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Plasma Estrogen Levels Are Associated With Severity of Injury and Outcomes After Aneurysmal Subarachnoid Hemorrhage

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Abstract

Background—Biochemical mediators alter cerebral perfusion and have been implicated in delayed cerebral ischemia (DCI) and poor outcomes after aneurysmal subarachnoid hemorrhage (aSAH). Estrogens (estrone [E1] and estradiol [E2]) are mediators with neuroprotective properties that could play a role in DCI. This study explored associations between plasma estrogen levels and outcomes following aSAH.

Methods—Plasma samples from 1–4, 4–6, and 7–10 days after hemorrhage from 99 adult aSAH patients were analyzed for estrogen levels using liquid chromatography tandem mass spectrometry. DCI was operationalized as radiographic/ultrasonic evidence of impaired cerebral blood flow accompanied by neurological deterioration. Outcomes were assessed using the Modified Rankin Scale at 3 and 12 months after hemorrhage. Statistical analysis included correlation, regression, and group-based trajectory.

Results—Higher E1 and E2 levels were associated with higher Hunt and Hess grade (E1, $p = .01$; E2, $p = .03$), the presence of DCI (E1, $p = .02$; E2, $p = .02$), and poor 3-month outcomes (E1, $p = .002$; E2, $p = .002$). Trajectory analysis identified distinct populations over time for E1 (61 % E1 high) and E2 (68% E2 high). Patients in higher trajectory groups had higher Fisher grades (E1, $p = .008$; E2, $p = .01$), more frequent DCI (E1, $p = .04$; E2, $p = .08$), and worse 3-month outcomes (E1, $p = .01$; E2, $p = .004$) than low groups.

Conclusions—These results provide the first clinical evidence that plasma E1 and E2 concentrations are associated with severity of injury and outcomes after aSAH.

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Keywords

subarachnoid hemorrhage; delayed cerebral ischemia; estrogen; outcomes

Aneurysmal subarachnoid hemorrhage (aSAH) is a sudden and devastating neurological event affecting approximately 33,000 individuals per year in the United States at a mean age of 55 years (with a higher incidence in women, Blacks, and Hispanics; de Rooij, Rinkel, Dankbaar, & Frijns, 2013). Despite advances in medical and surgical management, aSAH continues to be associated with complications necessitating vigilant monitoring and emergent interventions during the acute recovery period (Sehba, Pluta, & Zhang, 2011; Zubkov & Rabinstein, 2009). In addition to the initial bleed and despite early aneurysm repair, a large population of patients develops delayed neurological deficits and delayed cerebral ischemia (DCI; Cahill & Zhang, 2009). The rate of mortality and disability is greater than for other stroke etiologies, leaving survivors with profound physical and neuropsychological disability (de Rooij et al., 2013). Historically, prevention and recognition of cerebral vasospasm has been the primary target for improving outcomes after aSAH; however, clinical trials in which vasospasm was reduced have not resulted in improved outcomes (Ma et al., 2012; Vergouwten, Algra, & Rinkel, 2012). As a result, focus has shifted to early brain injury that may be precipitated by injury to the brain's neurovascular unit as a consequence of the hemorrhage and elevation in intracranial pressure. In particular, biochemical changes that alter the balance of vasoactive metabolites may impact recovery and outcomes following acute neurological insult (Caner, Hou, Altay, Fuj, & Zhang, 2012; Crago et al., 2011; Sehba et al., 2011).

