MANUSCRIPT

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BACKGROUND

Damage control laparotomy (DCL) has emerged as the standard of care for trauma patients requiring operative control of intra-abdominal and retroperitoneal injury(1-3). This advancement, which allows for the management of patients with previously lethal levels of hypothermia, acidosis, and coagulopathy, has also forced physicians to become comfortable with the management of an open abdomen. (4-6) Despite advances in the recognition of IAH/ACS and implementation of a multitude of devices for temporary abdominal closure (TAC) as many as 30% of patients will fail to achieve primary fascial closure (PFC).(7, 8) (9-11)

While the morbidity associated with the management of an open abdomen poses one threat, the trauma patient that has received a DCL is also susceptible to the effects of a massive inflammatory reaction and associated multifactorial edema; a risk factor for developing multiple organ dysfunction syndrome (MODS). (12) A variety of intravascular fluids have been investigated for their therapeutic effects against activation of neutrophils and the inflammatory cascade to improve clinical outcomes. (13-17). From these investigations it has been shown that HTS is superior to lactated ringer's solution (LR), and normal saline solution (NSS) at reducing neutrophil activation, apoptosis, bowel edema, and expression of inflammatory cytokines both *in-vitro* and *in-vivo*.(18-20)

More recently data has emerged demonstrating that the use of low-volume hypertonic saline infusion is effective at reducing the inflammatory cascade as a method of achieving earlier PFC.(12, 21-25). The data presented herein is a subset analysis of a larger multi-center randomized controlled trial analyzing the use of low-volume hypertonic saline to achieve early and higher rates of PFC among patients undergoing DCL. Specifically, this study investigated the levels of cytokines IL-6 and IL-8 as a marker for the inflammatory effect of HTS compared to NSS in this patient population. In line with previous literature, our hypothesis was that the HTS group would demonstrate reduced inflammatory cytokine levels.

METHODS

Patient data was analyzed from a single center as a part of a multi-center prospective randomized, double-blinded trial. The sample size for the overall study was based upon the primary outcome of the multicenter trial of the incidence of failure of PFC based on the retrospective review by Harvin et al. is 24%. We expected HTS to reduce closure failure incidence to 12%. Assuming two-sided testing on the equality of binomial proportions with a significance level of 5%, this study will achieve 80% power with n=139 subjects per group if the proportions of subjects experiencing failure of closure in the control and treated groups are 0.24 and 0.12 respectively [PASS Version 11, NCSS Kaysville Utah 2011]. The anticipated number

of subjects (280) is in range of referenced prospective observational studies. As the primary site, San Antonio Military Medical Center (SAMMC) enrollment was not to exceed 25% of total enrollment or 70 patients. Cytokine data was exclusively collected at SAMMC. Patients were identified between September 2014 through June 2017 who required admission to the Trauma Surgery service and underwent damage control laparotomy. Patients meeting the following criteria were excluded: (1) <18 years old, (2) pregnant, (3) traumatic loss of > 1/3 of the abdominal wall, (4) serum sodium <120mEq/L or >155mEq/L. Patients were required to be enrolled within 6 hours of receiving DCL. A total 70 patients meeting criteria were randomized to receive 30cc/hr of either 3% HTS or 0.9% NSS for the first 72 hours of their ICU admission following laparotomy.

The attending trauma surgeon and/or the surgery resident overseeing the trauma resuscitation notified the study personnel of patients who underwent a laparotomy. Upon Emergency Department (ED) admission, eligible patients who fulfill the inclusion criteria and who do not meet any of the exclusion criteria were be entered into the study after obtaining informed consent from the subject or legally authorized representative. The patient was randomized to either HTS or standard crystalloid at this point by an independent data collection specialist who used a computer program to carry out random allocation. The program printed out a randomization list in advance of study initiation and was prepared by someone not involved in the recruitment process. After the randomization list was prepared, it was be made available to the inpatient pharmacy in a binder with the study fluid coding in a separate letter. The pharmacist assigned the next fluid (A/B) on the list when the order was placed and prepared enough study fluid in plain bags for 72 hours and had the nurse pick it up. The study participant had to be consented, enrolled, and started on specified fluid within 6 hours of returning from the operating room. All patient care team members were blinded to the intervention arm.

