.00)	p=0.801
Other	1 (1.92)	0 (0.00)	p=1.000

SE, standard error; IQR, interquartile range; ISS, Injury Severity Score; MOI, mechanism of injury; MVA, motor vehicle accident; MSK, musculoskeletal; HEME, hematological; GI, gastrointestinal; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; DAI, diffuse axonal injury; EDH, epidural hematoma; IVH, intraventricular hemorrhage; ICH, intracerebral hemorrhage.

Bold indicates statistically significant finding comparison ($p < 0.05$).

addition of E2:A interaction term with GCS in the full logistic regression model resulted in an AUC of 81.3%. Interactions with E2 and GCS, as well as A and GCS, were explored and were non-significant (data not shown), demonstrating the specificity of E2:A ratios as a biomarker capturing unique mortality risk after TBI when paired with a well validated neurological severity measure.

Discussion

In the present study, we compared steroid levels, including E2:A ratio, in survivors and non-survivors of TBI. To our knowledge, this the first study to assess steroidogenesis during the acute response to injury, assess key steroid hormones involved in the aromatization pathway, and the capacity for an aromatization pathway biomarker to predict TBI mortality. These results have significant implications

for appreciating the systemic response to injury, and the data suggest E2:A may be a relevant systemic biomarker for mortality prediction. Our data show that individuals with a high post-injury aromatization state, indicated by E2:A ratios above the median, are more likely to die within the first 30 days of injury. Further, this work raises the question of how individuals in this high aromatization subgroup may respond to sex hormone supplementation with metabolic precursors such as progesterone, because previous work shows that serum progesterone relationships with mortality and poor outcome are mediated by E2.²⁶

E2 plays an important role in predicting outcome after severe TBI. The link between systemic E2 and mortality is not specific to TBI because it has been reported in both sepsis^{36,37} and major trauma,^{38,39} in addition to worse outcome in subarachnoid hemorrhage.²⁹ Our previous study investigating E2 levels in a cohort of

FIG. 2. Aromatization hormone profiles. Serum testosterone (T.), estradiol (E2), androstenedione (A), estrone (E1), and E1/T levels are graphed for days 0–3 post-injury by E2:A group. (a) T levels did not significantly differ between aromatization groups across all three days. (b) E2 levels were significantly greater in the high aromatization group for all three days ($p < 0.03$ all comparisons). (c) A levels differed significantly between aromatization group for day 0 ($p = 0.013$) and day 3 ($p = 0.0003$), where the low aromatization group had higher levels. (d) E1 levels were significantly greater in the high aromatization group across all three days ($p < 0.05$ all comparisons). (e) E1/T ratios significantly differed for days 2 ($p = 0.0136$) and 3 ($p = 0.001$), where the high aromatization groups had greater E1/T levels.

FIG. 3. Kaplan-Meier curve by aromatization group. Survival curves depicting significantly different time to 30-day mortality by aromatization group ($p=0.027$). The high estradiol/androstenedione group had a lower probability of surviving 30 days post-injury.

men and women with severe TBI showed elevated E2 levels were associated with poor outcome and mortality.²⁶ These findings, however, differed from other of our work in CSF showing elevated CSF E2:T ratios in a similar cohort were associated with *lower* mortality and *better* global outcomes.²³ The findings contrasted between these two studies require further investigation of the systemic and CNS aromatization pathways, with a focus on the biosynthesis and mechanistic impact of peripheral E2 production on CNS pathophysiology as well as non-neurological causes of morbidity and mortality after TBI.

Elevated serum E2 in acute illnesses are likely because of increases in extragonadal aromatization,^{40–42} marked by increased transcription of the aromatase gene.⁴³ In addition to other transcription factors,^{44,45} the inflammatory marker, TNF α , drives E2 production in adipose stromal cells by regulating the promoter region I.4 of the aromatase gene.⁴⁶ When biologically induced after TBI, the SNS and hypothalamic-pituitary-adrenal responses to injury lead primarily to amplification of aromatization pathways and elevated serum E2 levels that may represent a non-neurological contribution to mortality. Post-TBI overactivity of SNS-innervated