Estrogen has been widely studied in neurological disorders including both experimental ischemic and hemorrhagic insults (Azcoitia, Arevalo, De Nicola, & Garcia-Segura, 2011; Herson, Koerner, & Hum, 2009). E2 alters vascular reactivity, reduces tissue damage, improves functional recovery, and may stimulate repair processes in the central nervous system (Hum & Brass, 2003; Liu, Dziennis, Hum, & Alkayed, 2009). The absence of E2 has been linked to vasoconstriction, impaired cellular metabolism and cellular death in animal models (Azcoitia et al., 2011; Brown, Suzuki, Jelks, & Wise, 2009; Lin et al., 2009; Liu et al., 2009). In addition, researchers have attributed a lower incidence of ischemic neurologic insults and neurodegenerative diseases as well as improved outcomes after traumatic brain injury (TBI) in premenopausal females compared to men of equal age to higher estrogen levels (Liu et al., 2009; Miller & Cronin-Golomb, 2010; Sealy-Jefferson et al., 2012). E1 is a physiologic estrogen that is the predominant estrogen remaining after menopause. In experimental studies, E1 has been related to vascular reactivity and vasodilation through nitric oxide pathways (Rauschemberger, Sandoval, & Massheimer, 2011). It has also demonstrated neuroprotective properties in stroke, reperfusion injury, and TBI animal studies (Gatson et al., 2012).

In studies of patients with critical illness, associations between estrogen levels and improved outcomes have been inconsistent (Gatson et al., 2012; Selles, Polini, Alvarez, & Massheimer, 2005). No study to date has explored whether estrogen levels are related to the incidence of DCI or outcomes in patients after aSAH. The purpose of this study was to

determine the relationship between plasma E1 and E2 concentrations and outcomes (DCI and Modified Rankin Scale [MRS]) following aSAH.

Materials and Methods

Patient Sample

This study was a secondary analysis of prospectively collected data from aSAH patients admitted to the neurovascular intensive care unit who were enrolled in a National Institute of Health (NIH)-funded study (R01NR004339) exploring the relationships between biomarkers and complications of aSAH. For this analysis, we drew a convenience sample of eligible patients from the parent study between 2006 and 2011. Inclusion criteria for the parent study were (1) 21–75 years old, (2) verified aSAH, and (3) Fisher grade > 1 admitted within 3 days of aneurysm rupture. Exclusion criteria were (1) nonaneurysmal SAH, (2) mycotic aneurysm, or (3) history of preexisting debilitating neurologic disease. The sample was stratified to include women (both pre- and postmenopausal) and men. Patients taking any type of hormone or hormone replacement therapy were excluded. The parent study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from the patient or proxy prior to data collection.

Data Collection

We collected sociodemographic data, including gender, age, race, menopausal status, severity of injury (Fisher grade and Hunt and Hess [HIT] score), and clinical data from the patient/proxy and medical record.

Estrogen—As a part of the parent study, plasma samples were collected each morning after initial hemorrhage, centrifuged and aliquoted into cryovials within 60 minutes of collection, and stored at -80°C until analysis. To capture changes in circulating estrogen levels (E1 and E2) during the time period of highest risk for ischemic complications (Days 3–10 after hemorrhage), we pulled and analyzed three plasma samples per subject: the first available sample after consent (generally acquired Days 1–3 after hemorrhage), a Day 5 sample (or middle sample, Days 5–7), and a Day 10 sample (or last sample, Days 9–10).

E1 and E2 were assayed at the University of Pittsburgh (UP) Small Molecule Biomarker Core using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The UP LC-MS/MS method employs a validated liquid-liquid extraction, derealization, and detection with a triple quad mass spectrometer as previously described by Nelson et al. with modifications (Nelson, Grebe, O'Kane, & Singh, 2004). The calibration curves, obtained from extracting known concentrations of E1 and E2 from 2-hydroxypropyl- β -cyclodextrin (0.2% in water), ranged from 0.5 pg/ml (lower limit of quantitation) to 200 pg/ml. All back calculations of calibrators, interday and intraday precision and accuracy, and stability were within acceptable limits (<15% at all tested concentrations; Nelson et al., 2004).