The primary dependent variable of this study was the level of inflammatory cytokines IL-6 and IL-8. Serum samples for cytokine analysis were drawn daily at 0800 hours for all patients. Additional variables measured included vital signs (systolic blood pressure, heart rate), resuscitation fluid and volume (crystalloid, colloid, blood products), laboratory tests and temperature recorded every 6 hours (CBC, BMP, Coag panel, lactate, ABG, serum osmolality, amylase, lipase, thromboesastogram), bladder pressure, peak inspiratory pressure, ventilatory mode, and lasix administration dose and timing.

Demographic variables included age (years), initial Glasgow Coma Scale (GCS), BMI (kg/m^2), sex, and mechanism of injury. Additionally, trauma scores were assigned according to injury severity score (ISS),), Trauma Injury Severity Score (TRISS), Revised Trauma Score (RTS), and maximal Abbreviated Injury Score (AIS). Solid injury scoring scales were utilized according to AAST, 1994 revision criteria. Patient mortality and complications (VAP, wound dehiscence, SSI, intra-abdominal abscess, enterocutaneous fistula, hypernatremia, hyperchloremia, AKI, and ACS) were also tabulated, with the latter adhering to specifically documented criteria (Table 3).

Statistical analysis was performed using JMP 13 (SAS, Cary, NC). Fisher's exact test, Mann-Whitney U-test, or Student's t-test were used as appropriate. Statistical significance was set at $p<0.05$.

RESULTS

A total of 126 patients were assessed for eligibility for the study. Of these, 9 patients, the admitting service failed to notify study team within the appropriate time frame, 25 had no available legally available representative (LAR), 11 had LARs who declined, 4 had devastating brain injuries, and 7 met exclusion criteria (5 prisoners, 1 <18yoa, and 1 pregnant). Thus, 70 patients met inclusion criteria with 35 patients randomized to each arm. In each group 4 patients did not receive their intervention for the full 72 hours and were excluded from analysis. Within the NSS arm an additional 2 patients were excluded from analysis due to incomplete cytokine levels.

The HTS and NSS groups were similar across all demographics. Groups were also similar with regards to ISS, initial GCS, maximum AIS, TRISS, and RTS. There were more penetrating traumas in the NSS cohort (64% vs. 36%), but no difference in organ laceration, orthopedic injuries, abdominal trauma, or significant vascular injuries (data not published). Mean base deficit and lactate were not significantly different ($p>0.05$). Geometric means of IL-6 and IL-8 of both the HTS and NSS groups were calculated for each 24-hour period and compared. The geometric means of IL-6 and of IL-8 concentrations were significantly higher in the HTS group compared to the NSS group in the first 72 hours ($p=0.033$, $p=0.047$, respectively). However, when the geometric means of each trial group were compared using student's t-test, it becomes apparent that the statistical significance between the two groups disappears by the third day.

DISCUSSION

To date, this is the largest, human subject randomized controlled trial investigating the impact of HTS on inflammatory cytokines and clinical outcomes. A solution concentration and infusion rate of 3% HTS at 30cc/hr was chosen based off of previous trials that have shown this to be safe and efficacious for clinical outcomes. (24, 25) Additionally, 3% HTS is a readily and commercially available concentration as opposed to other concentrations that have been investigated such as 7.5% or 10%.(22) Harvin et. al and Loftus et. al illustrated that by using 3% HTS at 30cc/hr a statistically significant increase in early PFC could be achieved in both trauma patients as well as the acute care surgery population, respectively.

