Hydroxyethyl starch resuscitation downregulate pro-inflammatory cytokines in the early phase of severe acute pancreatitis: A retrospective study

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Abstract. In the present study, we investigated the effects of hydroxyethyl starch (HES) 130/0.4 on serum pro-inflammatory variables, immunologic variables, fluid balance (FB)-negative(-) rate and renal function in severe acute pancreatitis (SAP) patients. From October, 2007 to November, 2008, a total of 120 SAP patients were enrolled in this retrospective study. Fifty-nine patients in the HES group received 6% HES 130/0.4 combined with crystalloid solution for fluid resuscitation (HES group). In the control group, 61 patients received only crystalloid solution after admission. Interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor (TNF)-α levels in serum were measured on days 1, 2, 4 and 8. The peripheral blood $CD4^+CD8^+$ T lymphocyte rates, serum BUN and Cr values were also measured on days 1, 4 and 8. Patients with FB(-) rates were recorded from day 1 to 8. Interaction term analysis (hospital stay and fluid resuscitation methods) based on mixed-effects regression model revealed significantly lower levels of IL-1 and TNF-α in the HES group compared with the control group. The difference in curve's risk ratio was not significant for IL-6, $CD4^+CD8^+$ T lymphocyte rate, BUN and Cr values ($P>0.05$). In the HES group, we detected a significantly higher rate of patients with FB(-) from day 4 to 8 ($P<0.05$). Thus, HES 130/0.4 resuscitation could decrease the IL-1 and IL-8 levels, shorten the duration of positive FB, and preserve the patient's immune status as well as renal function during the early phase of SAP.

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Introduction

Severe acute pancreatitis (SAP) is an acute inflammatory process with variable involvement of local tissues and remote organs (1). The overexpression of inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor (TNF)-α, can seriously damage the microcirculatory system's endothelium and subsequently increase capillary permeability (2,3). Additionally, the persistent inflammatory state aggravates the existing hypoxia condition and systemic inflammatory response syndrome (SIRS), which eventually result in an increase in the mortality rate associated with SAP (4,5).

In addition to the cytokine cascade, the activation of the adaptive immune system, including $CD4^+CD8^+$ T lymphocytes is crucial in the development of SIRS and organ failure in SAP patients (6,7). Results obtained from prior studies reported a significant reduction in peripheral blood T-cell subgroups in the early phase of AP, which may correspond with patients' clinical outcome and disease severity (8,9). In a previous study, we reported that the reduction in $CD4^+$ T lymphocytes in peripheral blood was linked to organ failure and may act as a potential predictor of severity in AP patients (10). Effective fluid resuscitation and immune regulation therapy is significant for the prognosis of SAP in the early phase.

Among patients with AP, early fluid resuscitation is often associated with a reduced incidence of SIRS and organ failure (11,12). Currently, two types of fluids are frequently used for active resuscitation: Crystalloid and colloid fluids with large molecules that maintain the fluid intravascularly. Hydroxyethyl starch (HES) is a widely used colloid fluid in volume expansion and is a priming fluid in the early stage

of AP (13,14). Previous findings have shown that HES resuscitation in the early stages of SAP can decrease the acute physiology and chronic health evaluation II (APACHE II) score, reduce the need for mechanical ventilation, reduce the risk of intra-abdominal hypertension, and shorten the duration of positive fluid balance (FB) (15,16). However, recent studies indicated that HES may not provide a clinical benefit and may even cause a higher mortality rate as well as higher acute kidney injury rate in critically ill patients (17-20). This controversy aroused concerns regarding the safety of using HES resuscitation during the early phase of SAP. By contrast, other studies have shown that HES 130/0.4 can reduce the pro-inflammatory responses and regulate the $CD4^+CD8^+$ T lymphocyte subgroup ratio in cardiac surgery (21,22). However, whether HES 130/0.4 is effective for the reduction of pro-inflammatory cytokines and safe for renal function was the focus of the present study.

Materials and methods

Data source. All relevant clinical data were extracted from the database of a multi-center, randomized controlled trial (RCT) study, which was designed to observe the effects of early goal-directed fluid therapy with HES 130/0.4 on intra-abdominal hypertension (IAH), multiple organ dysfunction and FB in SAP patients. This RCT study was conducted from October, 2007 to November, 2008 and a total of 120 SAP patients from four sites were enrolled in the present study. There were no significant differences between the groups regarding demographic data (P>0.05) (Table I).

Patients. Participants were adult patients (aged 18-65 years) admitted within 3 days of disease onset. The diagnostic and classification criteria included two of the following features: i) Abdominal pain consistent with AP; ii) amylase activity at least 3-fold greater than the upper limit of normal; and iii) characteristic findings of AP on contrast-enhanced computed tomography CT) and, less commonly, on magnetic resonance imaging or transabdominal ultrasonography. Patients with confirmed SAP were considered eligible. The patients had at least one of the following criteria: i) Failure in at least one organ as defined by the Atlanta classification; ii) an APACHE II score ≥8; and iii) a Balthazar's CT grade classification score ≥7 (23). Patients with the following conditions were excluded: i) History of allergy to HES 130/0.4; ii) serious cardiac or renal failure; iii) serum albumin value <25 g/l; iv) blood coagulation dysfunction with international normalized ratio >3; v) history of other colloid intravascular volume-replacement regimens within 3 months prior to their enrollment in this study; vi) pregnant or lactating female patients; and vii) MOF needing continuous renal replacement treatment after admission.

