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GENDER BASED DIFFERENCES IN THE GENOMIC RESPONSE, INNATE IMMUNITY, ORGAN DYSFUNCTION AND CLINICAL OUTCOMES AFTER SEVERE BLUNT TRAUMATIC INJURY AND HEMORRHAGIC SHOCK

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Abstract

Introduction—The effect of gender on post-traumatic pathophysiology and outcomes after severe traumatic injury remains debated. We sought to determine the relationship of gender to the genomic and inflammatory responses, and clinical outcomes after hemorrhagic shock.

Methods—We analyzed blunt trauma patients in hemorrhagic shock from a prospective, multi-institutional cohort study to assess for gender based differences in the genomic response and clinical outcomes. Serially drawn blood samples were analyzed to evaluate peripheral leukocyte

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Author Contribution:

SCB, JC, RVM, FAM, LLM and PAE contributed to study design. SCB, MCL, HVM, TB, and JZ contributed to data analysis and interpretation. SCB prepared the manuscript. SCB, MCL, TB, JZ, JC, RVM, JM, FAM, HVB, LLM and PAE contributed to critical revision of the manuscript.

genome-wide expression and circulating inflammatory mediators at intervals between 0.5 and 28 days after injury. Multivariate logistic regression models were developed to assess the effect of gender on outcomes after controlling for age, injury and shock severity, blood transfusion, and comorbidities.

Results—The cohort consisted of 1,285 (67%) male and 643 (33%) female blunt trauma patients. Injury and shock severity were similar between the two groups. There were small but statistically significant differences between males and females regarding their age, BMI, and 12 hour blood and crystalloid administration. Organ failure was more severe in males, with slower recovery (9.0 vs. 6.5 days) in males compared to females ($p<0.01$). However, there were no differences between males and females in plasma levels of IL-6, IL-8, IL-10, IL-1 β , TNF- α and MCP-1. Multivariate analysis revealed that gender was not a significant independent risk factor for complicated recovery or 28-day mortality. Transcriptomic analysis revealed 333 genes with significant differential expression patterns between males and females ($FDR<0.001$), including genes associated with general inflammation, innate immunity, cell adhesion and cell signaling. None of the former genes were directly associated with sex hormones or X/Y chromosomes.

Conclusion—There are gender-specific differences in the leukocyte genomic response to severe injury that are associated with more robust and longer duration organ dysfunction. However, these expression patterns do not appear to be associated with sex-linked genes or circulating cytokine level differences, and do not translate to worsened gender-specific organ failure outcomes or inpatient mortality.

Keywords

Gender; trauma; hemorrhagic shock; leukocyte; genomics

Background

The effects of gender on differences in clinical outcomes after severe injury have been reported in a wide array of epidemiologic studies. Within these studies, there have been many reports of improved clinical outcomes for females as compared to males after severe traumatic injury.(1-5) However, published data remains conflicting on the magnitude and significance of these differences.(6-9) Results from varying animal models suggest that estrogen and testosterone influence the physiologic, immunologic and organ system responses to traumatic injury.(10, 11) While often attributed to differences in circulating sex hormone levels, our understanding of the underlying pathophysiology of gender-based differences in outcomes after severe injury remains incomplete. This is most exemplified in recent large, randomized controlled trials of sex hormone-based interventions after severe traumatic brain injury that, despite promising basic science and pre-clinical results, failed to show significant benefit in outcomes.(12, 13) The relationship of the sex hormone milieu is even less clear after hemorrhagic shock where, contrary to what epidemiologic data would suggest, higher levels of circulating estrogens have been shown to be associated with greater risk of poor clinical outcomes and mortality.(7, 14)

In this study, our goal was to determine the role of gender, and specifically the peripheral leukocyte genomic response, to the innate immune response, organ dysfunction and clinical

outcomes in a large, prospective cohort of severely injured blunt trauma patients in hemorrhagic shock. We hypothesized that there would be modest, if any, gender-specific differences in the innate immune response to injury and clinical outcomes in this patient population.

Methods

Study Cohort

We analyzed data from a prospective, multi-institutional cohort study of severely injured blunt trauma patients in hemorrhagic shock (The Inflammation and Host Response to Injury collaborative research program) in order to assess for gender-based differences in the innate immune response to injury and associated clinical outcomes including multiple organ failure (MOF), nosocomial complications and mortality. Institutional review board approval was obtained at each participating institution prior to initiation of the prospective cohort study. Severely injured blunt trauma patients with evidence of hemorrhagic shock were enrolled into the prospective, multi-center study protocol as previously described.(15, 16) The cohort consisted of all trauma patients (≥ 13 years of age), evaluated at five urban, academic Level 1 trauma centers. Inclusion criteria required a blunt traumatic mechanism with an abbreviated injury (AIS) score of 2 or greater outside the head region, base deficit of 6 mmol/L or greater, systolic blood pressure less than 90mm Hg in the pre-hospital setting or within 60 minutes of emergency department arrival, and blood product transfusion within 12 hours of injury. Exclusion criteria included a significant mortality risk from traumatic brain injury (AIS head >4), those evaluated at the enrolling trauma center more than 6 hours from the time of injury, cervical spinal cord injury, and thermal burns greater than or equal to 20% total body surface area.