The early increase in IL-6 and IL-8 of the HTS group relative to the NSS group seen early in the post-operative period was unexpected given prior literature expounding the anti-inflammatory effects of HTS. We believe this is multi-factorial. As part of a larger, multi-center RCT focused on increasing the rate of early PFC the timing and rate of HTS administration chosen for this study differs when compared to other literature investigating the anti-inflammatory effects of HTS. Most notably, our trial was specific to patients who underwent a DCL as opposed to other studies including a less restrictive demographic of trauma patients. Rizoli's double blinded RCT utilized a 250cc 7.5% HTS solution with 6% dextran-70 bolus initiated as patients were received in the emergency department. (26) Published in 2012, Junger et. al were also able to elucidate a reduction in the inflammatory response that predisposes the patient to MODS by utilizing pre-hospital, 250cc boluses of either 7.5% HTS or 7.5% HTS with 6% dextran-70; with dextran free

HTS achieving the superior anti-inflammatory effect.(12) Furthermore, as the ultimate focus of the data collected is on PFC cytokine data collection was delayed until 0800 of POD0/1 from the initial DCL. This is in contrast to the previously mentioned RCTs which provide data as early as 1 hour after initial evaluation. Finally, this trial presents novel data investigating specifically interleukins and their levels after trauma as opposed to neutrophil specific cell adhesion molecules and mediators.

There are obvious limitations to this study. Most obviously though adequately powered the data is representative of a single center and is subject to a sampling bias for any conclusions applied to other centers. Additionally, due to constrained resources despite the time of enrollment for each patient, serum samples for IL-6 and IL-8 cytokine analysis were drawn at 0800 each day. This equates to an uncontrolled variable for the time after DCL until IL-6 and IL-8 were measured. To try and account for this the geometric mean of each group was used as a measure that is less amenable to manipulation by outlying data points. Finally, the data analysis was not conducted under an intention-to-treat protocol and subject to skewing by the small number of patients who were randomized and did not survive or receive treatment for the full 72 hours.

This study is the first double blinded RCT to investigate the effects of dextran free HTS on inflammatory cytokines in patients receiving DCL. As such, it presents novel data to suggest that the inflammasome surrounding this intervention is still incompletely understood despite encouraging data that the addition of HTS may improve both early PFC rates and a reduction in MODS and associated comorbidities. We plan to continue with the analysis of this data as part of our larger multi-center RCT investigating the effect of HTS on PFC rates. Additionally, we believe the data presented here calls for additional clinical investigation optimized at decoding the traumatically induced inflammasome as a step towards decreasing inflammation reduced morbidity and mortality in the trauma patient. Specifically, we believe that future investigation should focus on determining the optimal timing, dose, concentration, and administration rate of HTS to reduce both neutrophil specific cell mediators and overarching cytokines such as IL-6 and IL-8.

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DISCLAIMER

The views expressed are those of the [author(s)] [presenter(s)] and do not reflect the official views or policy of the Department of Defense or its Components. The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DODI 3216.02_AFI 40-402.