Treatments. All the patients received specialized medical therapy for AP according to the United Kingdom, Chinese Medical Association, and International Association of Pancreatology guidelines (24-26). After admission, patients were randomly divided into 2 groups. One group received crystalloid (the control group) and the other group received crystalloid plus HES 130/0.4 resuscitation (the HES group). Patients in the two groups were infused with lactated Ringer's solution at a basic rate of 1-2 ml/kg/h. The control group received only Ringer's lactate and saline solution for resuscitation. In the treatment group, 6% HES 130/0.4 (Voluven; Fresenius Kabi, Bad Homburg, Germany) was infused at a volume ratio of 1:3 compared with saline solution. The volume of HES was maintained at <50 ml/kg. The total rate and volume of intravenous fluid was controlled to maintain hemodynamic stability. A stable hemodynamic status was defined as a central venous pressure (CVP) of 8-15 mmHg (1 mmHg=0.133 kPa), a urine output >0.5 ml/kg/h, a mean blood pressure >65 mmHg, a heart ratio of 80-100/min, a hematocrit >0.3 or a SpO_2 of CVP >0.7. Medication was planned to be administered for 7 days. After a negative FB emerged, the HES was disabled according to the patient's condition.

The study was approved by the Medical Ethics Committee of Union Hospital (Wuhan, China). The need for informed consent was waived by the Medical Ethics Committee because the study was a retrospective study using a database from which patients' identification information had been removed.

Measurement of parameters and detection methods. Factors included in this study were: Age, gender, height, CT grade, APACHE II scores, white blood cell counts, serum ALT, AST, BUN, Cr, Ca^{2+}, CRP and amylase. The intra-abdominal pressure (IAP) and CVP was measured at 10:00 a.m., 12:00 and 2:00 p.m. on the 1st day after admission according to the standard techniques established by the World Society of Abdominal Compartment Syndrome in 2006 (27). The mean IAP value was calculated and the CVP measurement protocols were defined as previously described (28).

Blood samples were obtained on days 1, 2, 4 and 8 after hospitalization and serum pro-inflammatory mediators including IL-1, IL-6, IL-8 and TNF-α levels were measured using enzyme-linked immunosorbent assays (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. Additionally, patients' blood samples on days 1, 4 and 8 were used to detect the rate of $CD4^+CD8^+$ T lymphocytes. The measurement protocols were described in a previous study (10).

Statistical analysis. Empower (R) (www.empowerstats.com; X&Y Solutions, Inc., Boston, MA, USA) and R (http://www.R-project.org) software were used for the statistical analysis. Missing data were described as 'NA' as per Empower (R) requirements. Data were presented as mean ± SD or proportions. Comparisons between groups were performed using the Chi-square test for categorical variables and two-sample t-test for continuous variables. P<0.05 was considered to indicate a statistically significant difference.

We compared the inter-group differences with regard to the patients' general condition, CT grade, APACHE II score, IAP value after admission, peritoneal drainage rate, inflammatory cytokine value, lymphocyte subsets rates and other biochemical values. In order to obtain a clear description of changes, we made line charts using the inflammatory cytokine values (including IL-1, IL-6, IL-8 and TNF-α) on days 1, 2, 4, and 8 after admission. Line charts were also made using lymphocyte subset rates (including $CD4^+CD8^+$) on days 1, 4, and 8 after admission.

Table I. Demographic information and baseline clinical characteristics in the 2 groups.

	HES group	Control group	P-value
No. (n)	59	61	-
Age (years)	47.98±12.48	46.43±11.54	0.479
Gender [n, (%)]			0.741
Male	36 (61.00%)	39 (63.90%)	
Female	23 (39.00%)	22 (36.10%)	
Height (cm)	165.85±7.73	167.05±7.00	0.374
CT grade [n, (%)]			0.991
3	35 (59.30%)	35 (58.30%)	
4	5 (8.50%)	5 (8.30%)	
5	19 (32.20%)	20 (33.30%)	
APACHE II score	13.03±5.18	12.87±5.41	0.865
IAP (mmHg)	9.26±2.15	9.68±2.70	0.433
CVP (cmH_2O)	10.44±3.28	9.92±3.23	0.379
WBC ($x10^9/l$)	14.51±4.43	14.13±4.67	0.646
ALT (U/l)	61.49±90.44	56.92±82.57	0.773
AST (U/l)	69.43±118.66	49.70±63.61	0.257
BUN (mmol/l)	5.59±3.64	6.43±3.52	0.201
Cr (μmol/l)	89.68±53.50	88.31±61.41	0.897
Ca^{2+} (mmol/l)	1.91±0.28	1.86±0.29	0.377
CRP (μg/ml)	197.56±108.96	190.57±92.24	0.718
Serum amylase (U/l)	789.69±661.13	696.23±594.54	0.417
Peritoneal drainage [n, (%)]			0.864
Yes	9 (15.3%)	10 (16.4%)	
Not	50 (84.7%)	51 (84.6%)	
IL-1 (pg/ml)	21.34±11.90	25.14±16.78	0.160
IL-6 (pg/ml)	51.89±44.55	57.85±68.09	0.576
IL-8 (pg/ml)	32.04±34.02	32.87±33.12	0.894
TNF-α (pg/ml)	45.52±29.78	40.27±16.12	0.234
$CD4^+$ T cell (%)	31.53±12.09	32.56±9.08	0.742
$CD8^+$ T cell (%)	21.00±8.01	19.82±6.74	0.590

HES group, patients received crystalloid solutions plus HES 130/0.4 for fluid resuscitation for day 1-8 after admission; control group, patients received only crystalloid solutions for fluid resuscitation for day 1-8 after admission. HES, hydroxyethyl starch; CT, computed tomography; APACHE II, acute physiology and chronic health evaluation II; IAP, intra-abdominal pressure; CVP, central venous pressure; IL, interleukin; TNF, tumor necrosis factor.

