Published in final edited form as:

J Infect. 2013 January ; 66(1): 87–94. doi:10.1016/j.jinf.2012.10.001.

INCREASED APOPTOSIS OF PERIPHERAL BLOOD NEUTROPHILS IS ASSOCIATED WITH REDUCED INCIDENCE OF INFECTION IN TRAUMA PATIENTS WITH HEMORRHAGIC SHOCK

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SUMMARY

Objective—We aimed to describe the relationship between early peripheral leukocyte apoptosis and incidence of subsequent infection in trauma patients with hemorrhagic shock (T/HS).

Methods—T/HS patients requiring emergency surgery were prospectively enrolled. Nucleosome ELISA and TUNEL staining were performed on peripheral blood drawn pre-operatively, postoperatively and at 24 hours. Subjects were followed for 30 days or until death or hospital discharge to record all episodes of infection.

Results—Forty-one subjects were enrolled. Six died within 24 hours of surgery and were not included in the analysis. Nucleosome levels peaked post-operatively and dropped to baseline levels at 24 hours ($p=0.03$). TUNEL analysis revealed that polymorphonuclear neutrophils (PMNs) accounted for 72% of apoptotic leukocytes; the remaining apoptotic cells were mainly lymphocytes. Increased post-operative leukocyte apoptosis was associated with decreased systemic inflammatory response syndrome (SIRS) severity. Seventeen of the 35 survivors (48.6%) developed infections, while 18 (51.4%) did not. Pre-operative and post-operative nucleosome levels were 2.5 and 3 times higher, respectively, in T/HS patients who did not develop infection compared to those who did. Increased nucleosome levels were associated in particular with protection against sepsis ($p=0.03$) and multiple infections ($p=0.01$).

Conclusion—Peripheral blood PMN apoptosis in the early resuscitative period is associated with decreased incidence of subsequent infection in T/HS patients.

Keywords

apoptosis; neutrophil; infection; trauma; shock; hemorrhage

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None of the authors have any conflicts of interest to declare.

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INTRODUCTION

Trauma continues to be an enormous public health problem and is a leading cause of death around the world (1). In the United States, trauma is the fifth leading cause of death overall and the number one cause of death for individuals between the ages of 1 and 45 years (2). With over 170,000 trauma deaths in the US in 2005, more Americans died of injuries than from cancers of the breast, colon, prostate, liver and pancreas combined (3). Historically, the majority of trauma victims die before reaching the hospital (4); however, more recent epidemiologic studies have shown that advances in trauma care systems and emergency medical services have resulted in a significantly larger percentage of patients who survive to hospital admission (5). Although exsanguination and head injury continue to account for a large proportion of early trauma deaths, the majority of late trauma deaths occur as a result of infection and/or multiple organ failure (MOF) (5).

The clinical association between late trauma deaths and the development of MOF and infections has been well established since the 1970's (6). However, it has primarily been in the past two decades or so that researchers have focused their investigations on the body's immunological and inflammatory responses to trauma-resuscitation at a molecular and cellular level in an effort to better understand MOF in this setting and how it predisposes to infections.

In addition to playing a key role in protection against acute bacterial infections, polymorphonuclear neutrophils (PMNs) modulate the innate immune response to non-infectious tissue insults such as trauma and shock. PMN apoptosis has been shown to be blunted in patients with SIRS (7) and sepsis (8–9) where it is thought to exacerbate tissue injury (10). Reduced PMN apoptosis has been attributed, at least in part, to production of pro-inflammatory cytokines such as G-CSF that prolong PMN survival (11–13). Further studies are needed to improve our understanding of the impact of altered PMN apoptosis on outcomes of trauma, particularly infections that complicate MOF.