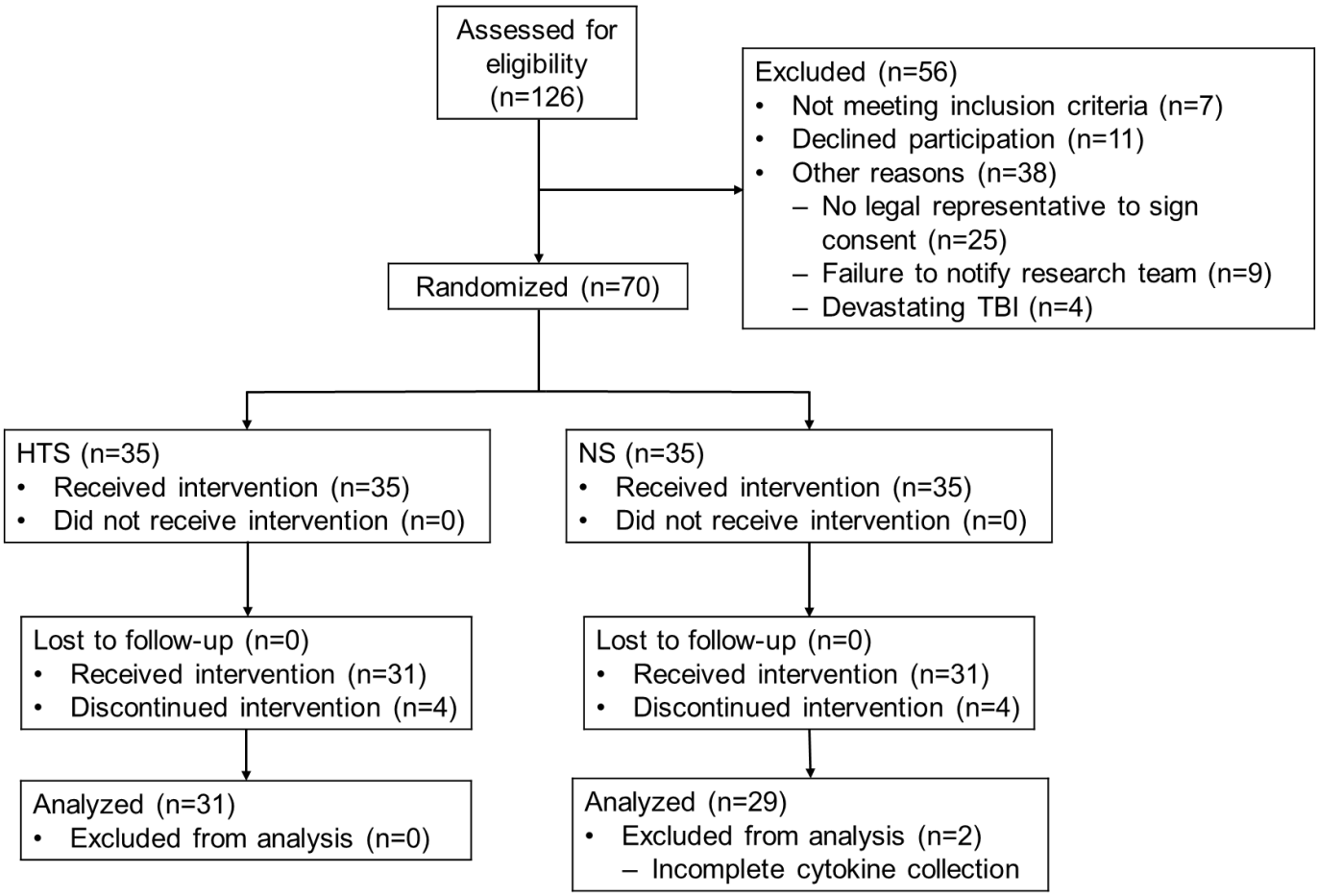

Figure 1. Flow diagram

TABLE 1. Sample Demographics

Variables		NS (n=35)	HTS (n=35)	*p*
Age-year	Median	34	36.5	.90
	Range	19-78	19-91	
GCS	Median	15	14	0.32
	Range	3-15	3-15	
BMI - kg/m^2	Median	29.39	28.24	0.20
	Range	18.62-52.8	20.15-52	
Sex	Male	28	22	0.10
	Female	6	12	
ISS	Median	19.5	23.5	0.42
	Range	5-41	1-57	
TRISS	Median	0.95	0.95	0.27
	Range	0.02-0.99	0.45-0.98	
RTS	Median	7.84	7.84	0.52
	Range	0-7.84	4.09-7.84	
Mechanism	Penetrating	21	12	0.03
	Blunt	13	22	