We evaluated the effect of the treatment on inflammatory cytokine levels and lymphocyte subset rates with regression analyses in the two groups. Additionally, a model adjusted for potential confounders [gender, age and peritoneal drainage (yes or not)] was established simultaneously. The longitudinal changes in inflammatory cytokine values and lymphocyte subset rates were analyzed with linear mixed-effects regression models in the two groups. We also calculated the effect of HES 130/0.4 on the serum BUN and Cr values based on the abovementioned model.

Changes in the inflammatory cytokine levels and lymphocyte subset rates were dependent on the treatment options and curing time. To reveal whether the effect of HES 130/0.4 was critical for the longitudinal changes in the inflammatory cytokine values and lymphocyte subset rates, a mixed-effects model was set-up to estimate the interaction between a fixed effect variable and hospital stay. First, the variable data were transformed into multiple records based on the admission time. Potential confounders [gender, age and peritoneal drainage (yes or not)] were also adjusted in these models. Then, the treatment effect (crystalloid plus HES 130/0.4 resuscitation or only crystalloid resuscitation), time effect (hospital stay) and the interaction between the treatment and time effects in both groups were evaluated in multiple regression models. Statistical analysis (P) elicited whether the fixed effect variable of the HES group was significant for the inflammatory cytokine values and lymphocyte subset rates when compared with the control group. We also calculated the effect of HES 130/0.4 on the serum BUN and Cr values based on the above model.

Table II. Regression analyses of the changes of $CD4^+CD8^+$ T lymphocyte rates, inflammatory cytokine and renal function values based on different analysis models.

	Model I			Model II		
	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value
$CD4^+$ T cell (%)						
HES group	0.78	0.40 to 1.16	<0.001	0.77	0.40 to 1.15	<0.001
Control group	0.71	0.35 to 1.07	<0.001	0.72	0.36 to 1.08	<0.001
$CD8^+$ T cell (%)						
HES group	-0.25	-0.47 to -0.04	0.027	-0.25	-0.47 to -0.04	0.027
Control group	0.04	-0.27 to 0.35	0.813	0.04	-0.27 to 0.35	0.798
IL-1 (pg/ml)						
HES group	-0.46	-0.71 to -0.20	<0.001	-0.46	-0.71 to -0.20	<0.001
Control group	-0.05	-0.34 to 0.24	0.741	-0.05	-0.34 to 0.24	0.733
IL-6 (pg/ml)						
HES group	-4.03	-5.01 to -3.05	<0.001	-4.03	-5.01 to -3.04	<0.001
Control group	-3.77	-5.13 to -2.41	<0.001	-3.76	-5.13 to -2.40	<0.001
IL-8 (pg/ml)						
HES group	-2.08	-2.87 to -1.29	<0.001	-2.08	-2.87 to -1.29	<0.001
Control group	-0.93	-1.78 to -0.08	0.034	-0.93	-1.78 to -0.08	0.034
TNF-α (pg/ml)						
HES group	-1.64	-2.40 to -0.87	<0.001	-1.64	-2.41 to -0.87	<0.001
Control group	-0.46	-1.00 to 0.07	0.093	-0.47	-1.00 to 0.07	0.090
BUN (mmol/l)						
HES group	-0.12	-0.25 to -0.02	0.090	-0.12	-0.25 to 0.02	0.090
Control group	-0.19	-0.27 to -0.12	<0.001	-0.19	-0.27 to -0.12	<0.001
Cr (μmol/l)						
HES group	-1.91	-3.43 to -0.39	0.015	-1.91	-3.43 to -0.39	0.015
Control group	-3.26	-4.54 to -1.98	<0.001	-3.28	-4.56 to -2.00	<0.001

Model I, mixed-effects regression model (no adjusted related risk factors); model II, mixed-effects regression model adjusted year, gender and peritoneal drainage (yes or not); HES group, patients received crystalloid solutions plus HES 130/0.4 for fluid resuscitation for day 1-8 after admission; control group, patients received only crystalloid solutions for fluid resuscitation for day 1-8 after admission. HES, hydroxyethyl starch; IL, interleukin; TNF, tumor necrosis factor.

Results

Scatter plots of $CD4^+CD8^+$ T lymphocyte rates and inflammatory cytokine value. Fig. 1 shows the difference in the lymphocyte subset rates and inflammatory cytokine levels between the two groups. $CD4^+CD8^+$ T-cell rates were comparable on days 1 and 8 after hospitalization, whereas the $CD4^+CD8^+$ T rate was higher on day 4 after hospitalization (Fig. 1A and B). Lower levels of IL-1, IL-6 and IL-8 were detected in the HES group compared with the control group on days 1, 2, 4 and 8 after hospitalization (Fig. 1C-E). Higher TNF-α level was detected in the HES group on day 1 (Fig. 1F). We also observed lower level of TNF-α on days 2, 4 and 8 in the HES group compared with the control group (Fig. 1F).