A total of 2,006 patients were enrolled into the overall prospective cohort study. Patients less than 18 years old were excluded for this analysis, leaving a total of 1,928 patients. Of these, a subset of 244 patients had undergone peripheral blood sampling at select time points between 12 hours and 28 days after injury for subsequent genomic and proteomic analyses. (17) Clinical care was standardized amongst participating centers via the implementation of clinical standard operating procedures (SOP) as previously described.(15) An extensive set of clinical data was prospectively collected, including demographics, injury and shock severity, organ dysfunction, infectious and non-infectious complications and 28-day mortality, and entered into the Inflammation and Host Response to Injury program's Trauma Related Database (TRDB).

Genomic expression and ontology analysis

Of the 1,928 adult patients enrolled in the prospective cohort study, 244 underwent peripheral blood sampling at 0.5, 1, 4, 7, 14 and 28 days after injury, while hospitalized. The sampling subgroup has previously been shown to be representative of the overall Inflammation and Host Response to Injury cohort.(16) Circulating leukocytes (neutrophils (PMNs), monocytes and t-lymphocytes) were isolated as previously described.(17-19) Total RNA extraction was performed and subsequently hybridized onto U133 GeneChips™ (Affymetrix; Santa Clara, CA). This expression data, focusing on the transcriptome of

circulating PMNs following severe injury and hemorrhagic shock, was then analyzed in order to determine individual gene expression differences (false discovery rate (FDR) <0.001) between male and female patients. Leave one out cross-validation was utilized to confirm that the differences in PMN gene expression between males and females could not be explained by chance alone. After the subset of trauma responsive genes with significant differential expression between males and females was identified, the overall change in gene expression in these gender-specific genome subsets was quantified via a modified difference from reference (DFR) metric, calculated as previously described.(20) Briefly, DFR summarizes all of the significant individual gene expression alterations, both up and down regulation as compared to baseline levels of uninjured control subjects. Therefore, the overall altered transcriptomic response of all gender-specific, trauma responsive genes of each patient in this analysis can be represented by a single DFR value. Additionally, this subset of significant genes were also analyzed for individual pathway (Gene Ontology and Biocarta), and functional pathway differences (IPA, Z-score (<-2, >2)) as previously described.(17)

Plasma cytokine/chemokine analysis

Assessments for circulating inflammatory mediators were also performed on selected plasma samples obtained from the sampling cohort at 0.5, 1, 4, 7, 14, 21 and 28 days after injury. Plasma samples were analyzed for multiple cytokine/chemokines, including interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 1 beta (IL-1 β), monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor alpha (TNF- α). These were identified and quantified utilizing the Luminex MAGPIX® xMap system (Luminex; Austin, TX, USA), using Milliplex® MAP multiplex kits (Millipore; Billerica, MA, USA) and Milliplex® Analyst 5.1 software. Mixed model analysis of longitudinal changes in cytokines was performed to properly account for correlations among repeated measurements for each patient. For each cytokine, change over time of log-transformed cytokine concentration was modeled, including gender and gender/time interaction in the model to examine whether the significant differences seen in plasma cytokine/chemokine concentrations were due to gender and/or time after injury. Similar models were fit also adjusting for age, new injury severity score (NISS), total blood transfusion in 12 hours, and maximum lactate in 24 hours. The Kenward-Roger method was used to calculate the denominator degrees of freedom owing to the unbalanced study design. Statistical analysis was performed using SAS (v.9.3, Cary, NC).

Clinical outcomes and multivariate risk factor analysis

All relevant clinical data utilized for analysis, including patient demographics, injury/shock severity, resuscitation parameters, organ dysfunction assessments, inpatient complications, length of stay, discharge disposition and inpatient 28-day mortality, were obtained for this analysis from the TRDB. Complicated recovery was defined as organ dysfunction recovery time greater than 14 days, no recovery, or death.(16, 17, 20) Univariate analyses were performed using either the students's t-test or Mann-Whitney signed rank test and Fisher's exact test, as appropriate. Logistic regression modeling was performed to develop multivariate risk-factor models in order to determine the independent effects of gender on complicated recovery and 28-day mortality. Covariates were selected based on known and

suspected confounding risk factors, as well as any significant risk factors identified by univariate analysis. Additionally, interaction analyses of gender with other significant predictors were performed to determine if gender modifies the effect of other risk factors. Statistical analyses were performed with SAS (v.9.3, Cary, NC, USA).

Results

Gender cohort demographics and clinical outcomes

The overall study cohort consisted of 1,928 severely injured patients with evidence of hemorrhagic shock. The percentage of males and females in the cohort was 67% and 33%, respectively. Due to the large sample size, there were statistically significant differences in age, body mass index, and 24-hour APACHE II scores between males and females, of questionable clinical significance (Table 1). The percentage of at least one major comorbidity between males and females were similar. Mechanism of injury was primarily motor vehicle crashes for women, while men had higher rates of motorcycle crashes, and falls. However, males and females had similar levels of injury severity, and rates of major acute surgical procedures performed. Overall, males and females had similar injury patterns. However, females had a higher severity of spine fractures, as reflected by maximum regional abbreviated injury scoring (AIS) spine. Female patients received lower amounts of blood transfusion and higher amounts of crystalloid volume resuscitation than male patients in the first 12 hours after injury (Table 1).