DCI—For this study, we defined DCI as neurological deterioration occurring within 12 hr of impaired cerebral blood flow (CBF). Neurological deterioration was defined by the persistent presence (at least 1 hr) of neurologic decline (an increase [>1 point] in score on

the National Institute of Health Stroke Scale [NIHSS]; decline [>1 point] in the Glasgow Coma Scale score; deterioration in pupil reaction; or documentation of a focal deficit and/or decline in level of consciousness [lethargy, agitation, and confusion] not associated with seizure, hydrocephalus, fever, or medication administration [e.g., sedatives or paralytics]). Impaired CBF was defined using surrogate measurements including cerebral angiography, transcranial Doppler (TCD), and computerized tomography (CT) or magnetic resonance (MR) perfusion scans. Angiographic vasospasm was determined from cerebral angiograms read and coded by neurosurgeons blinded to participant identity and dichotomized as either “negative” or “positive” ($\geq 25\%$ narrowing of cerebral blood vessels). Daily TCDs were coded as abnormal when a systolic middle cerebral artery velocity >200 ml/sec and/or a Lindegaard ratio > 3.0 was identified. Finally, head CT/MR and head CT/MR perfusion scans were reviewed by study personnel for the presence of cerebral ischemia, infarction, or low blood flow in the absence of hydrocephalus or rebleed. All patients received the study institution's standard therapy for SAH patients including strict blood pressure and central venous pressure parameters, nimodipine, hypertensive and hypervolemic therapy.

Outcomes—Outcomes were measured using the MRS at 3 and 12 months after aSAH. Assessments were completed as a part of the parent study during a face-to-face or telephone interview with the patient or primary caregiver. Mortality was determined from follow-up phone calls or hospital records.

Data Analysis

Neither E1 nor E2 data were normally distributed; therefore, we applied data transformation (natural and square root logarithmic). Since few metabolite measurements were not quantifiable or below limits of detection (E1: 1 sample [$<1\%$]; E2: 12 samples [4%]), we assigned those measurements a number at the lowest limit of detection. We dichotomized the 3- and 12-month MRS outcome variable to MRS poor (Score 3–6) and MRS good (Score 0–2). Using log-transformed E1 and E2 measurements, we employed correlations and regression models to evaluate the relationships between E1 and E2 measurements and outcomes while controlling for significant covariates such as demographic characteristics (age, gender, menopausal status, and severity of hemorrhage).

We used group-based trajectory analysis to identify the distinct short-term patterns (homogeneous latent trajectory classes) of E1 and E2 from Day 1–10 after initial hemorrhage. We conducted preliminary analyses to test models for each hormone and then tested and compared the models based on selection criteria such as statistical significance, entropy, the Bayesian information criterion, and the substantive utility of the classes along with clinical judgment to determine the optimal solution for the final number of trajectory groups. We used χ^2 test (weighted and nonweighted) and/or Fisher exact test to explore the associations between demographic and outcome variables and the different trajectory classes for both plasma E1 and E2 in the first 10 days after SAH.

Results

Patient Characteristics

The sample consisted of 99 patients being treated for aSAH, of whom 28 were premenopausal females, 34 were postmenopausal females, and 37 were men (Table 1). Subjects were predominately Caucasian (87%, $n = 86$) with a mean age of 50.4 years (standard deviation [SD] = 10. 1). Severity of injury by HH ranged from 1 (mild headache, no focal deficits) to 5 (deep coma), with 46% ($n = 45$) graded as HH 3. Fisher grades ranged from 2 (diffuse, thin layer of blood) to 4 (intracerebral or intraventricular clot, diffuse or no subarachnoid blood), with an average grade of 3 ($n = 45$, 46%). Of the patients who developed DCI (46%; $n = 45$), a combination of vasospasm demonstrated by cerebral angiography and/or TCD along with neurological deterioration was present in 38 patients. The presence of new cerebral ischemia or infarct along with neurological decline defined DCI in the remaining seven patients. We were unable to determine DCI for three patients (3%) due to poor neurological condition, which precluded our ability to derive neurologic deterioration, and we excluded these patients from analyses involving DCI. All patients had 12-month mortality data; however, long-term outcome data (MRS) for this set of subjects were available for only 65 (66%) subjects at 3 months and 63 (64%) at 12 months. Subjects were lost to follow-up due to patient or family refusal or inability to contact patient or family.