Regression analyses of the changes of $CD4^+CD8^+$ T lymphocyte rates, inflammatory cytokine and renal function values based on basic and adjusted models. To investigate the impact of different methods of fluid resuscitation on the $CD4^+CD8^+$ T lymphocyte rates, pro-inflammatory cytokine values and renal function, mixed-effects regression models were established. Model I (not adjusted for related risk factors) and model II [adjusted for year, gender and peritoneal drainage (yes or no)] were established simultaneously. Our results demonstrated the different fluid resuscitation methods elicited significantly elevated $CD4^+$ T-cell rates for model I (in the HES group: Coefficient=0.78, 95% CI=0.40 to 1.16, P-<0.001; and in the control group: Coefficient=0.71, 95% CI=0.35 to 1.07, P<0.001) (Table II). Correlation between fluid resuscitation and the $CD8^+$ T-cell rate in the HES group was significantly negative in model I (coefficient =-0.25, 95% CI=-0.47 to -0.04, P-value, 0.027), whereas the relationship was positive and not significant in the control group (coefficient=0.04, 95% CI=-0.27 to 0.35, P=0.813). Additionally, there was a similar tendency for the $CD4^+CD8^+$ T lymphocyte rates in models I and II.

In model I, IL-1, IL-6, IL-8 and TNF-α levels were reduced significantly in the HES group, while IL-6 and IL-8

Table III. Regression analyses of the impact of hospital stay, treatments and the interaction between hospital stay and different fluid resuscitation methods for the changes of $CD4^+CD8^+$ T lymphocyte rates, inflammatory cytokine and renal function value.

	Time			Treatment			Time:treatment		
	RR difference	SE	P-value	RR difference	SE	P-value	RR difference	SE	P-value
CD4 (%)	0.725	0.196	<0.001	-1.195	2.684	0.658	0.051	0.267	0.849
CD8 (%)	0.024	0.141	0.867	2.863	1.769	0.111	-0.251	0.192	0.193
IL-1 (pg/ml)	-0.050	0.139	0.718	-2.665	2.465	0.282	-0.406	0.197	0.041
IL-6 (pg/ml)	-3.761	0.604	<0.001	-3.698	6.249	0.555	-0.269	0.861	0.755
IL-8 (pg/ml)	-0.931	0.416	0.026	-3.360	5.760	0.561	-1.149	0.593	0.054
TNF-α (pg/ml)	-0.460	0.333	0.168	1.185	3.455	0.732	-1.189	0.474	0.013
BUN (mmol/l)	-0.192	0.055	<0.001	-0.489	0.589	0.408	0.076	0.078	0.328
Cr (μmol/l)	-3.268	0.717	<0.001	2.707	8.910	0.762	1.360	1.013	0.181

RR difference, revealed the curve's RR difference of observed variable (time, hospital stay; treatment, crystalloid plus HES 130/0.4 resuscitation or only crystalloid resuscitation; time:treatment, the interaction term effect between treatment and time) HES group and control group. SE, standard error; RR, risk ratio HES, hydroxyethyl starch; IL, interleukin; TNF, tumor necrosis factor.

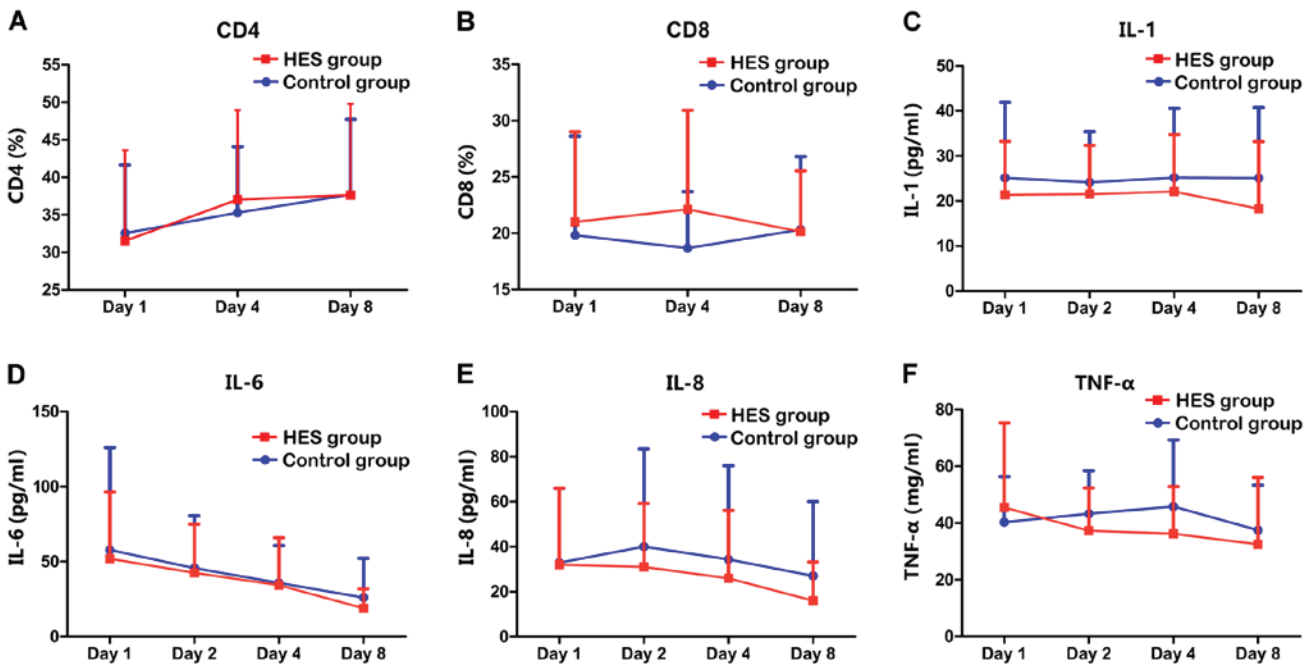

Figure 1. Scatter plots of $CD4^+CD8^+$ T lymphocyte rates and inflammatory cytokine values.