Unadjusted measures of organ dysfunction demonstrated greater peak levels of organ dysfunction and longer organ function recovery duration in males as compared to females (Table 2). Additionally, rates of ventilator associated pneumonia, non-infectious complications and an overall complicated recovery (organ recovery >14 days, no recovery or death) were higher in males than in females. However, there was no significant difference in intensive care unit length of stay, overall hospital length of stay, and 28-day mortality between males and females (Table 2). Overall, discharge disposition was similar between male and female patients. However, female patients were more likely than male patients to be discharged to a skilled nursing facility (27.7% vs. 21.8%), while male patients were more likely to be discharged home (25.5 vs. 20.9%) (Table 3). Multivariate analysis revealed that gender was not an independent predictor of either complicated recovery or 28-day mortality, after controlling for blood product transfusion, injury severity, shock severity, comorbidities, BMI and age (Table 4). Interaction analyses revealed that for complicated recovery, representing persistent organ dysfunction, there were no significant interactions of gender with other independent risk factors. However, for 28-day mortality, there were statistically significant interactions between gender, early massive transfusion, and hypotension upon emergency department presentation. Females presenting with systolic hypotension <90 mmHg but receiving <9.5 units PRBC had lower risk of mortality than males (OR 0.61, 95% CI 0.41-0.92, $p=0.015$), and males presenting without hypotension that received >9.5 units PRBC in the first 12 hours had a higher risk of mortality than females (OR 3.29, 95% CI 1.48-7.29, $p=0.127$). These interaction findings possibly indicate that male gender increases risk of 28-day mortality (but not persistent organ failure) in patients receiving >9.5 units PRBC in the first 12 hours.

Gender-specific peripheral leukocyte genomic response analysis

Microarray analysis of the polymorphonuclear leukocyte (PMN) transcriptomic response within the sampling cohort of injured patients revealed 333 genes with significant differential expression between males and females (false discovery rate <0.001) (Figure 1A). Analysis of the composite DFR expression metric, which quantifies overall expression aberrancy within this gender-specific gene subset, revealed an overall significant difference in the magnitude of the transcriptomic response in these trauma responsive genes between males and females over time, with females showing a more robust transcriptional response (Figure 1B). Expression changes began within 12 hours, and peaked between 5 and 7 days after injury in both males and females before returning towards uninjured control levels.

Based on gene ontology analysis of the 333 gender-specific, trauma-responsive genes, there were eight significant gene ontology pathways identified. These included pathways associated with negative regulation of lymphocyte regulation, response to TGF- β stimulus, ubiquitin dependent protein catabolic processes, and protein/macromolecule catabolic processing. Of the 333 genes differentially expressed by males and females, 8 were located on the X chromosome and 5 on the Y chromosome. However, none of these genes are directly associated with known sex hormonal ontology or pathways (Suppl. Table 1). The specific gene ontologies of the 10 most up and down regulated gender specific genes are associated with inflammation, innate immune function, cell adhesion and cell signaling. None of these 10 genes are directly associated with sex hormones or the sex chromosomes (Supp. Table 2). In addition, IPA Upstream Analysis predicted ($-2 > Z\text{-score} < 2$) different molecules related to immunity to be activated or inhibited based on the genomic expression profile of circulating neutrophils 7 days after injury (Supp. Table 3). Again, none of these are thought to be directly related to genomic expression from sex chromosomes.

Circulating cytokines/chemokine analysis

Plasma cytokine and chemokine analysis results are shown in Figure 2. Mixed model results revealed that there were significant decrease in the overall levels of IL-6, IL-8, and MCP-1 over the 28 day study period, as compared to peak levels, that occurred within the first 12 hours after injury ($p < 0.001$). However, while there appear to be trends of gender specific differences in early circulating levels of IL-6, IL-8, and late levels of TNF- α , these differences failed to reach statistical significance due to high variance (Figure 2).

Discussion

This analysis represents the largest prospective cohort study utilized in an attempt to clarify gender differences after severe blunt traumatic injury and hemorrhagic shock. Overall, the pattern of our results reflects many of the independently mixed results of the existing literature base on gender-based outcomes differences after injury. However, this study is the first to analyze several of these subsets of physiologic and immune responses, and clinical outcomes within a single, prospective patient population. We also believe there are specific novel findings within our study that will help reveal further insight into the underlying pathophysiology of how males and females respond differently to severe blunt injury and hemorrhagic shock.