Plasma Estrogen Levels Following aSAH

In addition to raw data, we calculated mean, minimum, and maximum E1 and E2 measurements for each subject and log transformed them for analysis. We employed correlations and regression models to evaluate the relationships between E1 and E2 concentrations and key demographic variables. Metabolite measurements were comparable to those of other studies using high-performance liquid chromatography measurements of E1 and E2 in human subjects (Rothman et al., 2011; Xu et al., 2007). There were no significant differences in E1 or E2 levels by gender or race. Increasing age was associated with higher E1 concentrations ($\beta = .35$, $p < .001$); however, there was no relationship between age and E2 concentrations (Table 2). Levels of both E1 and E2 increased with aSAH severity. Higher E1 and E2 concentrations were associated with higher HH scores ($\beta = .27$, $p = .01$ and $\beta = .21$, $p = .03$, respectively). When we compared E1 and E2 concentrations between dichotomized groups of HH scores, concentrations were higher in the HH 3–5 versus the HH 1 and 2 group ($\beta = .22$, $p = .03$ and $\beta = .23$, $p = .02$, respectively). There was a notable statistical increase in E1 concentrations between HH groups (HH 1 vs. HH 5, $p = .003$; HH 2 vs. HH 5, $p < .001$; HH 3 vs. HH 5, $p = .004$). We observed a similar trend in E2 concentrations; however, it was only statistically significant between HH 2 and HH 5 ($p = .04$). While there were no significant differences in overall Fisher grade for either E1 or E2 concentrations, patients with Grade 4 hemorrhage had significantly higher E1 and E2 levels than those with Fisher 2 (E1, $p = .008$; E2, $p = .08$) or 3 (E1, $p < .001$; E2, $p = .02$) grades.

Using log-transformed E1 and E2 measurements, group-based trajectory analysis (using censored normal model) identified two distinct populations over time for both E1 and E2 values. An estimated 61% ($n = 61$) of patients were in the E1 high and 38% ($n = 38$) in the

E1 low trajectories, with a significant declining pattern in both groups ($p < .001$; mean low = 3.9, $SD = .69$; mean high = 5.12, $SD = .66$). Weighted χ^2 analysis revealed that patients in the high E1 group were significantly more likely to be female ($p = .01$), postmenopausal ($p = .002$), and older ($p < .001$) and have a higher severity of injury as evidenced by Fisher grade ($p = .008$) than patients in the low E1 group (Table 3). An estimated 68% ($n = 68$) of patients were in the E2 high and 31% ($n = 31$) in the E2 low trajectories, with both groups displaying a significant declining pattern ($p = .01$). Using weighted χ^2 analysis, we found that patients in the high E2 trajectory group were more likely to have a higher severity of injury as scored by both HH ($p = .03$) and Fisher ($p = .01$) than patients in the low E1 group.

Plasma Estrogen Levels and Ischemic Outcomes

Using log-transformed mean E1 and E2 measurements, we created regression models to evaluate the relationships between E1 and E2 and DCI. The presence of DCI was significantly associated with higher levels of both plasma E1 ($\beta = .24$, $p = .02$) and E2 ($\beta = .24$, $p = .02$). Patients who developed DCI had a mean E1 of 140 pg/ml ($SD = 145.2$) and mean E2 of 18.3 pg/ml ($SD = 19.3$), as compared to patients who had no evidence of DCI (E1 = 90.6 pg/ml, $SD = 89.5$; E2 = 12.2 pg/ml, $SD = 13.4$), as shown in Table 2.

In addition to log-transformed E1 and E2 measurements, the initial univariate analysis included age, race, gender, HH, and Fisher. Using all variables with a maximum significance of $p = .10$ from the univariate analyses (age, HH), we created regression models to predict DCI. Mean log plasma E1 was significantly associated with DCI ($p = .02$, odds ratio [OR] 2.12, 95% confidential interval [CI]: [1.14, 4]) when we controlled for age and HH. Similarly, in a model with age and HH, mean log plasma E2 remained significantly associated with DCI ($p = .04$, OR 1.7, 95% CI: [1.02, 2.9]). Using trajectory analysis, we found that patients in the higher E1 and E2 trajectory groups were more likely to develop DCI (E1, $p = .04$; E2, $p = .08$) than those in the low groups; however, this result did not reach statistical significance (Table 3).