levels decreased significantly in the control group (P<0.05). In the control group, IL-1 and TNF-α levels were also reduced, but the reduction was not significant. Inflammatory cytokine distribution in model II was similar to that in model I. The Cr level decreased significantly in the two groups based on models I and II (P<0.001), while no obvious change in BUN value was detected in either group (P>0.05).

Regression analyses of the impact of hospital stay, resuscitation methods and the interaction between hospital stay and different fluid resuscitation methods for the changes of $CD4^+CD8^+$ T lymphocyte rates, inflammatory cytokines and renal function value. To investigate whether crystalloid plus HES 130/0.4 resuscitation is a critical factor for the reduction of the lymphocyte subset rates, inflammatory cytokines and renal function values compared in 2 groups. New multiple regression models were established to evaluate the impact of hospital stay, resuscitation methods and the relation between hospital stay and different fluid resuscitation methods. Additionally, its impact on the changes in the BUN and Cr values was analyzed in this model. Potential confounders, including gender, age and peritoneal drainage (yes or no), were also adjusted in this model.

As shown in Table III, there was a significant elevation in the $CD4^+$ T lymphocyte rates [risk ratio (RR) difference = 0.725, P<0.001] and reduction in the IL-6, BUN and Cr levels

Table IV. Percentage of patients with negative fluid balance within 8 days.

Negative FB [n, (%)]	HES group (n=50)	Control group (n=51)	P-value
Day 1	11 (22%)	7 (13.7%)	0.277
Day 2	14 (28%)	14 (27.5%)	0.95
Day 3	17 (34%)	12 (23.5%)	0.244
Day 4	13 (26%)	16 (31.4%)	0.55
Day 5	21 (42%)	12 (23.5%)	0.047
Day 6	36 (72%)	14 (28.6%)	<0.001
Day 7	36 (72%)	20 (40.8%)	<0.001
Day 8	38 (77.6%)	17 (35.4%)	<0.001

Negative fluid balance, the patients with out-put fluid volume exceed in-put fluid volume within 24 h; HES group, patients received crystalloid solutions plus HES 130/0.4 for fluid resuscitation for day 1-8 after admission; control group, patients received only crystalloid solutions for fluid resuscitation for day 1-8 after admission. HES, hydroxyethyl starch; FB, fluid balance.

(P<0.001) in the HES group compared with the control group. The use of crystalloid plus HES 130/0.4 resuscitation had no significant effect on the $CD4^+CD8^+$ T lymphocyte rates, inflammatory cytokines and renal function. The interaction between hospital stay and different fluid resuscitation methods was analyzed in this model, and results revealed the presence of cumulative effects in the impact of crystalloid plus HES 130/0.4 resuscitation. The RR difference of the observed variables with changes over time between the HES and control groups was significantly lower for IL-1 (RR=0.406, P=0.041) and TNF-α (RR=-1.189, P=0.013). It showed a marginally significant difference in case of IL-8 (RR=-1.149, P=0.054). The curve's RR difference was not significant for IL-6, the $CD4^+CD8^+$ T lymphocyte rate, BUN and Cr values (P>0.05).

Percentage of patients with negative FB at 8 days. We calculated the number of patients with negative FB(-) on a daily basis. Patients with peritoneal drainage were not included in this analysis. After excluding those patients with peritoneal drainage, 50 cases remained in the HES group and 51 cases in the control group. As shown in Table IV, there was a significantly higher number of patients with a negative FB from day 4 to 8 in the HES group (P<0.05).

Discussion

In the early phase of SAP, activation of inflammatory cells leads to the release of pro-inflammatory mediators which lie at the heart of the pathologic process and are involved in all aspects of the cascade leading to SIRS and multiple organ dysfunction syndrome (29). It has been established that the excessive production of inflammatory mediators is responsible for the escalation of localized pancreatic inflammation into a generalized systemic inflammatory response, irrespective of the initiating stimulus (30). Many studies have shown that plasma levels of pro-inflammatory cytokines are usually elevated early in the course of AP and are associated with severity of the disease (2,31-34). Thus, resolving the inflammatory status by downregulating the pro-inflammatory cytokines significantly improves the prognosis.

HES is a type of colloid solution that is widely used for fluid resuscitation in intensive care units. HES has the advantages of minimizing resuscitation volumes and the potential to sustain the intravascular volume for longer periods (35). Results obtained from our previous studies suggested that fluid resuscitation with HES in the early stages of SAP could improve the prognosis (16,36). Additionally, a recent study has indicated that HES resuscitation may attenuate SIRS by downregulating pro-inflammatory cytokine (21). However, the exact mechanism for the HES effect on cytokines is not well understood. Working on rat sepsis models, Feng *et al* reported that HES 130/0.4 inhibited the activation of nuclear factor-κB and neutrophil adhesion and migration, thus inhibiting cytokine production (37). Schäper *et al* demonstrated that HES prevented the inflammatory reaction by relieving ischemia reperfusion injury in the intestine (38). In the present study, we have shown that HES combined with crystalloid fluid resuscitation decreased IL-1 and TNF-α levels in peripheral blood.