TABLE 4. GLASGOW COMA SCALE BY MORTALITY STATUS AND AROMATIZATION GROUP

<i>Analysis variable: Glasgow Coma Scale</i>						
	<i>Mortality</i>	<i>N</i>	<i>Mean</i>	<i>Lower quartile</i>	<i>Median</i>	<i>Upper quartile</i>
E2:A Below 50th Percentile	Survivors	30	7.5	7	6	8
	Non-survivors	10	5.7	6	4	7
E2:A Above 50th Percentile	Survivors	20	6.1	6	5	7
	Non-survivors	19	6.2	6	5	7

E2, estradiol; A, androstenedione.

peripheral lymphoid tissue perpetuates the aseptic inflammatory response, including ongoing TNF α production,^{8,9} which, in addition to fueling neurological injury,¹⁰ can catalyze E2 production in adipose tissue because of its role as a tissue specific transcription factor for the aromatase gene.^{42–44} E2 production may have reciprocal effects on SNS-mediated systemic inflammation, through its ability to block adrenergic reuptake^{8,47} and through estrogen receptor-dependent processes,^{49–51} feeding forward the sympathetic response to injury.

In severe TBI, aromatization pathway biomarkers likely reflect a complex interplay between amplified aromatization, the peripheral inflammatory response, and their subsequent effects on peripheral vascular vasodilatation that accompanies systemic hypotension, the development of NNOD, and progression to multi-system organ failure. Approximately 40% of single cause acute mortality associated with TBI is because of NNOD, and 85% of TBI-related mortality is in part because of non-neurological conditions/complications,⁴ suggesting the systemic response to TBI may be just as important to consider as neurological injury when prognosticating mortality or poor global outcome. Patients with TBI are susceptible to sepsis, which may contribute to an exacerbated inflammatory response and lead to NNOD.⁵⁴ One particular study reported that in a population of men with severe TBI, the presence of septic shock and respiratory failure 72 h after initial injury was associated with higher mortality.⁵⁴

Our Kaplan-Meier curve indicates that there is a distinct drop in the survival curve one week after injury, suggesting that those who survived the first 72 h may be highly susceptible to hospital acquired infections and, in turn, NNOD. When taken together, E2:A ratios may serve as an early biomarker readout for NNOD-related mortality. Complications associated with NNOD include arrhythmias, myocardial ischemia, neurogenic pulmonary edema, congestive heart failure, sepsis, and acute kidney injury (AKI).^{3,4,51} These complications occur early and even among those with isolated head injuries,⁴ supporting the importance of the acute autonomic SNS response as a contributor to the systemic response to TBI.^{8,52,53}

As noted earlier, E2 production may have reciprocal effects on SNS-mediated inflammation, by blocking adrenergic reuptake^{8,47} and through estrogen receptor-dependent processes,^{49–51} suggesting the need to characterize the pathway of E2 production after injury as potentially explaining effects on outcome and mortality prediction. Most notably, our data show that in addition to overall amplified aromatization contributing to mortality status, the preferential metabolism of A to E1 may play an important role in discriminating survivors from non-survivors. These results implicate the potential use of a T-dependent pathway for survivors in our

TABLE 3. MULTIVARIATE COX PROPORTIONAL HAZARD MODEL

<i>Variable</i>	<i>Hazard ratio</i>	<i>Confidence interval</i>	<i>p</i>
Age	1.041	1.017–1.063	<0.001
GCS	0.532	0.353–0.801	0.003
E2:A	0.056	0.003–0.897	0.042
GCS*E2:A	1.784	1.130–2.816	0.013

GCS, Glasgow Coma Scale; E2:A, estradiol/androstenedione.

Bold indicates statistically significant finding comparison ($p < 0.05$).

cohort, and a T buildup among those individuals who do not survive, findings that may implicate aromatization pathways as modifiable endpoints, which could impact outcome.