Similar to previous studies, we did find gender-specific differences in certain clinical outcomes and physiologic responses to severe injury and hemorrhagic shock. Most specifically, male patients had slightly greater severity of organ dysfunction and longer organ failure recovery time, as compared to females. This more florid organ failure response by severely injured males is similar to previous findings as described by Sperry and others. (2, 5) While overall nosocomial infection rates were not different, ventilator associated pneumonia rates were significantly higher in males as opposed to females, which also has been shown in previous reports.(21-23) Additionally, our model interaction analysis suggested that male gender may confer greater risk of 28-day mortality, but not prolonged organ dysfunction, in patients receiving massive transfusion amounts of blood products in the first 12 hours. This could potentially represent a higher risk of early death from coagulopathy in men, which was suggested in a previous study analyzing outcomes effects of high-product ratio massive transfusion.(24) Interestingly, while we did find these differences in specific clinical outcomes, they did not translate to significant differences in the longer term outcomes of complicated recovery (organ recovery time >14 days, no organ recovery or death) and 28-day mortality. This is the first time that these gender-specific differences in clinical course have been shown to not translate to adverse longer term-inpatient outcomes and inpatient mortality. Again, while many of these findings have been described independently in the past, this is the first study to show the full spectrum of these outcomes within the same cohort.

Another novel finding of this study is the identification of a subset of trauma responsive genes that exhibit gender-specific differences in peripheral leukocyte expression. Results from previous genomic analysis on this Inflammation and Host Response to Injury cohort has shown that there are over 5,000 trauma responsive genes in circulating PMNs that undergo significant changes in expression when compared to uninjured controls.(16) In comparison, the fraction of these trauma responsive genes that exhibit gender-specific expression differences is relatively modest, at 333 genes. The gender-specific genomic responses in T-cell and monocyte population were even smaller (data not shown). Interestingly, a very small proportion of these genes were located on the X or Y chromosomes, and none were associated with known sex hormonal pathways via gene ontology analysis. Reports are conflicting on whether gender-specific outcomes differences after injury are influenced by pre or post-menopausal status, suggesting there may be other, non-hormonal mechanisms involved.(1, 2, 4) While this does not prove that downstream mechanisms of hormonal pathways are not influenced differentially after injury based on gender, it may offer some explanation why sex hormone based interventional therapies have not successfully translated to the injured clinical population, as illustrated in the PROTECT III and SYNAPSE trials for severe traumatic brain injury.(12, 13) Admittedly, there is currently no prospective clinical trial data to suggest these findings in severe traumatic brain injury are translatable to hemorrhagic shock. However, it lends credence to the statement that our understanding of the role of the sex hormones in potential gender outcomes disparities is incomplete, at best.

Previous genomic analysis of this cohort showed that the “genomic storm” of overall trauma responsive gene expression modulation peaks early after injury.(16) In contrast, the gender-specific subset of trauma responsive genes described in this analysis showed peak aberrancy

at 5-7 days after injury. Although ontology analysis revealed that many of these genes are involved in innate immunity and lymphocyte activation, this does not appear to directly coincide with the more florid and extended course of organ dysfunction in males, as organ dysfunction onset is early after injury, and in the majority of cases, resolves within 7-10 days (Table 2). Interestingly, while females showed a more robust aberrancy in expression as compared to males in this subset of trauma responsive genes, and the ontology of these differentially expressed genes reflect pathways regulating innate immunity, we were not able to demonstrate significant differences in either circulating cytokine and chemokine levels, nor composite organ failure or mortality outcomes, based on gender.

Despite relatively small differences in genomic expression between males and females shown in this analysis, it should be acknowledged that differences in the acute transcriptomic response within leukocytes do not rule out genomic phenotype variants as important in the activation of innate immunity after injury. This is borne out in this analysis in that gene ontology and pathway analysis predicted several upstream signal transduction and cytokine molecules which one would predict should lead to downstream differential circulating levels of associated cytokines. While severity of organ dysfunction did differ between females and males, we were unable to show similar differences in circulating cytokines in this population, suggesting that post-transcriptional, counter-regulatory, or non-cytokine dependent mechanisms may play key roles in these processes. Additionally, Sperry et al have shown that polymorphism in the IRAK-1 component of the toll-like receptor response is a predictor of organ failure and mortality after severe injury.(25) Therefore, there may be non-hormonal, non-transcriptionally dependent pathways in innate immune signaling that are responsible for differences in the post-injury immune response and subsequent associated organ dysfunction. Given the ontology analysis also identified gene pathways associated with TGF- β response and protein catabolic pathways, it is interesting to hypothesize that changes in these pathways potentially lead to more protein catabolism and lean muscle mass loss and delayed wound healing, which could potentially explain the higher rate of skilled care disposition shown by females in this cohort. However, further work in long-term functional outcomes after severe injury, and any potential gender-related outcomes difference, needs further investigation as this data is significantly lacking in the current literature base.