Plasma Estrogen Levels and Outcomes

Higher mean log estrogen measurements were associated with worse outcomes by MRS. Higher mean log E1 concentrations were correlated with poor MRS at 3 months ($\beta = .38$, $p = .002$) and 12-month mortality ($\beta = .15$, $p = .02$). Higher mean log E2 concentrations were significantly associated with poor outcomes by MRS at both 3 months ($\beta = .38$, $p = .002$) and 12 months ($\beta = .25$, $p = .05$). In a model with age and HH, mean log E1 remained significantly associated with poor 3-month MRS ($p = .02$, OR 2.68, 95% CI: [.78, 9.7]). However, this significance was blunted when DCI was added to the model with HH and age ($p = .07$, OR 2.7, 95% CI: [.73, 10.4]). Likewise, mean log E2 was significantly associated with poor outcome at 3 months by MRS when controlling for age and HH ($p = .03$, OR 2.6, 95% CI: [.79, 8.9]) and remained significant when DCI was included in the model ($p = .05$, OR 2.7, 95% CI: [.7, 10.1]). We identified no relationship between mean log E2 concentrations and outcomes at 12 months when controlling for HH and age with or without DCI ($p = .35$, OR .98, 95% CI: [.91, 1.02]). Mortality was not correlated with either mean E1 or E2 concentrations and was not significant in regression models with HH, age, and DCI (E1, $p = .36$; E2, $p = .28$).

Using trajectory groups, E2 levels were associated with outcomes at 3 and 12 months after aSAH. Patients in the low E2 group were significantly more likely to have good outcomes by MRS at 3 months ($p = .004$) and 12 months ($p = .02$) than patients in the high E2 group. Similarly, patients in the low E1 group were significantly more likely to have good outcomes by MRS at 3 months ($p = .01$) than patients in the high E1 group. In addition, mortality was significantly lower at 12 months in the low E2 trajectory group than in the high E2 group ($p = .04$). We found no significant relationship between mortality and E1 trajectory groups.

Discussion

This study is the first known clinical report of plasma E1 and E2 concentrations in a large cohort of aSAH patients. Given that E1 is the predominant estrogen after menopause and in males, the correlation between age, gender, and estrone levels was not unexpected. We found that higher plasma estrogen levels (both E1 and E2) were associated with a greater severity of injury, increased DCI, and poorer outcomes. Using correlation and regression analyses, we found that higher E1 and E2 concentrations were significantly associated with higher HH and Fisher scores and the development of DCI. Higher levels of E2 were significantly associated with poor outcomes by MRS at 3 and 12 months after hemorrhage. The correlation of E2 to poor outcomes at 3 months was independent of covariates including age, HH, and the presence of DCI. Likewise, E1 was significantly associated with poor MRS at 3 months. We observed comparable patterns using trajectory analysis, where higher E1 levels were associated with higher HH and Fisher scores, and higher E1 and E2 levels were significantly associated with DCI. Finally, patients in the lower trajectory groups for E1 and E2 were significantly more likely to have better outcomes at 3 months than those in the higher-trajectory groups.

The results of this study showed higher concentrations of both E1 and E2 in patients with larger hemorrhage burden and more serious clinical symptoms. In addition, estrogen levels were consistently higher immediately following aSAH. Certainly, we cannot draw definitive conclusions from this singular study; however, these correlations suggest that estrogen may be elevated in response to cerebral injury. Prior research has shown that higher estrogen levels are associated with markers of the stress response such as Cortisol, interleukins, and C-reactive protein (Kauffmann et al., 2011; Lerchbaum et al., 2011; May et al., 2008; Spratt et al., 2006). In these studies, investigators have considered stimulation of inflammatory cytokines to be a contributing factor for increased estrogen levels through increased peripheral aromatization (Kauffmann et al., 2011; May et al., 2008; Spratt et al., 2006; Wagner et al., 2011). Following aSAH, inflammatory mediators released in response to aneurysm rupture and blood in the arachnoid space (Sehba, Hou, Pluta, & Zhang, 2012) could result in a subsequent increase in the production of hormones, including E1 and E2.