The relative relationship of E2:A in the first three days post-injury provides a novel marker for quantifying the aromatization response after injury, as well as early mortality prediction after severe TBI. The ratio of E2:A in our model has the ability to predict 30-day mortality status, potentially even to a greater extent than standard neurological severity scores. In a logistic regression model, the addition of the ratio improved the model prognostication by 6.8%, suggesting the ratio paired with standard neurological severity measures, such as the GCS, may contribute to the possibility of E2:A ratios being a clinically useful biomarker. Elevated E2 levels are indicative of poor outcome, and the relationship between E2 and multi-system organ failure has been reported after severe polytrauma.³⁹ Further, the effects of E2 in AKI and mortality has been reported in sepsis,³⁶ suggesting peripheral E2 synthesis contributes to mortality status. Our cohort size is too small to tease out the relevance of specific complications and their relationship between hormone profiles and mortality. This work, however, specifically suggests the need to examine if/how mortality from systemic compromise after TBI is E2 related.

This study was not designed to identify baseline hormone measurements as a factor that might impact treatment response in the context of clinical trials. Yet our findings suggest that future studies designed to examine this issue might be warranted. The GCS still remains the most widely used inclusion criteria for clinical trials.⁵⁴ Criticism around the GCS is largely centered on the fact that while it is useful with regard to prognosis after TBI, it does not capture variability in the mechanistic impact of the secondary injury responses that contribute to neurological deficits.⁵⁴ A study investigating the predictive ability of the GCS after TBI found that it had the most accurate prognostication performance when paired with other factors such as age and pupillary response,⁵⁵ suggesting the need to assess GCS performance in the context of other clinical factors or biomarkers such as aromatization status.

Our work demonstrates that GCS discriminates mortality status among those in the lower aromatization state, but individuals in the high aromatization state have similar GCS regardless of mortality status. As such, these results indicate systemic aromatization and sex hormone production may provide added capacity for mortality prediction beyond neurological injury severity, particularly among those who are already severely injured.

Hormone treatments, such as progesterone, have had strong pre-clinical evidence for neuroprotection after TBI.^{56–60} Also, E2 is an end-product of progesterone metabolism, and animal models show neuroreparative associations with exogenous administration of E2.^{61–63} Two large independent Phase III randomized clinical trials, however, have failed to show any significant benefits to exogenous administration of progesterone,^{64,65} warranting a need to elucidate the effects of this putative neuroprotective treatment on acute systemic hormone physiology and aromatization pathway intermediates after TBI, as well as their systemic impacts on mortality. With progesterone being the synthetic precursor to A, T, E1, and E2, injury-induced aromatization, its role in the systemic response to TBI and mortality are important elements for understanding hormone pathophysiology and treatment in the setting of TBI.

This study begins to investigate E2:A as a systemic biomarker, reflective of the systemic response to TBI and informative with regard to one-month outcome. The relationship between GCS and E2:A provides potentially important insights about the contri-

bution of the systemic response post-TBI on mortality; in turn, considering how hormone therapies might impact the aromatization pathway and systemic response to TBI is warranted. This study is not without limitations. We cannot establish causality between E2:A and mortality in this study. The intermediate E1 was associated with mortality, and further work focusing on the role of E1 in mortality prediction and systemic response to TBI is needed. Also, there may be other unmeasured steroidogenic intermediates that may be relevant effectors on mortality status. Other demographic and clinical covariates such as sex and CT findings were assessed; however, they did not yield significant results in predictive models to 30-day mortality.

Thus, given the small sample size, a more parsimonious model is described. Given the small sample size of women in this study, a separate analysis on women was not performed. Subsequent studies, however, should investigate aromatization sex steroid hormones among women, and particularly by menopausal status. In addition, full sample collection was not complete for all patients because of clinical availability and stability of subjects. A descriptive missingness analysis (0–1 missing samples vs. 2–3 missing samples) was conducted, however, and neither E2:A ratios nor mortality was significantly different (data not shown). Sampling strategy along with missingness, however, does limit our ability to assess these markers with more temporal resolution in this cohort during the first 24 h, because we do not have the resolution to build predictive models in this time frame.

Future work should replicate these findings in larger cohorts, assess hormone relationships to NNOD, and characterize the early temporal dynamics of aromatization amplification that accompanies TBI. Further, this work does not include E1, T, or inflammatory/other aromatase transcription factors in predictive models. Future work, however, on acute hormone profiles after TBI has potential implications for understanding which individuals may benefit from early treatment interventions, including hormone therapies, which may impact injury-induced steroidogenesis and E2 production.

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