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Trends in Estradiol During Critical Illness are Associated with Mortality Independent of Admission Estradiol

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Abstract

Background—We have previously demonstrated that elevated serum estradiol (E2) upon ICU admission is associated with death in the critically ill, regardless of gender. However, little is known about how changes in initial E2 during the course of care might signal increasing patient acuity or risk of death.

Hypothesis—Changes from baseline serum E2 during the course of critical illness are more strongly associated with mortality than a single admission E2 level.

Methods—A prospective cohort of 1408 critically ill or injured non-pregnant, adult patients requiring ICU care for ≥ 48 hours with admission and subsequent E2 levels was studied. Demographics, illness severity, and E2 levels were examined and the probability of mortality was modeled with multivariate logistic regression. Changes in E2 were examined by both ANOVA and logistic regression.

Results—Overall mortality was 14.1% (95% confidence interval (CI) 12.3%–16%). Both admission and subsequent E2 levels were independently associated with mortality [admission estradiol odds ratio 1.1 (CI 1.0–1.2); repeat estradiol odds ratio 1.3 (CI 1.2–1.4)], with subsequent values being stronger. Changes in E2 were independently associated with mortality [odds ratio 1.1 (CI 1.0–1.16)] and improved regression model performance. The regression model produced an area under the ROC curve of 0.80 (CI 0.77–0.83).

Conclusions—While high admission levels of E2 are associated with mortality, changes from baseline E2 in critically ill or injured adults are independently associated with mortality. Future

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studies of E2 dynamics may yield new indicators of patient acuity and illuminate underlying mechanisms for targeted therapy.

Keywords

Estradiol; cytokines; critical illness; trends in estradiol

INTRODUCTION

The contribution of gender to outcome in the settings of sepsis, burns, and trauma has been extensively studied[1–17], but results remain inconclusive. Animal models have demonstrated that estradiol and the pro-estrous state are proinflammatory with improved survival after trauma-hemorrhage and sepsis, whereas testosterone and the pro-androgenic state are anti-inflammatory leading to immune depression and decreased survival [3,6,18–22]. These findings have led to a recent call for the administration of intravenous estrogen in critically ill and injured patients[23]. However, while animal data generally supports such therapy, observational clinical studies in humans are much less consistent. Two significant differences between animal models and the human clinical situation must be considered. First, although a robust inflammatory response conveys a survival advantage in animal models of untreated sepsis, in humans it contributes to the systemic inflammatory response syndrome and organ dysfunction *after* the septic insult has been treated and controlled with antibiotics. Second, estrogen biosynthetic pathways activated by the stress response are present in humans but *not* non-primate species. Primates possess peripheral aromatase enzyme activity (responsible for the conversion of androgens to estrogens) in adipocytes, fibroblasts, and osteoblasts which are stimulated by class I cytokines resulting in peripheral conversion of androgens to estrogens following stress [24–26]. In contrast, gonadal production in all species is inhibited by stress. These differences and other factors raise questions about the applicability of many animal studies to clinical practice.

Previously, we and others have demonstrated that serum estradiol (E2) levels are elevated in critical illness and that levels correlate with outcome regardless of gender [27–29]. The correlation of E2 obtained within 48 hrs of admission with mortality was stronger than TNF, IL-2, IL-6, or IL-10. However, the correlation of E2 levels and changes in E2 levels during critical illness has not previously been examined. We hypothesized that E2 levels obtained during the course of surgical critical illness and particularly changes in E2 would more strongly correlate with mortality than a single value obtained early in the course of illness. The objective of this study was to examine the correlation of serum E2 levels during the hospital course and changes in E2 with the in-hospital, 28-day, all-cause mortality in a population of critically ill and injured surgical patients.