IL-1 is an important mediator of inflammatory changes during pancreatitis. During the early phase of AP, IL-1 initiates the inflammatory cascade and activates the endothelium, allowing the migration of neutrophils into the post-venule and resulting in neutrophil degranulation, adhesion molecule expression, and chemokine activity. Additionally, TNF-α derived from macrophages and monocytes interacts with a number of other cytokines such as IL-1, IL-6 and platelet activation factor, which participate in this process simultaneously (39). The present study demonstrated that IL-1 and TNF-α levels decreased significantly in the HES group (P<0.05) while the IL-8 level decreased only marginally in this group compared with the control group (P=0.054). These results suggested that HES combined with colloid fluid resuscitation decreased the pro-inflammatory cytokine concentration and improved the SIRS status.

In addition to the pro-inflammatory cytokine cascade, the activated adaptive immune system including $CD4^+CD8^+$ T lymphocytes are central to the development of SIRS and organ failure in AP patients (40,41). Previous findings have shown that a significant reduction in the proportion of $CD4^+$ T cells is correlated with the severity of AP (8,9,42). In a previous study, we showed that the reduction of peripheral blood $CD4^+$ T lymphocytes was associated with persistent organ failure (10). Ozturk *et al* reported a higher $CD4^+$ T lymphocyte level and $CD4^+$:$CD8^+$ T-cell ratio, in coronary surgery patients, in the HES 130/0.4 group compared with the crystalloid group (21). Differences in the $CD4^+CD8^+$ lymphocyte subset rates between the HES and control groups were not significant in this study. This phenomenon may be explained by the fact that the immune system in SAP patients is affected by multiple organs and colloid resuscitation alone is insufficient to influence patients' adaptive immune system. The mechanism by which HES may affect $CD4^+CD8^+$ lymphocyte subsets is still unclear.

Early effective fluid resuscitation is recommended to shorten the duration of SIRS and reduce morbidity and mortality among AP patients (43). However, higher capillary permeability

results in loss of fluid from the intravascular space and fluid sequestration into the third space, which facilitates the deficiency in blood volume. Excess fluids may be harmful for effective organ perfusion, in critically ill patients and can increase the mortality rate and cause various complications, including IAH and abdominal compartment syndrome, which are associated with a poor prognosis for SAP patients (44). Previous findings have shown that FB-positive(+) status was associated with the poor prognosis of critically ill patients (45,46). Barmparas *et al* reported that the early attainment of FB(-) status was associated with a nearly 70% reduction in the risk of mortality in critically ill surgical patients (47). Therefore, maintaining colloid osmotic pressure and achieving FB(-) status earlier are important factors for the prognosis of SAP patients.

This study presented significantly higher rates of patients with FB(-) from day 4 to 8 in the HES group after excluding those patients with peritoneal drainage, which indicated HES 130/0.4 combined crystalloid resuscitation could significantly shorten FB(+) duration. This can be explained by the fact that HES 130/0.4 belongs to a family of polydispersed colloid solutions with polymerized amylopectin molecules which do not leak from capillary vessels. This characteristic makes HES to sustain its colloid osmotic pressure longer than crystalloid solutions alone (48). These results also suggested that HES combined with crystalloid fluid resuscitation could negatively affect the release of pro-inflammatory cytokines, which may be another cause for the effect of HES on FB.

Recently, safety concerns for the clinical use of HES 130/0.4 for acute volume resuscitation have attracted the attention of the researchers in this field. The most important concern was the effect of HES 130/0.4 on renal function (17,18,20). However, we did not observe any HES related effects on renal function for SAP patients in this study. However, no patients with renal failure were included in this study. Due to potential acute kidney injury, HES 130/0.4 should be used with extreme caution for SAP patients with renal failure.

There are certain limitations to our study. First, this was an observational study that revealed the effect of HES 130/0.4 on pro-inflammatory cytokines, FB status and renal function. Whether HES 130/0.4 may provide a better prognosis merits further investigation. Second, our study only included data obtained from the first 8 days after hospitalization. Third, previous studies reported that HES 130/0.4 changed the $CD4^+$ T lymphocytes and the $CD4^+$:$CD8^+$ T cell ratio compared with those patients who only received crystalloid resuscitation after coronary surgery (21). However, we did not observe this effect in this study. This may be due to the fact that HES 130/0.4 was only used in the 1st week, which did not produce any changes in patient's immune status. The exact mechanism for the effect of HES 130/0.4 on $CD4^+$:$CD8^+$ T lymphocytes should be investigated in future.

In conclusion, we identified that HES treatment could decrease IL-1 and IL-8 levels, shorten the duration of positive FB, and preserve the patient's immune status and renal function during the early phase of SAP.