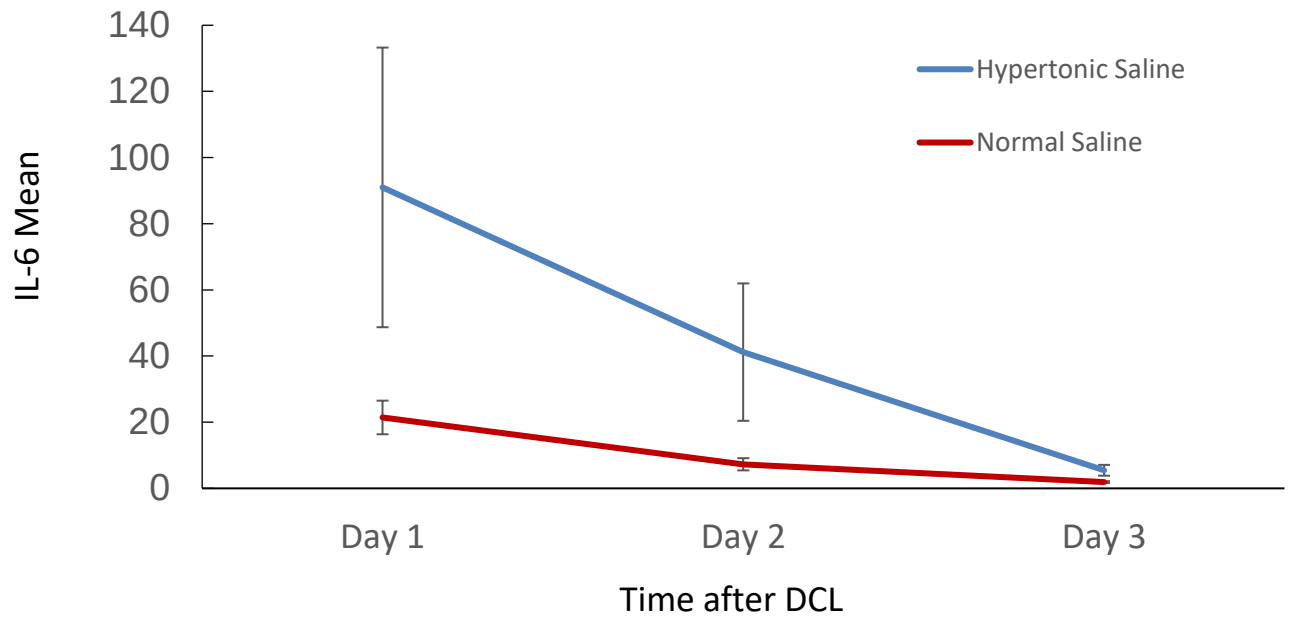

Figure 2. IL-6 levels

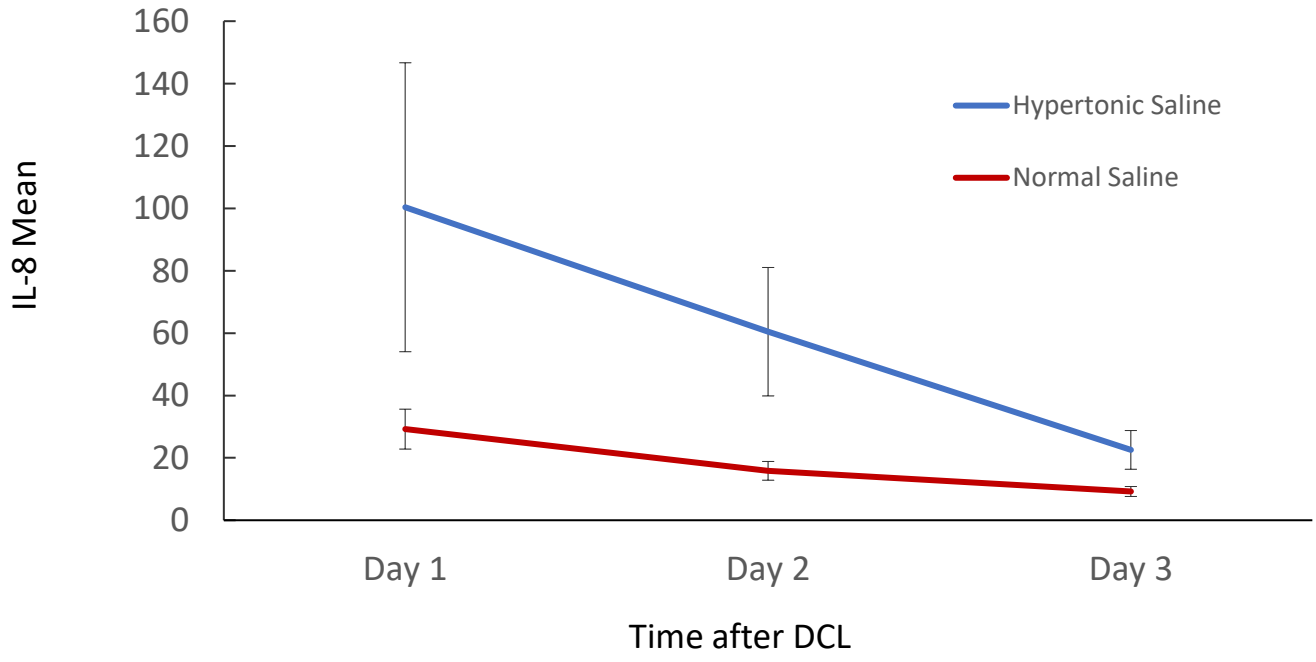

Figure 3. IL-8 levels

TABLE 2. Mortality, Morbidity, and Selected Laboratory Values

Variable	NS (n=35)	HTS (n=35)	*p*
Mortality	2	4	0.22
Complications			
VAP	3	4	0.69
Dehiscence	1	1	1.0
Surgical site infection	0	3	0.08
Intra-abdominal Abscess	2	1	0.56
Enterocutaneous Fistula	0	1	0.31
Hypernatremia	4	1	0.16
Hyperchloremia	5	1	0.088
AKI	3	5	0.45
ACS	6	3	0.28
INR			
Max	1.64 (1.51-1.76)	1.66 (1.52-1.79)	0.82
Min	1.18 (1.13-1.22)	1.16 (1.11-1.20)	0.52
BD			
Max (+)	1.94 (0.81-3.06)	1.39 (0.41-2.37)	0.46
Min (-)	7.42 (8.68-6.16)	8.34 (9.59-7.10)	0.29
Lactate (mg/dL)			
Max	4.51 (3.99-5.03)	4.98 (4.16-5.79)	0.34
Min	0.92 (0.77-1.05)	1.03 (0.88-1.18)	0.26

TABLE 3. Definitions

1. Hours to primary fascial closure= time of end of initial OR until end of OR during which fascia is closed, rounded to the nearest 30 minute interval
2. Ventilator associated pneumonia= pneumonia that develops 48 hours or longer after mechanical ventilation
    a. pulmonary infection: signs include fever, purulent secretions, and leukocytosis
    b. bacteriologic evidence of pulmonary infection
    c. radiologic suggestion of pulmonary infection
3. Dehiscence= partial or complete separation of the midline abdominal fascia
4. Surgical site infection= infection occurs within 30 days after exploratory laparotomy
    a. involves only skin and subcutaneous tissue of the incision
    b. patient has at least one of the following:
        i. purulent drainage from the superficial incision.
        ii. organisms isolated from an aseptically-obtained culture from the superficial incision or subcutaneous tissue.
        iii. superficial incision that is deliberately opened by a surgeon, attending physician or other designee and is culture positive or not cultured