METHODS

Study design and participating centers

A multi-institutional, prospective, longitudinal cohort study was conducted at the University of Virginia Health System and Vanderbilt University Medical Center after approval of each Institutional Review Board. Patients 18 years and older admitted for at least 48 hours to the Surgical-Trauma Intensive Care Unit (ICU) at the University of Virginia Health System, or the Surgical ICU or Trauma ICU at Vanderbilt University Medical Center from October 2001 to May 2006 were eligible for enrollment. A minimum ICU stay of 48 hours was required to exclude postoperative surgical patients with short observational stays and early deaths. Patients with burns as the primary indication for ICU admission and pregnant patients were excluded.

Data collection

Clinical data were prospectively collected by full-time research nurses at both facilities. Information sources included patients and their families, nursing flow sheets, paper and electronic medical records, and healthcare providers. Gender, age, body mass index (BMI), date of hospital and ICU admission, and hospital mortality were recorded. Severity of illness scores and prognostic predictors including the Acute Physiology and Chronic Health Evaluation (APACHE) II score [30] and the Multiple Organ Dysfunction (MOD) score [31] were calculated at the time of study entry and were based on the worst physiologic parameters in the first 24 hours after hospital admission. The Injury Severity Score (ISS) [32] and Trauma Score-Injury Severity Score (TRISS) probability of survival [33] were also calculated for trauma patients following discharge. Patient or family reported menopausal status was recorded when available. When not available by interview, menopausal status was estimated by patient age (<45 years premenopausal, 45 to 55 years perimenopausal, >55 years postmenopausal). Post-study analysis revealed that this age group categorization accurately predicted menopausal status in 86% of cases where menopausal status was known. Patient care was at the discretion of the attending physician according to established critical care protocols in the respective ICUs.

Hormonal and cytokine assays

One 10 mL blood sample was collected at study entry for analysis of hormone and cytokine levels. Serial hormone and cytokine levels were collected twice weekly until death, discharge, or day 28, whichever came first. Plasma was separated from whole blood and stored at -70°C until analysis. Assays were performed in the General Clinical Research Center at the University of Virginia, General Laboratory at Vanderbilt University Medical Center, or the University of Alabama at Birmingham. Estradiol was measured by kit EIA utilizing a competitive immunoassay strategy and alkaline phosphatase/p-Npp chromogenic reaction (Estradiol, catalog # 90108, Assay Designs, Inc., Ann Arbor, MI). Samples were also assayed for IL-1, 2, 4, 6, 8, 10, 12, and TNF- α . This was accomplished by using a LINCoplexTM custom kit, following the manufacturer's instructions (Linco Research, Inc., St. Charles, MO). Twenty-five μL of human serum from each collection time point on all patients was run on duplicate on a Luminex 100 System[®] (Miraibio, Inc., Alameda, CA) to determine the concentration of cytokines of interest. The Luminex[®] system is an ELISA based technology allowing multi-analyte detection in a single well. Data reduction was performed with STATLia[®] (Brendan Scientific) and subsequently incorporated into the database. Blood samples for hormone and cytokine analysis were prospectively drawn from each patient twice weekly, and were separate from blood samples drawn in the course of patient care.

Statistical analysis

Statistical analysis was performed using STATA version 11.0 (STATA Corp., College Station, TX, USA). Continuous variables with normal distributions were summarized by reporting the mean and standard deviation and survivors vs. non-survivors were compared using two sample *t*-tests for independent samples. Other continuous variables were presented by reporting their median and interquartile range (IQR) and compared using the Wilcoxon Rank Sum tests. Differences in proportions were compared using a chi-square or Fisher's exact test. Comparisons among more than two groups were made using one-way Analysis of Variance (ANOVA), with Bonferroni correction. All confidence intervals are at the 95% level.