We previously demonstrated in a rat model of trauma and hemorrhagic shock that cardiac (14), hepatocyte (15), alveolar epithelial cells (16) and PMN apoptosis (Tweardy et al 2006, unpublished findings) are increased as a function of the duration of hypotensive phase and that apoptosis peaks approximately 4 hours following initiation of resuscitation. In the current study, we examined the hypotheses that increased peripheral PMN apoptosis occurs early in the resuscitative period in trauma/hemorrhagic shock (T/HS) patients and is associated with a decreased incidence of infections. Our findings show that peripheral leukocyte apoptosis occurs in T/HS patients, achieves maximum levels immediately post-operatively and normalizes by 24 hours. PMN were the predominant cell within the leukocyte population undergoing apoptosis. In addition, the degree of leukocyte apoptosis was inversely proportional to systemic inflammatory response syndrome (SIRS) severity and the risk of infection. These findings support the hypothesis that neutrophil apoptosis limits early tissue injury thereby decreasing subsequent susceptibility to infection in hospitalized T/HS patients.

MATERIAL AND METHODS

Patient description and enrollment

This research was conducted at Ben Taub General Hospital, a level-one trauma center located in Houston, TX. Trauma patients arriving consecutively at the Emergency Center (EC) with a systolic blood pressure less than 90 mm Hg who required emergent laparotomy or thoracotomy were enrolled and brought immediately to the operating room (OR) where they underwent simultaneous fluid resuscitation and repair of their injuries. All samples and

outcomes data were collected prospectively as part of a separate, ongoing randomized controlled clinical trial at our institution comparing hypotensive resuscitation to standard fluid resuscitation for T/HS patients (17). Inclusion criteria for the study included traumatic injury to the chest and/or abdomen requiring emergent laparotomy or thoracotomy and at least one documented systolic blood pressure less than 90 mm Hg. Exclusion criteria included any of the following: 1) age >45 years or <14 years; 2) pregnancy; 3) incarceration; 4) known history of coronary artery disease, renal disease or cerebrovascular disease; 5) patients in whom traumatic brain injury could not be definitively ruled out based upon mechanism of injury and/or negative CT scan of the head. Patients enrolled in the clinical trial that fulfilled all the inclusion criteria and did not fulfill any exclusion criterion were enrolled in the study. In just over half the cases, emergent surgery was initiated before collection of the pre-operative blood sample could be done. Such patients were excluded from the study. Patients were followed daily for 30 days or until death or hospital discharge. Patients' vital signs and all incidences of infection were recorded.

Venous blood sampling and leukocyte apoptosis studies

Peripheral venous blood samples were drawn at three time-points: pre-operatively, post-operatively, and at 24 hours. Pre-operative samples were collected either in the EC or in the OR immediately preceding the start of the case; post-operative samples were drawn upon transfer from the OR to the surgical intensive care unit (SICU); and 24-hour samples were drawn at 24 hours after admission to the SICU. Timing of the immediate post-operative blood sample was based upon our previous finding that peak levels of cell apoptosis typically occur within 4 hours of initiating fluid resuscitation following hemorrhagic shock in animals (14–16). Five mL of blood was collected in 2 heparinized tubes. Peripheral blood leukocytes were harvested by dextran sedimentation, as previously described (18), and the cell pellets immediately frozen for protein extraction. Protein was extracted using lysis buffer (Roche) and quantified using the Bradford method. Nucleosome ELISA was then performed using the Cell Death Detection ELISApplus® kit (Roche). In addition to the nucleosome ELISA assay, cytopins were prepared from leukocytes isolated from 8 of the postoperative blood samples and TUNEL stained as described (14). The percentage of TUNEL-positive leukocytes, polymorphonuclear leukocytes (PMN) and mononuclear cells within each sample was enumerated microscopically within 20 random 1,000x fields by one of the authors (AM) experienced in blood cell histology.

Clinical data

Vital signs (heart rate, temperature, respiratory rate and blood pressure) were recorded for the entire study duration of 30 days or till discharge or death. Clinical outcomes of mortality and infection were also recorded. Infection was defined according to the criteria described in Table 1. Patient had to exhibit all the listed criteria in order to be diagnosed with infection. Culture positivity was included as one of the criteria for more stringent and accurate diagnosis. The association of peak post-operative nucleosome level with SIRS data collected at three definitive time points, namely pre-operative, post-operative and 24 hours was analyzed for each participating patient in the study.