References

1. Mitchell RM, Byrne MF and Baillie J: Pancreatitis. Lancet 361: 1447-1455, 2003.
2. Zhang J, Niu J and Yang J: Interleukin-6, interleukin-8 and interleukin-10 in estimating the severity of acute pancreatitis: an updated meta-analysis. Hepatogastroenterology 61: 215-220, 2014.
3. Wang XY, Tang QQ, Zhang JL, Fang MY and Li YX: Effect of SB203580 on pathologic change of pancreatic tissue and expression of TNF-α and IL-1β in rats with severe acute pancreatitis. Eur Rev Med Pharmacol Sci 18: 338-343, 2014.
4. Singh VK, Wu BU, Bollen TL, Repas K, Maurer R, Mortele KJ and Banks PA: Early systemic inflammatory response syndrome is associated with severe acute pancreatitis. Clin Gastroenterol Hepatol 7: 1247-1251, 2009.
5. Sathyanarayan G, Garg PK, Prasad H and Tandon RK: Elevated level of interleukin-6 predicts organ failure and severe disease in patients with acute pancreatitis. J Gastroenterol Hepatol 22: 550-554, 2007.
6. Liu Y, Wang L, Cai Z, Zhao P, Peng C, Zhao L and Wan C: The decrease of peripheral blood $CD4^+$ T cells indicates abdominal compartment syndrome in severe acute pancreatitis. PLoS One 10: e0135768, 2015.
7. Pezzilli R, Billi P, Gullo L, Beltrandi E, Maldini M, Mancini R, Incorvaia L and Miglioli M: Behavior of serum soluble interleukin-2 receptor, soluble CD8 and soluble CD4 in the early phases of acute pancreatitis. Digestion 55: 268-273, 1994.
8. Pezzilli R, Billi P, Beltrandi E, Maldini M, Mancini R, Morselli Labate AM and Miglioli M: Circulating lymphocyte subsets in human acute pancreatitis. Pancreas 11: 95-100, 1995.
9. Uehara S, Gothoh K, Handa H, Tomita H and Tomita Y: Immune function in patients with acute pancreatitis. J Gastroenterol Hepatol 18: 363-370, 2003.
10. Yang Z, Zhang Y, Dong L, Yang C, Gou S, Yin T, Wu H and Wang C: The reduction of peripheral blood $CD4^+$ T cell indicates persistent organ failure in acute pancreatitis. PLoS One 10: e0125529, 2015.
11. Lipinski M, Rydzewska-Rosolowska A, Rydzewski A and Rydzewska G: Fluid resuscitation in acute pancreatitis: normal saline or lactated Ringer's solution? World J Gastroenterol 21: 9367-9372, 2015.
12. de-Madaria E, Soler-Sala G, Sánchez-Payá J, Lopez-Font I, Martínez J, Gómez-Escolar L, Sempere L, Sánchez-Fortún C and Pérez-Mateo M: Influence of fluid therapy on the prognosis of acute pancreatitis: a prospective cohort study. Am J Gastroenterol 106: 1843-1850, 2011.
13. Mao EQ, Tang YQ, Fei J, Qin S, Wu J, Li L, Min D and Zhang SD: Fluid therapy for severe acute pancreatitis in acute response stage. Chin Med J (Engl) 122: 169-173, 2009.
14. Freitag M, Standl TG, Kleinhans H, Gottschalk A, Mann O, Rempf C, Bachmann K, Gocht A, Petri S, Izbicki JR, *et al*: Improvement of impaired microcirculation and tissue oxygenation by hemodilution with hydroxyethyl starch plus cell-free hemoglobin in acute porcine pancreatitis. Pancreatology 6: 232-239, 2006.
15. Du XJ, Hu WM, Xia Q, Huang ZW, Chen GY, Jin XD, Xue P, Lu HM, Ke NW, Zhang ZD, *et al*: Hydroxyethyl starch resuscitation reduces the risk of intra-abdominal hypertension in severe acute pancreatitis. Pancreas 40: 1220-1225, 2011.
16. Yang ZY, Wang CY, Jiang HC, Sun B, Zhang ZD, Hu WM, Ou JR and Hou BH: Effects of early goal-directed fluid therapy on intra-abdominal hypertension and multiple organ dysfunction in patients with severe acute pancreatitis. Zhonghua Wai Ke Za Zhi 47: 1450-1454, 2009 (In Chinese).
17. Haase N, Perner A, Hennings LI, Siegemund M, Lauridsen B, Wetterslev M and Wetterslev J: Hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis: systematic review with meta-analysis and trial sequential analysis. BMJ 346: f839, 2013.
18. Zarychanski R, Abou-Setta AM, Turgeon AF, Houston BL, McIntyre L, Marshall JC and Fergusson DA: Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation: a systematic review and meta-analysis. JAMA 309: 678-688, 2013.
19. Perner A, Haase N, Winkel P, Guttormsen AB, Tenhunen J, Klemenzson G, Müller RG, Aneman A and Wetterslev J: Long-term outcomes in patients with severe sepsis randomised to resuscitation with hydroxyethyl starch 130/0.42 or Ringer's acetate. Intensive Care Med 40: 927-934, 2014.
20. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Åneman A, Madsen KR, Møller MH, Elkjær JM, Poulsen LM, *et al*; 6S Trial Group; Scandinavian Critical Care Trials Group: Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 367: 124-134, 2012.