The effects of baseline E2, change in E2, APACHE II, age, trauma status, gender by menopause status, and gender on mortality were assessed by univariate logistic regression models. These covariates (other than change in E2) were chosen based upon known

associations with mortality in this population.. Change in E2 was defined as the difference between the first and second E2 levels. Admission E2 and difference in E2 were chosen for inclusion in the regression models since the use of absolute difference is the statistically preferable means by which to measure change in a parameter[34], and the inclusion of admission E2 controls for baseline level. A model with baseline E2 and difference in E2 was also considered. This model is preferable to one containing admission E2 and repeat E2 as measures of trend, since such a model would not account for correlation between admission E2 and repeat E2 values. A computation of the area under the receiver operating characteristic (AUROC) curve for multiple variables was performed. The ROC curve is a graphical representation of the sensitivity and specificity of a given diagnostic test. As the AUROC approaches 1.0, it becomes more accurate; as the area approaches 0.5, it becomes random and of little diagnostic value. The area under the ROC curve was used as an overall assessment of each model's predictiveness. Differences between AUROC curves for nested models were compared using a likelihood ratio test. Tests for statistical significance were two-sided with an alpha of 0.05.

Restricted cubic splines with 3 knots[35] were examined for each continuous covariate in an effort to enable the model to accurately detect non-linear relationships. Nested models were compared with a likelihood ratio test. In several cases, the simple logistic regression model did not fit the data well (i.e., using a likelihood ratio test, the null hypothesis that the relationship between the log-odds of mortality and the variable of interest could be rejected). In the case of E2 and difference in E2, the likelihood ratio test suggested that the relationship between these covariates and the mortal log-odds may be better described by a non-linear model. However, when restricted cubic splines were used for these variables and entered into the regression model, the improvement in model fit was primarily due to a nonlinear relationship between the log-odds of death and change in E2. For this reason, E2 data was kept in the model as a continuous covariate, and a restricted cubic spline with 3-knots was fit and entered into the logistic regression model for the difference in E2 variable only[35].

The effects on mortality of baseline and change in E2 in combination with the other covariates listed above were also assessed with a multiple logistic regression model.

RESULTS

Patient Demographic and Clinical Characteristics

A total of 2,289 patients were enrolled in the entire study. To study the correlation of E2 levels during the hospital course and changes in E2 with mortality, patients with an admission E2 and a repeat E2 drawn 2–5 days later were included in the cohort for analysis of serial hormones. Any patient without a subsequent draw or without an initial E2 within 48 hours of enrollment was excluded from analysis. Excluded patients represented those who either died or were discharged from the ICU before a repeat E2 level could be drawn. A total of 1408 patients met inclusion criteria and had complete E2 data, with an overall 28-day mortality of 14.1% (CI 12.3%–16%). The included and excluded cohorts had similar mortality (Figure 1). The demographics, clinical characteristics, initial hormone and cytokine levels and repeat hormone and cytokine levels by outcome are displayed in Table I. Non-survivors were older [59.5 years (+/- 16.6) vs. 47.8 (+/- 17.6) for survivors] with higher APACHE II scores [22.14 (+/- 6.7) vs. 17.18 (+/- 6.1) for survivors]. Most deaths occurred in non-trauma patients (65.7%, CI 58.6%–72.2%). The overall mortality for trauma patients was 7.8% (CI 6.1%–9.8%) and 23.9% (CI 20.4%–27.7%) for non-trauma patients. Survivors and non-survivors did not differ by gender, BMI, or white blood cell (WBC) count. Median admission E2 and second E2 levels were significantly higher in non-survivors, regardless of gender or trauma status.

We then sought to determine the risk of mortality by serum E2 level. The normal physiologic range of E2 for men is ≤ 54 pg/mL and ≤ 75 pg/mL for women (although this is affected by timing of menstruation and menopause). We therefore dichotomized patients into one of two groups, based upon admission and repeat E2 levels. Group 1 was comprised of men and women whose admission E2 was in the normal range and stayed in the normal range at their repeat E2 level, and men and women whose admission E2 was above the normal range but trended to normal levels by the time of their repeat E2 level. Group 2 was comprised of men and women whose admission E2 was above normal and stayed above normal when their repeat E2 was drawn, and men and women whose admission E2 was in the normal range but trended to above normal by the time of their repeat E2 level. Mortality in group 1 was 9.8% and mortality in group 2 was 32.8% ($p < 0.001$), indicating that as E2 trends up, the risk of mortality increases.