The National Institutes of Health has made the inclusion of females (and minorities) a requirement for research involving human subjects (http://grants.nih.gov/grants/funding/women_min/women_min.htm) and animal studies (http://orwh.od.nih.gov/about/director/director_nature_2014.asp) a priority. In addition, although trauma has traditionally been considered a disease of young males, females contributed to 1/3 of our study population. Finally, the role of the females in combat in militaries such as the United States is expanding (<http://www.gao.gov/assets/680/671507.pdf>). Thus, studies such as ours help to fill the necessity of understanding how gender can influence the host to have a unique response to the same insult. Previous work by our group has identified how age can alter the immune response to severe injury and shock.(17, 26) Gender appears to also be associated with an altered and unique peripheral leukocyte genomic response to trauma. Currently, there are significant efforts to not only create methodology that can identify those patients who are destined to have poor outcomes, but to be able to appropriately immunomodulate those

patients in order to improve patient outcomes.(27-29) Most of the immunomodulation studies to date in trauma and sepsis have been unsuccessful, which is thought to be due to multiple issues.(29, 30) As we progress forward, it will be very important to understand the specific role gender (or age) on the immune response in order to personalize our medical treatment of these individuals.

There are several areas and questions that this study fails to address. Differences in the leukocyte genomic response to injury between males and females does not necessarily reflect pre-existing hormone levels or sex hormone related cellular responses. Because circulating sex hormone levels were not measured in the sampling cohort, we cannot infer on how these gender-specific expression changes within leukocytes affect peripheral sex hormone levels or gonadal production. In fact, it is likely that while the circulating transcriptomic response in circulating leukocytes very well represents the cellular and downstream innate immune response to severe injury, it is unlikely to accurately represent or have relevance to the genomic response within other tissue types, such as endocrine and gonadal organs. Indeed, it's been shown that higher levels of estrogens after injury are associated with worse outcomes, and therefore increases in sex hormone levels after injury may be merely an early marker, as opposed to directly related to, poor clinical outcomes.(14) Of note, we also do not know what the proportion of post-menopausal women is in this cohort. However, we have previously published data on this cohort showing that age range by gender is similar, approximately 31% of females in the cohort were above an age likely to be post-menopausal (>55 years).(17) Although the gender cohorts are balanced by age, it is possible this proportion of post-menopausal women is contributing to a diluting effect to any hormone-based gender differences. One final finding of note that must be discussed is that previous preliminary analyses from smaller subsets of the Inflammation and Host Response to Injury cohort, prior to its completion of enrollment, revealed gender based differences in organ failure and circulating levels of IL-6, which were not replicated with this analysis of the completed cohort.(2, 31) These findings should be more broadly taken into consideration when evaluating apparently significant findings in individual studies of relatively small sized cohorts. This is further argument that findings of any kind should be replicated amongst multiple, large cohort studies prior to developing wide ranging inferences based on individual published results.

Overall, we have shown that there are gender-specific genomic responses within the circulating leukocyte population of severely injured patients in hemorrhagic shock, which are associated with more florid and longer episodes of multiple organ dysfunctions. However, these changes do not appear to translate to significant differences between males and females with regards to longer term complicated organ dysfunction outcomes or 28-day inpatient mortality. The role of gender with regards to the innate immune response, multiple organ failure and overall clinical outcomes remains incompletely elucidated and warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Frink M, Pape HC, van Griensven M, Krettek C, Chaudry IH, Hildebrand F. Influence of sex and age on mods and cytokines after multiple injuries. *Shock*. 2007; 27(2):151–6. [PubMed: 17224789]
2. Sperry JL, Nathens AB, Frankel HL, Vanek SL, Moore EE, Maier RV, Minei JP. Characterization of the gender dimorphism after injury and hemorrhagic shock: are hormonal differences responsible? *Crit Care Med*. 2008; 36(6):1838–45. [PubMed: 18496363]
3. Sperry JL, Vodovotz Y, Ferrell RE, Namas R, Chai YM, Feng QM, Jia WP, Forsythe RM, Peitzman AB, Billiar TR. Racial disparities and sex-based outcomes differences after severe injury. *J Am Coll Surg*. 2012; 214(6):973–80. [PubMed: 22521668]
4. Yang KC, Zhou MJ, Sperry JL, Rong L, Zhu XG, Geng L, Wu W, Zhao G, Billiar TR, Feng QM. Significant sex-based outcome differences in severely injured Chinese trauma patients. *Shock*. 2014; 42(1):11–5. [PubMed: 24667619]
5. Trentzsch H, Lefering R, Nienaber U, Kraft R, Faist E, Piltz S. The role of biological sex in severely traumatized patients on outcomes: a matched-pair analysis. *Ann Surg*. 2015; 261(4):774–80. [PubMed: 25029437]
6. Summers JJ, Ziembicki JA, Corcos AC, Peitzman AB, Billiar TR, Sperry JL. Characterization of sex dimorphism following severe thermal injury. *J Burn Care Res*. 2014; 35(6):484–90. [PubMed: 24823341]
7. Dossett LA, Swenson BR, Heffernan D, Bonatti H, Metzger R, Sawyer RG, May AK. High levels of endogenous estrogens are associated with death in the critically injured adult. *J Trauma*. 2008; 64(3):580–5. [PubMed: 18332796]
8. Schoeneberg C, Kauther MD, Hussmann B, Keitel J, Schmitz D, Lendemann S. Gender-specific differences in severely injured patients between 2002 and 2011: data analysis with matched-pair analysis. *Crit Care*. 2013; 17(6):R277. [PubMed: 24289182]
9. Brown JB, Cohen MJ, Minei JP, Maier RV, West MA, Billiar TR, Peitzman AB, Moore EE, Cuschieri J, Sperry JL. Characterization of acute coagulopathy and sexual dimorphism after injury: females and coagulopathy just do not mix. *J Trauma Acute Care Surg*. 2012; 73(6):1395–400. [PubMed: 23064602]
10. Sperry JL, Minei JP. Gender dimorphism following injury: making the connection from bench to bedside. *J Leukoc Biol*. 2008; 83(3):499–506. [PubMed: 17895398]
11. Doucet D, Badami C, Palange D, Bonitz RP, Lu Q, Xu DZ, Kannan KB, Colorado I, Feinman R, Deitch EA. Estrogen receptor hormone agonists limit trauma hemorrhage shock-induced gut and lung injury in rats. *PloS one*. 2010; 5(2):e9421. [PubMed: 20195535]
12. Skolnick BE, Maas AI, Narayan RK, van der Hoop RG, MacAllister T, Ward JD, Nelson NR, Stocchetti N. A clinical trial of progesterone for severe traumatic brain injury. *N Engl J Med*. 2014; 371(26):2467–76. [PubMed: 25493978]
13. Wright DW, Yeatts SD, Silbergleit R, Palesch YY, Hertzberg VS, Frankel M, Goldstein FC, Caveney AF, Howlett-Smith H, Bengelink EM, et al. Very early administration of progesterone for acute traumatic brain injury. *N Engl J Med*. 2014; 371(26):2457–66. [PubMed: 25493974]
14. Zolin SJ, Vodovotz Y, Forsythe RM, Rosengart MR, Namas R, Brown JB, Peitzman AP, Billiar TR, Sperry JL. The early evolving sex hormone environment is associated with significant outcome and inflammatory response differences after injury. *J Trauma Acute Care Surg*. 2015; 78(3):451–7. discussion 7–8. [PubMed: 25710413]