Statistical analysis

Statistical analysis was performed using STATA® statistical software package, version 10.0 (StataCorp, College Station, TX). Comparisons of continuous, independent variables were performed using the Wilcoxon Mann-Whitney test. Comparisons of continuous, paired variables were performed using the Wilcoxon Signed Rank test.

RESULTS

Peripheral blood leukocyte apoptosis peaks immediately post-operatively and consists mostly of neutrophils

41 patients had post-operative blood samples drawn and were included in the study. The subjects' baseline characteristics and intra-operative fluid requirements are shown in Table 2. The high prevalence of racial minorities noted in this study roughly reflects the racial breakdown of penetrating trauma victims treated at our urban, county hospital. The mean time from presentation to the emergency center to arrival in the OR was 15.6 minutes. The mean duration of surgery was 114 minutes.

Nucleosome levels in study subjects peaked at the post-operative time point, and then dropped to pre-operative levels at 24 hours (Figure 1A). The 41% decrease of nucleosome levels at 24-hour vs. the post-operative time point was statistically significant ($p=0.03$); the 1.7-fold increase in post-operative nucleosome levels vs. pre-operative levels nearly reached statistical significance ($p=0.06$).

TUNEL analysis was performed on 8 consecutive post-operative peripheral blood samples in order to quantify the percent of leukocytes undergoing apoptosis and to identify the type of leukocytes undergoing apoptosis (polymorphonuclear neutrophils [PMNs] vs. mononuclear cells). Overall, 9% of all peripheral leukocytes were apoptotic; PMNs accounted for 72% of the apoptotic cells with remaining cells consisting of lymphocytes.

Peripheral blood leukocyte apoptosis is inversely correlated with SIRS criteria during early resuscitation

Using a linear regression model, we found that post-operative nucleosome levels were inversely correlated to heart rate at 24 hours (Figure 1B; correlation coefficient $r = -0.36$, $p=0.02$) and at 48 hours (Figure 1C; $r = -0.39$, $p=0.01$). Post-operative nucleosome levels also were inversely correlated to temperature at 48 hours (Figure 1D; $r = -0.38$, $p=0.01$). No such correlations were seen when comparing pre-operative or 24-hour nucleosome levels to heart rate or temperature. Respiratory rate was not included in this analysis since the vast majority of patients were sedated and intubated and many of these patients had no spontaneous respirations over the ventilator settings.

Higher peripheral blood PMN apoptosis in the early resuscitative period is associated with incidence of subsequent infection

Of the 41 subjects in whom post-operative samples were obtained, 6 died within 24 hours of surgery. Of the 35 survivors, 17 (48.6%) developed an infection over the next 30 days, 3 of whom subsequently died. Infections were defined as listed in Table 1; the incidence of each type of infection is listed in Table 3. Nine of the 17 subjects who developed an infection had multiple (≥ 2) infections. The mean number of infections in those who developed any infection was 1.8 (range 1 to 5). Eighteen subjects who survived past the initial 48 hours never developed any type of infection during the next 30 days. Of these patients, one subsequently died.

There were no statistically significant differences at baseline between those who developed an infection vs. those who did not develop any infection with regards to each of the characteristics shown in Table 2 ($p>0.05$ for all comparisons), with the exception of colloid administration. Patients who developed infection received more colloid intra-operatively than those who did not develop infection (910 ml vs. 530 ml; $p=0.01$). There was no significant difference between the infected and uninfected patient groups regarding pre or peri-operative antibiotic use. Also there was no significant association between the risk of

developing infection and randomization group for the trial determining the impact of hypotensive resuscitation vs. standard fluid resuscitation.