    c. patient has at least one of the following signs or symptoms: pain or tenderness; localized swelling; erythema; or heat. A culture negative finding does not meet this criterion.
    d. diagnosis of a superficial incisional SSI by the surgeon or attending physician or other designee.
5. Intra-abdominal abscess= infection occurs within 30 days after the exploratory laparotomy
    a. infection involves any part of the body deeper than the fascial/muscle layers, that is opened or manipulated during the operative procedure
    b. patient has at least one of the following:
        i. purulent drainage from a drain that is placed into the organ/space (e.g., closed suction drainage system, open drain, T-tube drain, CT guided drainage)
        ii. organisms isolated from an aseptically-obtained culture of fluid or tissue in the organ/space
        iii. an abscess or other evidence of infection involving the organ/space that is detected on gross anatomical or histopathologic exam, or imaging test
6. Enterocutaneous fistula (ECF)= an abnormal communication between the small or large bowel and the skin or atmosphere.
7. Abdominal compartment syndrome (ACS)= organ dysfunction caused by intra-abdominal hypertension (IAH) as documented by bladder pressure of at least 15cmH2O.
8. Hypernatremia= serum sodium level >155
9. Hyperchloremia= serum chloride level >118
10. Ventral hernia= incisional hernia of abdominal wall where the fascia does not heal properly.
11. Anastamotic leak= intestinal wall defect with communication of the intraluminal and extraluminal compartments (at site of anastomosis) that is diagnosed by CT scan as a result of symptoms to include ileus, leukocytosis, fever, vomiting, or diarrhea/constipation.
12. Acute kidney injury= defined by RIFLE criteria
13. RIFLE Score: Risk, Injury, Failure, Loss of function, End stage kidney disease (appendix F)
14. Death (either yes or no), if yes then was it outside of the 72hour study protocol (yes or no), and select only one cause of death.