15. Cuschieri J, Johnson JL, Sperry J, West MA, Moore EE, Minei JP, Bankey PE, Nathens AB, Cuenca AG, Efron PA, et al. Benchmarking outcomes in the critically injured trauma patient and the effect of implementing standard operating procedures. *Ann Surg.* 2012; 255(5):993–9. [PubMed: 22470077]
16. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, Hayden DL, Hennessy L, Moore EE, Minei JP, et al. A genomic storm in critically injured humans. *J Exp Med.* 2011; 208(13): 2581–90. [PubMed: 22110166]
17. Vanzant EL, Hilton RE, Lopez CM, Zhang J, Ungaro RF, Gentile LF, Szpila BE, Maier RV, Cuschieri J, Bihorac A, et al. Advanced age is associated with worsened outcomes and a unique genomic response in severely injured patients with hemorrhagic shock. *Crit Care.* 2015; 19:77. [PubMed: 25880307]
18. Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W, et al. Whole blood and leukocyte RNA isolation for gene expression analyses. *Physiol Genomics.* 2004; 19(3):247–54. [PubMed: 15548831]
19. Laudanski K, Miller-Graziano C, Xiao W, Mindrinos MN, Richards DR, De A, Moldawer LL, Maier RV, Bankey P, Baker HV, et al. Cell-specific expression and pathway analyses reveal alterations in trauma-related human T cell and monocyte pathways. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103(42):15564–9. [PubMed: 17032758]
20. Cuenca AG, Gentile LF, Lopez MC, Ungaro R, Liu H, Xiao W, Seok J, Mindrinos MN, Ang D, Baslanti TO, et al. Development of a genomic metric that can be rapidly used to predict clinical outcome in severely injured trauma patients. *Crit Care Med.* 2013; 41(5):1175–85. [PubMed: 23388514]
21. Sharpe JP, Magnotti LJ, Weinberg JA, Brocker JA, Schroepfel TJ, Zarzaur BL, Fabian TC, Croce MA. Gender disparity in ventilator-associated pneumonia following trauma: identifying risk factors for mortality. *J Trauma Acute Care Surg.* 2014; 77(1):161–5. [PubMed: 24977772]
22. Croce MA, Brasel KJ, Coimbra R, Adams CA Jr. Miller PR, Pasquale MD, McDonald CS, Vuthipadadon S, Fabian TC, Tolley EA. National Trauma Institute prospective evaluation of the ventilator bundle in trauma patients: does it really work? *J Trauma Acute Care Surg.* 2013; 74(2): 354–60. discussion 60–2. [PubMed: 23354225]
23. Haider AH, Crompton JG, Oyetunji T, Stevens KA, Efron DT, Kieninger AN, Chang DC, Cornwell EE 3rd, Haut ER. Females have fewer complications and lower mortality following trauma than similarly injured males: a risk adjusted analysis of adults in the National Trauma Data Bank. *Surgery.* 2009; 146(2):308–15. [PubMed: 19628090]
24. Sperry JL, Zolin S, Zuckerbraun BS, Vodovotz Y, Namas R, Neal MD, Ferrell RE, Rosengart MR, Peitzman AB, Billiar TR. X chromosome-linked IRAK-1 polymorphism is a strong predictor of multiple organ failure and mortality postinjury. *Ann Surg.* 2014; 260(4):698–703. discussion -5. [PubMed: 25203887]
25. Nacionales DC, Szpila B, Ungaro R, Lopez MC, Zhang J, Gentile LF, Cuenca AL, Vanzant E, Mathias B, Jyot J, et al. A Detailed Characterization of the Dysfunctional Immunity and Abnormal Myelopoiesis Induced by Severe Shock and Trauma in the Aged. *J Immunol.* (Baltimore, Md : 1950). 2015
26. Gentile LF, Cuenca AG, Efron PA, Ang D, Bihorac A, McKinley BA, Moldawer LL, Moore FA. Persistent inflammation and immunosuppression: a common syndrome and new horizon for surgical intensive care. *J Trauma Acute Care Surg.* 2012; 72(6):1491–501. [PubMed: 22695412]
27. Vanzant EL, Lopez CM, Ozrazgat-Baslanti T, Ungaro R, Davis R, Cuenca AG, Gentile LF, Nacionales DC, Cuenca AL, Bihorac A, et al. Persistent inflammation, immunosuppression, and catabolism syndrome after severe blunt trauma. *J Trauma Acute Care Surg.* 2014; 76(1):21–9. discussion 9–30. [PubMed: 24368353]
28. Efron PA, Mohr AM, Moore FA, Moldawer LL. The future of murine sepsis and trauma research models. *J Leukoc Biol.* 2015
29. Gentile LF, Nacionales DC, Lopez MC, Vanzant E, Cuenca A, Cuenca AG, Ungaro R, Baslanti TO, McKinley BA, Bihorac A, et al. A better understanding of why murine models of trauma do not recapitulate the human syndrome. *Crit Care Med.* 2014; 42(6):1406–13. [PubMed: 24413577]