The mortality associated with serum E2 levels grouped into quartiles was then determined for both admission and repeat E2 levels. The median admission E2 level for the entire population was 29 pg/mL (IQR 10–67). Admission E2 was divided into quartiles as follows: undetectable < 10 pg/mL (mortality 9.1%, 95% CI 6.5%–12.2%), low ≥ 10 pg/mL to < 29 pg/mL (mortality 10.5%, 95% CI 7.2%–14.6%), intermediate ≥ 29 pg/mL to < 67 pg/mL (mortality 11.2%, 95% CI 8.1%–14.9%), high ≥ 67 pg/mL (mortality 25.2%, 95% CI 20.8%–30.2%). Repeat E2 was divided into quartiles as follows: undetectable < 10 pg/mL (mortality 7.4%, 95% CI 5.4%–9.9%), low ≥ 10 pg/mL to < 18 pg/mL (mortality 10.4%, 95% CI 5.8%–16.9%), intermediate ≥ 18 pg/mL to < 48 pg/mL (mortality 10.9%, 95% CI 7.8%–14.5%), and high ≥ 48 pg/mL (mortality 28.9%, 95% CI 24.3%–33.9%). When grouped by admission E2 quartile, patients in the highest E2 quartile had a mortality rate of 25.2% (CI 20.8%–30%) compared to a mortality of 9.1% (CI 6.5%–12.2%) in patients in the lowest quartile ($p < 0.0005$). Patients with a repeat E2 in the highest quartile demonstrated a higher mortality rate of 28.9% (CI 24.3%–33.9%) compared to 7.4% (CI 5.4%–9.9%) in patients with a repeat E2 in the lowest quartile ($p < 0.001$).

We hypothesized that the changes in E2 (magnitude and direction of E2) would be more strongly associated with mortality than a static admission value alone. To examine the relationship between changes in E2 and mortality, four groups were created relative to differences in admission and repeat E2 quartiles. Mortality rates of patients in these groups are displayed in Table II. Mortality was significantly greater in groups trending to the highest quartile or remaining high than for those values trending to the lowest quartile or remaining in the lowest quartile. There was no significant difference in mortality between patients whose admission and repeat E2 levels were in the lowest quartile compared to patients whose admission E2 was in the highest quartile and trended to the lowest quartile. Risk of death by admission E2 quartile and by trend in E2 quartile is demonstrated in Figures 2 and 3.

The Effect of Change in E2 on Mortality

Univariate regression models—The probability of death by difference in E2 is shown in Figure 4. The probability of death increases markedly with positive elevations in serum E2 levels. A large drop in serum E2 was also associated with a non-significant elevation in mortality. This appeared to be due to the high severity of illness in these patients and their corresponding high E2 levels upon admission. Repeat E2 and difference in E2 were included to assess the association between changes in E2 over time and mortality. Univariate logistic regression was performed to determine the association between each covariate and the risk of death. The receiver operating characteristic (ROC) curve for the unadjusted covariates was derived and the odds ratio (OR) and area under the ROC (AUROC) curve with corresponding 95% CI for each covariate are displayed in Table III. APACHE II yielded the

largest AUROC curve (0.71, CI 0.67–0.75). Of the single variables, repeat E2 was the most predictive (0.69, CI 0.65–0.73), followed by age (0.68, CI 0.64–0.72) and initial E2 (0.64, CI 0.59–0.68). Other variables providing no predictive value were BMI (0.49, CI 0.45–0.54), gender (0.48, CI 0.45–0.52), and diabetes (0.52, CI 0.49–0.55). Both unadjusted admission (OR for 75th vs. 25th percentile 1.1, 95% CI 1.0–1.2, *p* 0.002) and repeat (OR for 75th vs. 25th percentile 1.3, 95% CI 1.2–1.4, *p* 0.005) E2 levels were significantly associated with mortality, with the second E2 value being more strongly associated with mortality (Figure 5).