Of note, subjects who did not develop any infection had 3-fold higher leukocyte nucleosome levels pre-operatively (40.2 mU/mg; Figure 2) than those who did develop an infection (13.6 mU/mg; $p=0.04$). In addition, those who did not develop infection had 2.5-fold higher leukocyte apoptosis level post-operatively (49.8 mU/mg; Figure 2 and Table 4) compared to those who did develop infection (19.8 mU/mg, $p=0.02$). Twenty-four-hour leukocyte apoptosis levels in the two groups were similar (Figure 2). These results suggest that leukocyte apoptosis is associated with protection against subsequent infection. The finding of increased leukocyte apoptosis pre-operatively also suggests the possibility of a genetic component to apoptosis of an individual's leukocytes in response to severe trauma.

To determine if increased leukocyte apoptosis is associated with protection from a particular type of infection or from more severe infection, we compared post-operative nucleosome levels within subgroups of T/HS patients who developed particular types of infections, had multiple infections, or had bacteremia/sepsis with those who did not develop these complications (Table 4). Post-operative nucleosome levels in patients who did not develop bacteremia/sepsis were 4.2 fold higher than in those who did develop bacteremia/sepsis ($p=0.03$), while post-operative nucleosome levels in patients who did not develop multiple infections were 4.6 fold higher than in those patients who did develop multiple infections ($p=0.01$). These findings suggest that leukocyte apoptosis post-operatively is associated with protection from bacteremia/sepsis and multiple infections, in particular. Similar results suggest that higher leukocyte apoptosis pre-operatively is associated with protection from bacteremia/sepsis (data not shown).

DISCUSSION

In this study, we demonstrated that peripheral leukocyte apoptosis occurs in T/HS patients, peaks immediately post-operatively, consists mostly of PMN, is inversely correlated with heart rate and fever at 24 and 48 hours, and is higher pre-operatively and post-operatively in those destined to not develop infections, especially multiple infections and bacteremia plus sepsis. These results suggest that apoptosis of peripheral blood leukocytes, in particular PMN, early in hospitalized T/HS patients may protect patients from subsequent infection perhaps through reducing early PMN-mediated tissue injury.

Much of the disagreement in the literature over whether leukocyte apoptosis increases or decreases the risk of infection following hemorrhagic shock depends upon which leukocyte population is being examined. Studies involving lymphocytes have correlated increased levels of apoptosis within primary lymphoid organs such as the thymus and spleen with elevated risk of subsequent infection (19–22). This is presumably due to the direct immunosuppressive effect of depleting functional lymphocytes within these organs. Regarding circulating blood lymphocytes, Teodorczyk-Injeyan et al suggested that injury related immune-deficiency correlated to increased T cell apoptosis (23). Another recent study showed that high injury scores (SOFA) were linked to elevated circulating lymphocyte apoptosis markers (sFas, sFas/FasL ratio) in patients with bacteremia (24).

On the other hand, several studies of neutrophils in rodents have convincingly shown that increased levels of PMN apoptosis may actually reduce the severity of subsequent infection (25–26) and MOF (10, 27). Henrich et al suggest that trauma-activated PMNs release inflammatory mediators and contribute to tissue injury and delayed wound healing (28).

Data from human studies are limited, and much remains to be learned. A recent study in trauma patients demonstrated an inverse correlation between degree of late neutrophil

apoptosis and severity of end-organ dysfunction, however there was no significant difference between septic and non-septic patients (29). The current study, to our knowledge, is the first to examine the impact of early peripheral blood PMN apoptosis on infectious outcomes in trauma patients with hemorrhagic shock. TUNEL staining of peripheral blood leukocyte cytopins in our current study demonstrated that 72% of the apoptotic leukocytes in the early post-operative period were PMNs. In keeping with these findings, and based upon other results presented in this paper, we propose that increased peripheral blood PMN apoptosis demonstrated early in the course of trauma-hemorrhagic shock may protect the severely-injured trauma patient from developing subsequent infection, by one or both of two mechanisms. The first mechanism is through reducing immune-mediated tissue injury; the second mechanism involves restricted immunosuppression associated with clearance of apoptotic PMNs.