30. Sperry JL, Friese RS, Frankel HL, West MA, Cuschieri J, Moore EE, Harbrecht BG, Peitzman AB, Billiar TR, Maier RV, et al. Male gender is associated with excessive IL-6 expression following severe injury. *J Trauma*. 2008; 64(3):572–8. discussion 8-9. [PubMed: 18332795]

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Figure 1.

Gender-specific leukocyte transcriptomic response of 333 genes differentially expressed between females and males after severe injury and hemorrhagic shock. A, Expression heat map of 333 trauma responsive genes with gender-specific, differential expression (false discovery rate <0.001) from 0.5 to 28 days after injury. B, Differences in gender-specific trauma responsive gene expression as represented by the summary difference from reference (DFR) metric revealed significant differences between males and females from 0.5 to 28 days after injury ($p < 0.001$).

Figure 2.

Circulating levels of cytokines and chemokines in severely injured female and male patients. Plasma levels of interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 1 beta (IL-1B), tumor necrosis factor alpha (TNF α), and monocyte chemoattractant protein 1 (MCP-1). Point values represent mean values at each timepoint (0.5, 1, 4, 7, 14, 21 and 28 days), with error bars representing standard error of the mean. Mixed modeling analysis revealed significant changes in plasma levels over time in circulating plasma levels of IL-6, IL-8 and MCP-1 ($p < 0.001$). However, there were not statistically significant differences between males and females, across all measured circulating mediators and timepoints.

Table 1

Demographics - Gender differences in severely injured blunt trauma patients.

	Male (n=1285)		Female (n=643)		(p)
	(median)	(IQR)	(median)	(IQR)	
Age (yrs)	42	(27-55)	44	(28-58)	0.01
NISS	34	(27-48)	34	(27-48)	0.16
AIS Head	0	(0-3)	0	(0-3)	0.65
AIS Face	0	(0-1)	0	(0-1)	0.20
AIS Neck	0	(0-0)	0	(0-0)	0.87
AIS Thorax	3	(0-4)	3	(0-4)	0.47
AIS Abdomen	2	(0-3)	2	(0-3)	0.60
AIS Spine	0	(0-2)	2	(0-2)	0.026
AIS Upper extremity	1	(0-2)	1	(0-2)	0.41
AIS Lower extremity	3	(0-3)	3	(2-3)	0.36
AIS External/skin	0	(0-0)	0	(0-0)	0.37
BMI	27	(24-31)	26	(23-31)	0.001
Injury to ED arrival (hrs.)	1.3	(0.8-2.5)	1.3	(0.8-2.5)	0.41
Lowest pre-hospital SBP	86	(71-104)	87	(73-100)	0.89
Lowest ED SBP	82	(70-95)	83	(70-96)	0.69
Max. APACHE II score 0-24 hours	29	(25-34)	29	(24-33)	0.03
Max. Lactate 12-24 hours	3.0	(2.1-4.4)	2.7	(1.9-4.2)	0.07
Total Blood 0-12 hours (U)	5.0	(2.6-9.4)	4.0	(2.0-8.0)	0.002
Total crystalloid 0-12 hours (L)	6.0	(7.0-14.4)	7.0	(6.1-11.9)	<0.001
	(n)	(%)	(n)	(%)	(p)
1 Major medical comorbidity	380/1285	(29.6)	192/643	(29.9)	0.92
Mechanism - Fall	119/1285	(9.3)	43/643	(6.7)	0.06
Mechanism - MVC	596/1285	(46.4)	443/643	(68.9)	<0.001
Mechanism - MCC	266/1285	(20.7)	34/643	(5.3)	<0.001
Mechanism - MPC	173/1285	(13.5)	95/643	(14.8)	0.44
Mechanism - Other	131/1285	(10.2)	28/643	(4.4)	<0.001
Major acute surgical procedures	1196/1285	(93.1)	591/643	(92.0)	0.36