Construction of multivariate regression model—To determine whether baseline serum E2 levels repeat E2 levels were independently associated with mortality, we performed a multiple regression analysis that also included age, trauma status, APACHE II score, gender, menopause status, and diabetes as covariates. Admission and repeat E2 levels remained significantly associated with mortality when adjusted for age, gender, trauma status, diabetes and APACHE II score (admission E2 OR for 75th to 25th percentile 1.1, CI 1.03–1.17, *p* 0.002 vs. repeat E2 OR for 75th to 25th percentile 1.2, CI 1.1–1.3 *p* <0.001). The difference in E2 covariate was added to the model containing admission E2 and the covariates previously identified to estimate the independent effect of change in E2 on mortality. The adjusted OR, 95% confidence intervals and corresponding *p*-values for these variables are displayed in Table III. Admission E2 was significantly associated with mortality. When the difference in E2 as a variable for trend was included in the logistic regression model, it was a strong predictor of mortality, both in size of effect and statistical significance. Gender was not statistically associated with mortality. Addition of a term for E2 trend using a restricted cubic spline improved logistic regression model performance compared to the model containing E2 alone (*p* 0.005). The overall predictive value for the logistic regression model including admission E2 and a variable for trend in E2 was 0.80 (CI 0.77–0.83) vs. 0.78 (CI 0.75–0.81) for the logistic regression model including admission E2 but not trend in E2 (*p* 0.001).

Cytokine subgroup analysis—Because the role of serum cytokines in the modulation of the inflammatory cascade and their association with mortality are both well accepted, we performed a subgroup analysis to compare the strength of association of E2 with mortality with that of various cytokines, including IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12, measured at the same time point. Cytokine levels were ordered on all 1408 study subjects at the baseline and twice weekly blood draws. However, due to laboratory problems in handling these samples including loss during labeling, thawing, or shipping, complete cytokine data was missing on 399 patients. The unadjusted AUROC for each of the cytokines upon admission and repeat blood draw are displayed in Table IV. For comparison, unadjusted AUROCs for E2 values derived from the 1010 patients with complete cytokine data are also included in this Table. Comparing these AUROCs with the corresponding AUROCs in Table III shows very similar results for E2 AUROCs based on complete data and those based on patients with complete cytokine values. After adjusting for age, trauma status, APACHE II score, diabetes and menopause status, neither the admission cytokines nor changes in cytokines were independently associated with mortality with the exception of IL-6 and IL-8. Table V shows the AUROCs obtained from logistic regression models that included a baseline cytokine, the change in this cytokine, age, trauma status, APACHE II score, diabetes and menopause status. For comparison, the AUROC from the analogous model using baseline E2 and change in E2 are also included in this Table. This AUROC is also derived from the 1010 patients with complete cytokine data. When the same model was run on all 1408 patients, the E2 AUROC was virtually unchanged (AUROC = 0.80, 95% CI 0.77–0.83). The adjusted AUROC for the model containing admission E2 and changes in E2 was virtually identical to those based on cytokines. As indicated in Table V, estradiol and

changes in estradiol appear to be equally effective as cytokines and changes in cytokines in predicting overall mortality.