It is well established that PMNs play an integral role in the body's initial inflammatory response to trauma and are recruited to sites of injury (6, 10). Once sequestered, these cells possess tremendous potential to induce additional injury to the tissues to which they are recruited. Apoptotic PMNs within the circulation are removed by macrophages within the liver and spleen rather than infiltrating into organs and contributing to inflammation and further tissue injury. Preventing tissue injury and necrosis in this manner may reduce the risk that the organ will become a subsequent site of infection as well as the likelihood it will fail.

Tissue injury from infiltrating PMNs is not limited only to organs that have been directly injured from trauma. Ischemia-reperfusion injury (which occurs after successful resuscitation from hemorrhagic shock) also results in the recruitment of neutrophils to the reperfused organs. In fact, neutrophil-mediated organ injury following resuscitation from hemorrhage has been demonstrated in liver, heart, kidney, and intestine (6). Additionally, activated PMNs stimulate macrophages to release inflammatory cytokines and chemotactic substances which further amplify the inflammatory response and increase the risk of developing MOF (30). The presence of large numbers of activated PMNs has been repeatedly associated with pathogenesis of SIRS (6–7, 27), which itself is associated with increased risk of MOF and infection (27, 30). Patients with SIRS/MOF have been shown to have delayed PMN apoptosis (7), enhanced PMN oxidative burst activity (7, 10) and increased end organ sequestration (10). It therefore follows that if PMNs could be removed from the circulation before end-organ infiltration and therefore without inciting an inflammatory response, the risk of tissue injury and susceptibility to infection might be reduced (26–27).

An alternative mechanism to explain our findings involves apoptosis-mediated immunosuppression. It is well established that apoptosis of PMN within tissue is accompanied by localized immunosuppression (31–32). Ingestion of apoptotic cells by macrophages results in the release of anti-inflammatory mediators, including TGF- β and PGE₂ (33) and suppresses the production of proinflammatory cytokines such as IL-8 and TNF- α , as well as other proinflammatory mediators, including TXA₂ (32). Clearance of circulating apoptotic PMNs by the liver and spleen may restrict immunosuppression to these organs thereby sparing other organs such as the lung, which is among the most common sites of infection following serious trauma that leads to MOF and death.

It is clear from the above discussion that neutrophils are a critical component of the innate host defense and their apoptosis and removal are essential for efficient resolution of inflammation. What remains unclear, however, is how PMN apoptosis is regulated in humans. A recent study demonstrated delayed spontaneous, as well as microbe-related apoptosis, of neutrophils in 4 patients with auto-inflammatory disease, leukocytosis, and single-nucleotide polymorphisms (SNPs) in two genes encoding proteins that contribute to

the formation of the caspase-1-activating NALP3 inflammasome complex, *NLRP3* (Q705K) and *CARD8* (C10X) (34). These findings implicate these two genes as part of the genetic program controlling PMN apoptosis in response to stress and infection. A notable finding in our study was that infected and uninfected patients segregated early in the pre-operative course and exhibited a significant difference in pre-operative leukocyte apoptosis following severe injury. This finding suggests that it might be desirable to intervene early in those individuals with low pre-operative neutrophil apoptosis in an effort to increase it. However, such an intervention would need to be selective for neutrophils and otherwise safe.