Contrary to our expectation, higher levels of estrogen in patients in this analysis did not translate into protection from DCI and did not improve long-term outcomes. In preclinical studies, increased estrogen levels have been consistently associated with decreased ischemia and infarct after hemorrhagic, traumatic, and ischemic neurological insults (Alkayed et al., 1998; Auriat, Plahta, McGie, Yan, & Colbourne, 2005; Lin et al., 2009; Zhang et al., 2009).

Analogous positive associations have not been commonly reported in studies of critical illness in humans. High estrogen levels, primarily E2, have been associated with greater severity of injury and poor outcomes after TBI (May et al., 2008; Wagner et al., 2011), sepsis, surgery, and critical illness (Kauffmann et al., 2011; Lerchbaum et al., 2011; May et al., 2008). In a study of over 300 postmenopausal female patients with acute stroke, researchers found an independent association between endogenous estradiol levels and stroke severity (NIHSS), short-term mortality and functional outcome (MRS; Pappa et al., 2012).

We can derive several explanations for these findings from the literature. The disparities in the outcomes of preclinical and clinical studies may result from the condition of the cells and/or host at the time of estrogen exposure. Studies that have demonstrated beneficial effects from estrogen have predominately involved healthy cells including neurons, glia, and endothelium, either before injury or at the time of injury. In the presence of age, disease or neurologic compromise and injury, the effect of estrogen may exacerbate neuronal injury in large part through dysfunction of cellular calcium regulation (Brinton, 2008). Factors necessary for estrogen-supported neuroprotection may be absent or interfered with at the time of neurologic insult. First, insulin growth factor (IGF-1) facilitates the neuroprotective actions of estrogen and other hormones. In the absence of IGF-1, estradiol may be neurotoxic (May et al., 2008). IGF-1 levels decline with age and investigators have posited these decreased levels as a factor for poor outcomes in older females treated with hormone therapy (Azcoitia et al., 2011). Researchers have also demonstrated associations between low IGF-1 levels and stroke severity in older females and increased morbidity and mortality in ischemic heart disease and stroke (Aberg et al., 2011; Bendel et al., 2010; Bondanelli et al., 2006; Johnsen et al., 2005). Bendel et al. (2010) reported low IGF-1 concentrations following ictus in a small cohort of aSAH patients ($N = 30$); levels returned to baseline at 3 months post hemorrhage. Second, the balance between estrogens and other neurosteroids may play a role in ischemia and outcomes (Vergouwen, Vermeulen, & Roos, 2009). Specifically, Garringer et al. (2013) found that a lower ratio of estradiol to testosterone was associated with mortality and poor outcomes after traumatic and ischemic brain injury. Finally, estrogen receptors ER α and ER β , necessary for neuroprotective responses, may be suppressed in such a manner that these mechanisms are diminished. ER α has been implicated in anti-inflammatory and protective mechanisms, while ER β may be more active in recovery after insult. Yi et al. (2011) showed that selective estrogen receptor modulators (SERMs) reduce brain inflammation and neurodegeneration and promote positive cognitive outcomes in animal studies (Yi et al., 2011).

The results from this study demonstrate relationships between E1 and E2 levels and injury severity, DCI and outcomes in aSAH patients. The physiologic explanation for these findings is not clear. The relationships may be due to increases in estrogen levels that result from pathogenic mechanisms such as inflammation, thereby representing an adaptive response. However, in the impaired brain, cells may be negatively affected by higher estrogen levels, or factors necessary for estrogen to play a neuroprotective may be impacted resulting in unexpected deleterious outcomes. Further validation of these findings is

necessary to determine how estrogen may impact response to injury and outcomes after aSAH.