REFERENCES

1. Burch JM, Ortiz VB, Richardson RJ, Martin RR, Mattox KL, Jordan GL, Jr. Abbreviated laparotomy and planned reoperation for critically injured patients. Ann Surg. 1992;215(5):476-83; discussion 83-4.
2. Morris JA, Jr., Eddy VA, Blinman TA, Rutherford EJ, Sharp KW. The staged celiotomy for trauma. Issues in unpacking and reconstruction. Ann Surg. 1993;217(5):576-84; discussion 84-6.
3. Rotondo MF, Schwab CW, McGonigal MD, Phillips GR, 3rd, Fruchterman TM, Kauder DR, Latenser BA, Angood PA. 'Damage control': an approach for improved survival in exsanguinating penetrating abdominal injury. The Journal of trauma. 1993;35(3):375-82; discussion 82-3.
4. Meldrum DR, Moore FA, Moore EE, Franciose RJ, Sauaia A, Burch JM. Prospective characterization and selective management of the abdominal compartment syndrome. Am J Surg. 1997;174(6):667-72; discussion 72-3.
5. Shapiro MB, Jenkins DH, Schwab CW, Rotondo MF. Damage control: collective review. The Journal of trauma. 2000;49(5):969-78.
6. Ertel W, Oberholzer A, Platz A, Stocker R, Trentz O. Incidence and clinical pattern of the abdominal compartment syndrome after "damage-control" laparotomy in 311 patients with severe abdominal and/or pelvic trauma. Crit Care Med. 2000;28(6):1747-53.
7. Miller PR, Meredith JW, Johnson JC, Chang MC. Prospective evaluation of vacuum-assisted fascial closure after open abdomen: planned ventral hernia rate is substantially reduced. Ann Surg. 2004;239(5):608-14; discussion 14-6.
8. Weinberg JA, George RL, Griffin RL, Stewart AH, Reiff DA, Kerby JD, Melton SM, Rue LW, 3rd. Closing the open abdomen: improved success with Wittmann Patch staged abdominal closure. The Journal of trauma. 2008;65(2):345-8.
9. Hatch QM, Osterhout LM, Ashraf A, Podbielski J, Kozar RA, Wade CE, Holcomb JB, Cotton BA. Current use of damage-control laparotomy, closure rates, and predictors of early fascial closure at the first take-back. The Journal of trauma. 2011;70(6):1429-36.
10. Burlew CC, Moore EE, Biffl WL, Bensard DD, Johnson JL, Barnett CC. One hundred percent fascial approximation can be achieved in the postinjury open abdomen with a sequential closure protocol. J Trauma Acute Care Surg. 2012;72(1):235-41.
11. Dubose JJ, Scalea TM, Holcomb JB, Shrestha B, Okoye O, Inaba K, Bee TK, Fabian TC, Whelan J, Ivatury RR, et al. Open abdominal management after damage-control laparotomy for trauma: a prospective observational American Association for the Surgery of Trauma multicenter study. J Trauma Acute Care Surg. 2013;74(1):113-20; discussion 1120-2.
12. Junger WG, Rhind SG, Rizoli SB, Cuschieri J, Shiu MY, Baker AJ, Li L, Shek PN, Hoyt DB, Bulger EM. Resuscitation of traumatic hemorrhagic shock patients with hypertonic saline-without dextran-inhibits neutrophil and endothelial cell activation. Shock. 2012;38(4):341-50.
13. Mattox KL, Maningas PA, Moore EE, Mateer JR, Marx JA, Aprahamian C, Burch JM, Pepe PE. Prehospital hypertonic saline/dextran infusion for post-traumatic hypotension. The U.S.A. Multicenter Trial. Ann Surg. 1991;213(5):482-91.
14. Rhee P, Burris D, Kaufmann C, Pikoulis M, Austin B, Ling G, Harviel D, Waxman K. Lactated Ringer's Solution Resuscitation Causes Neutrophil Activation after Hemorrhagic Shock. Journal of Trauma and Acute Care Surgery. 1998;44(2):313-9.