A limitation of this study is the relatively small sample size, which makes it difficult to determine significant differences between subgroups of infected patients. In several instances, differences just barely failed to reach statistical significance, and it is possible that with a larger sample size, these differences might become statistically significant. Almost half of the patients in the parent trial were excluded from this study because surgery was initiated before collection of a pre-operative blood sample. This could be regarded as a potential limitation of the study, even though how it may have biased the results is unclear. Another limitation is the presence of numerous potential confounders, which could not be controlled for. Also, multiple comparisons between the infected and non-infected groups were made for the sake of completeness and the differences observed in neutrophil apoptosis between the groups may be due to the increased chance of a difference between groups emerging in the setting of multiple comparisons. The significance of identifying 72% of apoptotic cells as PMNs may have been enhanced if complete blood counts and differentials were also performed. Clearly, PMNs predominated as the most common apoptotic cell in the peripheral blood, but in the absence of peripheral blood leukocyte counts differentials, it is not possible to determine the absolute number of apoptotic PMN and lymphocytes.

In conclusion, this study demonstrates that increased peripheral blood PMN apoptosis in the early post-operative period is associated with decreased risk of developing subsequent infection in severely injured trauma patients requiring emergency laparotomy or thoracotomy. These findings add additional support to the hypothesis that strategies aimed at limiting PMN number and function at an early point in the course of resuscitation period may be beneficial in this patient population.

Acknowledgments

This work was supported, in part, by grant DM090899 from the Department of Defense to D.J.T.

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Figure 1A.

Figure 1B.

C.

Figure 1C.

D.

Figure 1D.

Figure 1. Peripheral blood leukocyte apoptosis peaks immediately post-operatively (A) and is inversely correlated with heart rate at 24 and 48 hours (B, C) and temperature at 48 hours (D) during early resuscitation

In panel A, peripheral blood was collected from patients at three time points—pre-op, post-op and 24 hours—for isolation of leukocytes by dextran sedimentation. Cells were lysed using buffer provided in the Cell Death Detection ELISApplus® kit (Roche) and protein quantified using the Bradford method. Nucleosome ELISA was then performed as described by the manufacturer (Roche) and each value normalized to total protein content with lysate. Data plotted are the median value for each time point (actual value indicated within each bar). Statistical differences between groups were determined using the Wilcoxon signed rank test; p values are indicated by asterisks: (*) 0.06 and (**) 0.03. In panels B and C, post-operative peripheral blood leukocyte nucleosome levels were plotted as a function of heart rate at 24 hours (B) or 48 hr (C) for each patient. Post-operative nucleosome levels were inversely related to heart rate at 24 hours ($r=-0.36$, $p=0.02$) and 48 hr ($r=-0.39$, $p=0.01$; linear regression modeling). In panel D, post-operative peripheral leukocyte nucleosome

levels were plotted as a function of temperature at 48 hours for each patient. Post-operative peripheral leukocyte nucleosome levels were inversely related to temperature at 48 hours ($r = -0.38$, $p = 0.01$; linear regression modeling).

Figure 2. Peripheral blood leukocyte nucleosome levels in patients with and without infection
Peripheral blood was collected from patients at three time points—pre-op, post-op and 24 hours—for isolation of leukocytes by dextran sedimentation. Cells were lysed using buffer provided in the Cell Death Detection ELISApplus® kit (Roche) and protein quantified using the Bradford method. Nucleosome ELISA was then performed as described by the manufacturer (Roche) and each value normalized to total protein content with lysate. Patients were followed for 30 days, or until death or hospital discharge for development of infection. Pre-operative, post-operative and 24 hours median nucleosome levels of patients who developed infection were compared to patients who did not develop any infection. Statistical differences between groups were determined using the Wilcoxon signed rank test; p values are indicated by asterisks: (*) 0.04, (**) 0.02, and (***) >0.05.

Table 1

Types of infections and criteria for their diagnosis.

Pneumonia
1. Infiltrate on chest x-ray
2. Positive sputum culture
3. Fever and/or leukocytosis
Intra-abdominal Infection
1. Fluid collection requiring drainage
2. Fluid described as purulent
3. $\pm$ positive culture
Bacteremia and Sepsis
1. Positive blood cultures
2. Meets 2 SIRS criteria
Urinary Tract Infection
1. Positive urine culture and/or urinalysis
2. Fever and/or leukocytosis
Wound Infection
1. Erythema and/or wound drainage
2. Fever and/or leukocytosis
3. $\pm$ positive wound culture

* Patients must exhibit all of the criteria listed for each diagnosis.