Limitations

In this study, we analyzed E1 and E2 levels on a representative set of samples in aSAH patients. We selected samples based on their availability for specific time points during the time of highest neurologic risk. Analyzing daily levels might provide a more robust trajectory analysis as well as the opportunity to evaluate temporal levels of estrogen and the timing of DCI. If variations in daily estrogen levels after hemorrhage were correlated with estrous cycle information in premenopausal females, it would be possible to examine the potential confounding effect of gonadal hormone surges on outcomes. Because we used retrospective data, specific information regarding estrous cycle in premenopausal females and onset of menopause in postmenopausal females was not available. Researchers should consider including other neurosteroids, growth hormones, SERMs, and inflammatory markers in future studies to elucidate the associations among these markers and outcomes after aSAH. We acknowledge that the definition of DCI is fluid and debated. We defined DCI via components of surrogate blood flow measurements in the presence of sustained neurological deterioration. Although this definition is similar to accepted criteria for DCI, it does use TCD information in addition to CBF and CT information. Since follow-up angiograms are performed as a result of clinical changes rather than research protocols, angiographic vasospasm information was not available on all subjects.

Conclusion

This study provides the first known evidence evaluating the relationships between estrogen concentrations and injury severity and outcomes in patients after aSAH. Our findings are contrary to those of preclinical studies, which have reported neuroprotective effects of estrogen after neurological insult and ischemia. Increased estrogen may be a response to injury resulting in consequential pathologic mechanisms that impact outcomes, or estrogen response may be impacted by the injured cells and lack of mediators necessary to promote neuroprotection. Further work is necessary to better understand the role of estrogen in cerebral protection in humans in order to identify potential pathways for nurses and clinicians to target for intervention to prevent or treat the cascade of events that produce adverse outcomes after aSAH.

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Table 1

Demographic Data and Plasma Estrogen Analysis.

Variable	Female		Male (<i>n</i> = 37)
	Premenopausal (<i>n</i> = 28)	Postmenopausal (<i>n</i> = 34)	
Age, years (mean [SD])	43.54 (5.97)	59.1 (5.78)	47.5 (10.2)
Race			
Caucasian	22 (79)	31 (91)	33 (89)
Other	6 (21)	3 (9)	4 (11)
Hunt and Hess			
1	2 (7)	2 (6)	1 (3)
2	9 (32)	5 (15)	11 (30)
3	12 (43)	17 (50)	16 (43)
4	4 (14)	7 (21)	9 (24)
5	1 (4)	3 (9)	0 (0)
Fisher			
2	14 (50)	8 (24)	14 (38)
3	10 (36)	16 (47)	19 (51)
4	4 (14)	10 (29)	4 (11)
DCI	16 (57)	15 (44)	14 (38)
DCI vasospasm sources	14 (50)	12 (35)	12 (32)
DCI cerebral infarction	2 (7)	3 (9)	2 (5)
MRS 3 month (<i>n</i> = 65)			
Good	12 (63)	13 (57)	13 (57)
Poor	7 (37)	10 (43)	10 (44)
MRS 12 month (<i>n</i> = 63)			
Good	14 (78)	17 (68)	14 (70)
Poor	4 (22)	8 (32)	6 (30)
Mortality (12 month)			
Alive	24 (86)	26 (76)	31 (84)
Dead	4 (14)	8 (24)	6 (16)
Samples			
E1 detected	84 (100)	102 (100)	110 (97)
E2 detected	83 (99)	96 (94)	106 (95)

Note. *N* = 99. Data are reported as *n*(%) unless otherwise indicated DCI = delayed cerebral ischemia; E1 = estrone; E2 = estradiol; MRS = Modified Rankin Scale.

Table 2

Correlation Analysis of Mean Plasma Estrogen Measurements.