IQR, interquartile range; ISS, injury severity score; AIS, abbreviated injury score; ED, emergency department; SBP, systolic blood pressure; U, units; L, liters; hrs, hours; MVC, motor vehicle collision; MCC, motorcycle collision; MPC, motor pedestrian collision.

Table 2

Unadjusted Clinical Outcomes - Male vs. Female severely injured blunt trauma patients

	Male		Female		
	(n=1285)		(n=643)		
	(median)	(IQR)	(median)	(IQR)	(p)
MOF day of onset	2	(2-4)	2	(2-3)	0.60
Max. Marshall MOF score	5.2	(3.5-7.1)	4.4	(3.0-6.1)	<0.001
Max. Denver 2 MOF score	2	(1-4)	2	(0-3)	<0.001
MOF recovery day	9	(4-19)	6.5	(4-19)	0.01
Ventilator days (d)	6	(2-14)	6	(2-12)	0.05
ICU LOS (d)	10	(4-19)	9	(4-17)	0.10
Hospital LOS (d)	19	(10-31)	18	(9-29)	0.06
	(n)	(%)	(n)	(%)	(p)
Non-infectious complications	620/1285	(48.2)	249/643	(38.7)	<0.001
Nosocomial infection	568/1285	(44.2)	263/643	(40.9)	0.17
SSI	182/1285	(14.2)	81/643	(12.6)	0.36
VAP	382/1285	(29.7)	122/643	(19.0)	<0.001
ICU readmission	128/1285	(10.0)	56/643	(8.7)	0.41
Tracheostomy	311/1285	(24.2)	142/635	(22.3)	0.39
Chronic Critical Illness	496/1285	(38.5)	221/635	(34.8)	0.11
Complicated recovery	535/1285	(41.6)	226/643	(35.2)	0.007
Withdrawal of active care	109/1285	(8.5)	62/643	(9.6)	0.40
28-day Mortality	210/1285	(16.3)	98/643	(15.2)	0.55

IQR, interquartile range; MOF, multiple organ failure; LOS, length of stay; d, days; SSI, surgical site infection; VAP, ventilator associated pneumonia.

Table 3

Discharge disposition - Male vs. Female Severely injured blunt trauma patients.

	Male		Female		(p)
	(n)	(%)	(n)	(%)	
<u>Discharge disposition</u>					
Home	327/1285	(25.5)	134/643	(20.9)	0.03
Inpatient Rehab	313/1285	(24.4)	140/643	(21.8)	0.21
Other acute care facility	57/1285	(4.4)	33/643	(5.1)	0.49
Skilled Nursing Facility	280/1285	(21.8)	178/643	(27.7)	0.005
Other	98/1285	(7.6)	60/643	(9.3)	0.22
Death (inpatient)	210/1285	(16.3)	98/643	(15.2)	0.55

Table 4**Multivariate outcomes analysis**

<i>Complicated recovery</i> ¹	OR	95% CI	p
Total blood (U) 0-12 hours	1.08	1.05-1.10	<0.0001
Lactate (mmol/L) 0-6 hours	1.15	1.10-1.20	<0.0001
NISS	1.04	1.03-1.05	<0.0001
Age (y)	1.03	1.02-1.04	<0.0001
BMI	1.04	1.03-1.06	<0.0001
ED SBP <90 (mmHg)	1.50	1.15-1.96	0.003
Male Gender	1.16	0.89-1.50	0.27
<i>28-day Mortality</i>²			
	OR	95% CI	p
Total blood >9.5 (U) 0-12 hours	3.63	2.70-4.89	<0.001
Lactate >6 (mmol/L) 0-6 hours	2.25	1.68-3.01	<0.001
Age (y)	1.03	1.02-1.04	<0.001
NISS	1.03	1.02-1.04	<0.001
1 major medical comorbidity	1.60	1.13-2.26	0.008
ED SBP <90 (mmHg)	1.61	1.16-2.24	0.005
Male Gender	1.04	0.78-1.39	0.8

U, units; y, years; BMI, body mass index; ED, emergency department; SBP, systolic blood pressure; OR, odds ratio; NISS, new injury severity score; CI, confidence interval.

¹Model Parameters; AUC (c)=0.799, 95% CI 0.776-0.822

²Model Parameters; AUC (c)=0.793, 95% CI 0.766-0.820