Table 2

Patient characteristics (n=41).

Demographics			
	Infection	No infection	Total
Age (mean $\pm$ SD)	31.6 $\pm$ 10.1	33.8 $\pm$ 8.6	32.5 $\pm$ 9.3
% Male	92%	94%	93%
Black	47%	54%	51%
Hispanic	53%	42%	46%
Asian	0%	4%	2%
Mechanism			
Blunt trauma	0%	4%	2%
Gunshot wound	94%	63%	76%
Stab wound	6%	33%	22%
Presenting Vital Signs			
	Mean $\pm$ SD		
Systolic BP	80 $\pm$ 20	71.9 $\pm$ 26.8	75 $\pm$ 24
Diastolic BP	49 $\pm$ 20	34.7 $\pm$ 16.9	40 $\pm$ 19
Pulse	113 $\pm$ 18	98.7 $\pm$ 43	104 $\pm$ 36
Baseline Labs			
	Mean $\pm$ SD		
Base Deficit	-9.2 $\pm$ 4.7	-13.8 $\pm$ 7.7	-12.0 $\pm$ 7.0
Hemoglobin	29.9 $\pm$ 4.3	31.8 $\pm$ 7.1	31.1 $\pm$ 6.2
Glucose	201.8 $\pm$ 58.5	223 $\pm$ 99	214 $\pm$ 84
Injury Severity Score			
	Mean $\pm$ SD		
RTS *	10.2 $\pm$ 1.8	9.8 $\pm$ 6.2	10 $\pm$ 4.6
ISS **	23.8 $\pm$ 10.8	20.7 $\pm$ 12.9	22.0 $\pm$ 12
TRISS ***	0.97 $\pm$ 0.02	0.81 $\pm$ 0.33	0.88 $\pm$ 0.27
IV Fluids			
	Mean $\pm$ SD		
Crystalloid (mL)	3588 $\pm$ 2039	3350 $\pm$ 1861	3449 $\pm$ 1915
Colloid (mL)	912 $\pm$ 404	458 $\pm$ 405	646 $\pm$ 464
Transfusions			
	Mean $\pm$ SD		
PRBC's (mL)	1515 $\pm$ 1937	2521 $\pm$ 2725	2100 $\pm$ 2400
FFP (mL)	383 $\pm$ 559	516 $\pm$ 943	460 $\pm$ 800
Platelets	50 $\pm$ 140	118 $\pm$ 943	100 $\pm$ 210
Total Transfusions	1985 $\pm$ 2419	3115 $\pm$ 3696	2660 $\pm$ 3250
Total Inputs	6456 $\pm$ 3803	6963 $\pm$ 4640	6750 $\pm$ 4270

* Revised Trauma Score.

** Injury severity score.

*** Trauma-Injury Severity Score.

Table 3

Number of patients per infection category.

	n	% of total subjects (N=35)
Pneumonia	11	31
Intra-abdominal Abscess	9	26
Bacteremia and Sepsis	5	14
Urinary Tract Infection	4	11
Wound Infection	4	11
Other (Empyema)	1	3

Table 4
Correlation between post-operative nucleosome levels in peripheral blood leukocytes and type of infection.

	Infection		No infection		p-value
	n	Median Nucleosome Level	n	Median Nucleosome Level	
Any Infection	17	19.8	18	49.8	0.02 *
Pneumonia	11	19.8	24	46.8	0.051
Abdominal Abscess	9	26.1	26	39.0	0.57
Sepsis	5	10.2	30	42.8	0.03 *
Urinary Tract Infection	4	14.2	31	39.8	0.07
Wound Infection	4	27.4	31	39.8	0.19
Multiple Infections	9	10.2	26	46.8	0.01 *

* Analysis was performed using the Wilcoxon-Mann-Whitney test.