Variable	E1			E2		
	Mean (SD)	β	p Value	Mean (SD)	β	p Value
Gender		.18	.07		.11	.25
Male	100.9 (108.1)			13.7 (14.0)		
Female	122.3 (125.1)			15.8 (17.7)		
Race		.02	.88		-.05	.61
Caucasian	115.9 (125.2)			15.2 (16.2)		
Other	106.8 (73.4)			14.4 (18.5)		
Age		.35	<.001		.11	.26
Hunt and Hess		.27	.01		.21	.03
HH 1	63.7 (46.5)			8 (7.1)		
HH 2	92 (112.5)			12.65 (14.3)		
HH 3	118.3 (131.2)			15.7 (17)		
HH 4	143.2 (116.5)			18.6 (20)		
HH 5	138.4 (53.0)			13.6 (7)		
Dichotomized Hunt and Hess		.22	.03		.23	.02
1,2	87.3 (104.8)			11.9 (13.4)		
3-5	126.7 (123.9)			16.4 (17.5)		
Fisher		.18	.08		.16	.12
2	111.1 (100.5)			15.2 (16.7)		
3	98.5 (115.8)			13.6 (16.5)		
4	162.6 (150.5)			18.4 (15.8)		
DCI		.24	.02		.24	.02
No	90.6 (89.5)			12.2 (13.4)		
Yes	140 (145.2)			18.3 (19.3)		
Menopause		-.12	.22		-.14	.17
Premenopause	111.5 (152.7)			16.2 (20.5)		
Postmenopause	135.1 (86.2)			15.4 (14.4)		
Male	100.9 (108.1)			13.7 (14.0)		

Variable	E1			E2		
	Mean (SD)	β	p Value	Mean (SD)	β	p Value
MRS, 3 month		.38	.002		.38	.002
Good	97 (120.7)			12.9 (16.1)		
Poor	156.3 (104.4)			22.2 (18.7)		
MRS, 12 month		.17	.18		.25	.05
Good	116.4 (128.6)			15.2 (18)		
Poor	150.6 (107.4)			20.5 (18.2)		
Mortality at follow-up		.15	.13		.15	.12
Alive	108.27 (90.34)			14.16 (13.47)		
Dead	143.78 (80.49)			19.95 (12.37)		
E1/E2					.78	<.001

Note. Estrogen measures are mean for all three-study time points combined. DCI = delayed cerebral ischemia; E1 = estrone; E2 = estradiol; HH = Hunt and Hess; MRS = Modified Rankin Scale; SD = standard deviation.

Table 3

Trajectory Analysis of (Log) Plasma Estrogen Measurements.

Variable	E1			E2		
	High n = 61	Low n = 38	p Value	High n = 68	Low n = 31	p Value
Gender			.01			.9
Male	16	21		25	10	
Female	45	17		43	21	
Race			.25			
Caucasian	51	35		59	27	.86
Other	10	3		9	4	
Age			<.001			.99
Hunt and Hess			.29			.21
Hunt and Hess dichotomized			.12			.03
1,2	15	15		16	14	
3–5	46	23		52	17	
Fisher			.008			.01
2	24	12		22	14	
3	21	24		28	17	
4	16	2		18	0	
DCI			.04			.08
No	26	25		31	20	
Yes	32	13		34	11	
Menopause			.04			.99
Premenopause	19	12		21	10	
Postmenopause	26	5		21	10	
Male	16	21		26	21	
MRS, 3 month			.01			.004
Good	19	19		21	17	
Poor	22	5		24	3	
MRS, 12 month			.31			.02
Good	26	14		23	17	

Variable	E1			E2		
	High <i>n</i> = 61	Low <i>n</i> = 38	<i>p</i> Value	High <i>n</i> = 68	Low <i>n</i> = 31	<i>p</i> Value
Poor	18	5		20	3	
Mortality at follow-up			.14			.04
Alive	47	34		52	29	
Dead	14	4		16	2	

Note. DCI = delayed cerebral ischemia; E1 = estrone; E2 = estradiol; MRS = Modified Rankin Scale.