DISCUSSION

This study not only confirms previous work demonstrating that admission E2 levels are associated with mortality but also supports our hypothesis that E2 levels during the course of critical illness correlate more strongly with mortality than admission values. First, for the repeat E2 value, there was a larger separation in the median E2 levels for survivors versus non-survivors and a greater separation in mortality between the lowest and highest quartiles relative to the admission value. In addition, mortality for patients in whom E2 was in the highest quartile at repeat analysis was significantly greater than those in the lowest quartile at the second analysis, regardless of the admission quartile. Finally, the unadjusted AUROC for the repeat E2 was greater than the AUROC for the admission E2 and adding the trend in E2 to multivariate regression models including admission E2, APACHE II, age, trauma status, gender, and diabetes improved the model's performance. Although the association of cytokines with outcome of critical illness has been studied to a greater extent than sex hormones, E2 correlated at least as strongly with mortality whether obtained upon study enrollment or during the hospital course. This may be explained in part by the relatively short half-life of cytokines relative to serum E2, making serum E2 a clinically preferable indicator of mortal threat. Whether E2 is actually a mediator of the inflammatory process and contributing to outcome or simply a marker of disease severity is not known[36] and cannot be determined from these data. Answering such questions demands further investigation regarding the contribution of sex hormones to the outcome of critically ill and injured patients and, until such work is performed, calls for estrogen supplementation in critically ill patients are premature.

While this study did not investigate the source of E2 in this patient population, previous studies demonstrate that increased levels are related to the increased peripheral conversion of androgens to estrogens by the peripheral aromatase enzyme under the stimulatory effect of class I cytokines and TNF[24]. Furthermore, E2's potential to contribute to inflammatory processes in critical illness has been established. It is a well-documented immunomodulator[37,38] altering monocyte and macrophage function and life span[39] and cytokine and chemokine production. These changes have translated into early survival in animal models, but could lead to an exaggerated inflammatory response later in the clinical course. In addition to its inflammatory properties, estradiol has also been shown to induce and activate nitric oxide synthase in endothelial cells which may contribute to low peripheral resistance in response to shock[40]. Estrogens are also known to modulate insulin resistance, an important contributor to outcomes after critical injury.

The strengths of this study include its large sample size, prospective data collection by trained experts, and multi-institutional nature. There are several important limitations however. The initial admission E2 level was obtained within 48 hours of study enrollment, introducing the possibility of sampling bias, and possibly missing early elevations in E2. Additionally, patients who died or were discharged from the ICU within 48 hours were excluded, limiting the ability to generalize data to these patients. Finally, nearly 30% of patients in our cohort lacked complete cytokine data. Therefore, comparisons made between models containing E2 and changes in E2 vs. models containing cytokines and changes in cytokines based only upon available data in the subgroup analysis may not be generalizable to the larger cohort. Potential bias resulting from missing cytokine data is minimized however by the prospective study design, in which blood samples were drawn from all patients twice weekly, regardless of changes in clinical status or risk of death. Hence, the lack of cytokine data was not in any way affected by decisions made by the patient's

clinicians. Additionally, the adjusted AUROC for E2 in the entire 1408 patient cohort is nearly identical to the AUROC for E2 in the 1010 patient subgroup with complete cytokine data. This reduces the concern that our cytokine results were biased by missing data.

Conclusions

Admission E2 and changes in serum E2 over the course of critical illness or injury are more strongly associated with mortality than a single admission E2 level. Furthermore, the association of E2 and changes in E2 with mortality appear to be similar to the mortal association with cytokines and changes in cytokines. In view of the short half-life of cytokines, the fact that E2 and changes in E2 appear to perform at least as well as cytokines in predicting mortality makes E2 a clinically useful predictor of mortal outcome. Given the evidence presented herein and elsewhere, additional clinical investigations are urgently needed to identify underlying mechanisms linking elevated or up-trending serum E2 to mortality in critically ill patients. An interventional trial of anti-E2 therapy may be warranted.

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ABBREVIATIONS

ANOVA	Analysis of Variance
APACHE	Acute Physiology and Chronic Health Evaluation
BMI	Body Mass Index
CI	Confidence Interval
E2	17 β -Estradiol
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ICU	Intensive Care Unit
IQR	Interquartile Range
ISS	Injury Severity Score
MODS	Multiple Organ Dysfunction Score
ROC	Receiver Operator Characteristic
TRISS	Trauma Injury Severity Score
WBC	White Blood Cell

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Figure 1. Flow diagram of method for cohort selection with mortality rates and corresponding 95% confidence intervals (CI) for entire population, included cohort, and excluded cohort.