15. Deb S, Martin B, Sun L, Ruff P, Burris D, Rich N, DeBreux S, Austin B, Rhee P. Resuscitation with lactated Ringer's solution in rats with hemorrhagic shock induces immediate apoptosis. The Journal of trauma. 1999;46(4):582-8; discussion 8-9.
16. Deb S, Sun L, Martin B, Talens E, Burris D, Kaufmann C, Rich N, Rhee P. Lactated ringer's solution and hetastarch but not plasma resuscitation after rat hemorrhagic shock is associated with immediate lung apoptosis by the up-regulation of the Bax protein. The Journal of trauma. 2000;49(1):47-53; discussion -5.
17. Koustova E, Stanton K, Gushchin V, Alam HB, Stegalkina S, Rhee PM. Effects of lactated Ringer's solutions on human leukocytes. The Journal of trauma. 2002;52(5):872-8.
18. Alam HB, Stegalkina S, Rhee P, Koustova E. cDNA array analysis of gene expression following hemorrhagic shock and resuscitation in rats. Resuscitation. 2002;54(2):195-206.
19. Balogh Z, McKinley BA, Cox CS, Jr., Allen SJ, Cocanour CS, Kozar RA, Moore EE, Miller IC, Weisbrodt NW, Moore FA. Abdominal compartment syndrome: the cause or effect of postinjury multiple organ failure. Shock. 2003;20(6):483-92.
20. Gushchin V, Alam HB, Rhee P, Kirkpatrick JR, Koustova E. cDNA profiling in leukocytes exposed to hypertonic resuscitation fluids. J Am Coll Surg. 2003;197(3):426-32.
21. Attuwaybi B, Kozar RA, Gates KS, Moore-Olufemi S, Sato N, Weisbrodt NW, Moore FA. Hypertonic saline prevents inflammation, injury, and impaired intestinal transit after gut ischemia/reperfusion by inducing heme oxygenase 1 enzyme. The Journal of trauma. 2004;56(4):749-58; discussion 58-9.
22. Gonzalez EA, Kozar RA, Suliburk JW, Weisbrodt NW, Mercer DW, Moore FA. Conventional dose hypertonic saline provides optimal gut protection and limits remote organ injury after gut ischemia reperfusion. The Journal of trauma. 2006;61(1):66-73; discussion -4.
23. Shah SK, Uray KS, Stewart RH, Laine GA, Cox CS, Jr. Resuscitation-induced intestinal edema and related dysfunction: state of the science. J Surg Res. 2011;166(1):120-30.
24. Harvin JA, Mims MM, Duchesne JC, Cox CS, Jr., Wade CE, Holcomb JB, Cotton BA. Chasing 100%: the use of hypertonic saline to improve early, primary fascial closure after damage control laparotomy. J Trauma Acute Care Surg. 2013;74(2):426-30; discussion 31-2.
25. Loftus TJ, Efron PA, Bala TM, Rosenthal MD, Croft CA, Smith RS, Moore FA, Mohr AM, Brakenridge SC. Hypertonic saline resuscitation after emergent laparotomy and temporary abdominal closure. J Trauma Acute Care Surg. 2018;84(2):350-7.
26. Rizoli SB, Rhind SG, Shek PN, Inaba K, Filips D, Tien H, Brenneman F, Rotstein O. The immunomodulatory effects of hypertonic saline resuscitation in patients sustaining traumatic hemorrhagic shock: a randomized, controlled, double-blinded trial. Ann Surg. 2006;243(1):47-57.