Figure 2.
Risk of death by Admission Estradiol Quartile

Figure 3.
Risk of death by trend in estradiol quartile

Figure 4.
Probability of Death by Unadjusted Difference in Estradiol

Figure 5.
Probability of Death by Unadjusted Admission and Repeat Estradiol

Table I

Demographics, clinical characteristics, hormone and cytokine levels by outcome

VARIABLE	Units	Survivors (n=1210)	Non-Survivors (n= 198)	p value
Age [^]	years	47.8 (17.6)	59.5 (16.6)	<0.001
Males [#]	n (%)	795 (65.6)	134 (67.7)	0.577
Non-trauma patients [#]	n (%)	413 (34.1)	130 (65.7)	<0.001
Diabetes [#]	n (%)	201 (16.6)	41 (20.7)	0.155
BMI	kg/m ²	29.4 (8.7)	29.4 (9.3)	0.996
APACHE II [^]	NA	17.18 (6.1)	22.14 (6.7)	<0.001
MODS [*]	NA	8 (6–10)	9 (5–12)	0.006
WBC [^]	X 10 ⁹ /L	15.9 (9.3)	16.24 (9.0)	0.658
Initial estradiol [*]	pg/mL	25.8 (10.0–60.8)	56.5 (13.0–114.6)	<0.001
Second estradiol [*]	pg/mL	14.8 (10.0–40.6)	50.8 (14.1–99.4)	< 0.001
Difference in estradiol [^]	NA	–14.7 (82.4)	–16.1 (111.6)	0.836
Initial IL-1 [*]	pg/mL	2.7 (2.7–4.6)	2.7 (2.7–6.5)	0.875
Second IL-1 [*]	pg/mL	2.7 (2.7–4.1)	2.7 (2.7–5.6)	0.190
Initial IL-2 [*]	pg/mL	3.7 (2.7–14.0)	3.3 (2.7–18.1)	0.658
Second IL-2 [*]	pg/mL	3.6 (2.7–14.8)	5.1 (2.7–17.9)	0.479
Initial IL-4 [*]	pg/mL	39.7 (5.8–175.4)	106.1 (12.0–355.0)	<0.001
Second IL-4 [*]	pg/mL	30.5 (6.4–164.7)	76.8 (10.5–244.3)	0.007
Initial IL-6 [*]	pg/mL	150.3 (47.7–369.9)	217.9 (65.2–618.9)	0.005
Second IL-6 [*]	pg/mL	107 (40.7–225.5)	181.9 (42.4–429.5)	<0.001
Initial IL-8 [*]	pg/mL	34.2 (13.2–73.7)	66.6 (16.1–134.9)	0.001
Second IL-8 [*]	pg/mL	28.4 (10.6–63.2)	53.2 (19.3–126.7)	<0.001
Initial IL-10 [*]	pg/mL	59.6 (25.4–163.6)	94.5 (39.2–236.9)	<0.001
Second IL-10 [*]	pg/mL	47 (21.6–129.2)	73.8 (34.7–252.2)	<0.001
Initial IL-12 [*]	pg/mL	3.35 (2.7–11.8)	2.7 (2.7–10.4)	0.163
Second IL-12 [*]	pg/mL	3.05 (2.7–11.55)	2.7 (2.7–9.5)	0.376

[^] mean (SD); compared using two sample t-test,[#] proportion; compared using Chi-squared test^{*} Median (IQR); compared using Wilcoxon Rank sum test

Table II

Percentage of mortality by trend in estradiol between undetectable and high quartiles

Group	Serum estradiol values	Survivors (N)	Non-survivors (N)	Percent Mortality
A (N=334) Admission quartile 1 to Repeat quartile 1	Undetectable (<10 pg/mL) to Undetectable (<10 pg/mL)	311	23	6.9 (CI 4.4%–10.1%)
B (N= 35) Admission quartile 4 to Repeat quartile 1	High (>=67 pg/mL) to Undetectable (<10 pg/mL)	30	5	14.3 (CI 4.8%–30.2%)
C (N= 14) Admission quartile 1 to Repeat quartile 4	Undetectable (<10 pg/mL) to High (>=48 pg/mL)	8	6	42.9 (CI 17.7%–71.1%)
D (N= 224) Admission quartile 4 to Repeat quartile 4	High (>=67 pg/mL) to High (>=48 pg/mL)	151	73	32.6 (CI 26.5%–39.1%)

Table III

Variables included in the multivariate regression model with corresponding p-values, odds ratios and unadjusted covariate AUROC

		Unadjusted AUROC values (95% CI) (univariable model)	Adjusted Odds Ratio (95% CI) (multivariable model)	p-value (multivariable model)
Covariates independently associated with mortality	APACHE II *	0.71 (0.67–0.75)	2.2 (1.8–2.8)	<0.001
	Repeat estradiol *§	0.69 (0.65–0.73)	1.2 (1.1–1.3)	<0.001
	Age *	0.68 (0.64–0.72)	2.0 (1.5–2.8)	<0.001
	Trauma status	0.34 (0.31–0.38)	0.4 (0.3–0.6)	<0.001
	Initial Estradiol *	0.64 (0.59–0.68)	1.3 (1.1–1.5)	0.001
	Difference in estradiol *†	0.51 (0.46–0.56)	1.1 (1.05–1.3)	0.003
Covariates not independently associated with mortality	Gender by menopausal status (relative to male)			
	Pre-menopausal	0.48 (0.45–0.52)	0.5 (0.2–1.2)	0.135
	Peri-menopausal		0.7 (0.4–1.0)	0.049
	Post-menopausal		0.5 (0.2–1.2)	0.103
	Diabetes	0.52 (0.49–0.55)	0.7 (0.5–1.1)	0.087
	BMI *	0.49 (0.45–0.54)	1.0 (0.8–1.2)	0.832

* Odds ratios are reported for the 75th percentile vs. 25th percentile

† restricted cubic spline of variable included in multivariable model

§ Odds ratio for repeat estradiol in multivariable model represents logistic regression model with repeat estradiol, controlling for APACHE II score, age, trauma status, gender and diabetes.

Table IV

Unadjusted AUROCs of estradiol and individual cytokines (95% CI)*

PARAMETER	ADMISSION AUROC	REPEAT AUROC
Estradiol	0.64 (0.59–0.69)	0.70 (0.65–0.75)
IL-2	0.51 (0.46–0.56)	0.52 (0.47–0.57)
IL-4	0.60 (0.54–0.65)	0.56 (0.51–0.61)
IL-6	0.57 (0.52–0.62)	0.60 (0.54–0.65)
IL-8	0.61 (0.55–0.66)	0.63 (0.58–0.68)
IL-10	0.59 (0.54–0.64)	0.60 (0.55–0.65)
IL-12	0.54 (0.49–0.59)	0.53 (0.48–0.57)

* derived from 1010 patient subgroup with complete cytokine data

Table V

Adjusted AUROCs of regression models containing E2 and changes in E2 or cytokines and trends in cytokines (95% CI)

Parameter included in model*	AUROC
Estradiol*	0.80 (0.77–0.84)
IL-2	0.78 (0.75–0.82)
IL-4	0.79 (0.75–0.82)
IL-6	0.81 (0.77–0.84)
IL-8	0.79 (0.75–0.83)
IL-10	0.79 (0.75–0.82)
IL-12	0.79 (0.75–0.82)

* model includes age, trauma status, APACHE II score, diabetes, menopause status, admission and absolute difference between initial and repeat value, run on 1010 patient subgroup