21. Öztürk T, Onur E, Cerrahoğlu M, Çalgan M, Nizamoglu F and Çivi M: Immune and inflammatory role of hydroxyethyl starch 130/0.4 and fluid gelatin in patients undergoing coronary surgery. Cytokine 74: 69-75, 2015.
22. Choi YS, Shim JK, Hong SW, Kim JC and Kwak YL: Comparing the effects of 5% albumin and 6% hydroxyethyl starch 130/0.4 on coagulation and inflammatory response when used as priming solutions for cardiopulmonary bypass. Minerva Anestesiol 76: 584-591, 2010.
23. Balthazar EJ: Acute pancreatitis: assessment of severity with clinical and CT evaluation. Radiology 223: 603-613, 2002.
24. Working Party of the British Society of Gastroenterology; Association of Surgeons of Great Britain and Ireland; Pancreatic Society of Great Britain and Ireland; Association of Upper GI Surgeons of Great Britain and Ireland. UK guidelines for the management of acute pancreatitis. Gut 54 (Suppl 1): i1-i9, 2005.
25. Group of Pancreas Surgery, Chinese Society of Surgery, Chinese Medical Association: The guideline of diagnosis and treatment of severe acute pancreatitis. Zhonghua Wai Ke Za Zhi 45: 727-729, 2007 (In Chinese).
26. Uhl W, Warshaw A, Imrie C, Bassi C, McKay CJ, Lankisch PG, Carter R, Di Magno E, Banks PA, Whitcomb DC, *et al*; International Association of Pancreatology: IAP guidelines for the surgical management of acute pancreatitis. Pancreatology 2: 565-573, 2002.
27. Malbrain ML, Cheatham ML, Kirkpatrick A, Sugrue M, Parr M, De Waele J, Balogh Z, Leppäniemi A, Olvera C, Ivatury R, *et al*: Results from the international conference of experts on intra-abdominal hypertension and abdominal compartment syndrome. I. Definitions. Intensive Care Med 32: 1722-1732, 2006.
28. Yang C, Yang Z, Chen X, Liu T, Gou S, Chen C, Xiao J, Jin X, He Z, Dong L, *et al*: Inverted U-shaped relationship between central venous pressure and intra-abdominal pressure in the early phase of severe acute pancreatitis: a retrospective study. PLoS One 10: e0128493, 2015.
29. Makhija R and Kingsnorth AN: Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg 9: 401-410, 2002.
30. Zyromski N and Murr MM: Evolving concepts in the pathophysiology of acute pancreatitis. Surgery 133: 235-237, 2003.
31. Fink GW and Norman JG: Specific changes in the pancreatic expression of the interleukin 1 family of genes during experimental acute pancreatitis. Cytokine 9: 1023-1027, 1997.
32. Park J, Chang JH, Park SH, Lee HJ, Lim YS, Kim TH, Kim CW and Han SW: Interleukin-6 is associated with obesity, central fat distribution, and disease severity in patients with acute pancreatitis. Pancreatology 15: 59-63, 2015.
33. Chen CC, Wang SS, Lee FY, Chang FY and Lee SD: Proinflammatory cytokines in early assessment of the prognosis of acute pancreatitis. Am J Gastroenterol 94: 213-218, 1999.
34. Exley AR, Leese T, Holliday MP, Swann RA and Cohen J: Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. Gut 33: 1126-1128, 1992.
35. Bunn F and Trivedi D: Colloid solutions for fluid resuscitation. Cochrane Database Syst Rev 7: CD001319, 2012.
36. Zhao G, Zhang JG, Wu HS, Tao J, Qin Q, Deng SC, Liu Y, Liu L, Wang B, Tian K, *et al*: Effects of different resuscitation fluid on severe acute pancreatitis. World J Gastroenterol 19: 2044-2052, 2013.
37. Feng X, Yan W, Liu X, Duan M, Zhang X and Xu J: Effects of hydroxyethyl starch 130/0.4 on pulmonary capillary leakage and cytokines production and NF-kappaB activation in CLP-induced sepsis in rats. J Surg Res 135: 129-136, 2006.
38. Schäper J, Ahmed R, Schäfer T, Elster A, Enigk F, Habazettl H, Mousa S, Schäfer M and Welte M: Volume therapy with colloid solutions preserves intestinal microvascular perfusion in endotoxaemia. Resuscitation 76: 120-128, 2008.
39. Lane JS, Todd KE, Gloor B, Chandler CF, Kau AW, Ashley SW, Reber HA and McFadden DW: Platelet activating factor antagonism reduces the systemic inflammatory response in a murine model of acute pancreatitis. J Surg Res 99: 365-370, 2001.
40. Oiva J, Mustonen H, Kylänpää ML, Kyhälä L, Kuuliala K, Siitonen S, Kemppainen E, Puolakkainen P and Repo H: Acute pancreatitis with organ dysfunction associates with abnormal blood lymphocyte signaling: controlled laboratory study. Crit Care 14: R207, 2010.
41. Nakayama S, Nishio A, Yamashina M, Okazaki T, Sakaguchi Y, Yoshida K, Fukui T, Uchida K and Okazaki K: Acquired immunity plays an important role in the development of murine experimental pancreatitis induced by alcohol and lipopolysaccharide. Pancreas 43: 28-36, 2014.
42. Curley PJ, McMahon MJ, Lancaster F, Banks RE, Barclay GR, Shefta J, Boylston AW and Whicher JT: Reduction in circulating levels of CD4-positive lymphocytes in acute pancreatitis: relationship to endotoxin, interleukin 6 and disease severity. Br J Surg 80: 1312-1315, 1993.
43. Warndorf MG, Kurtzman JT, Bartel MJ, Cox M, Mackenzie T, Robinson S, Burchard PR, Gordon SR and Gardner TB: Early fluid resuscitation reduces morbidity among patients with acute pancreatitis. Clin Gastroenterol Hepatol 9: 705-709, 2011.
44. Al-Bahrani AZ, Abid GH, Holt A, McCloy RF, Benson J, Eddleston J and Ammori BJ: Clinical relevance of intra-abdominal hypertension in patients with severe acute pancreatitis. Pancreas 36: 39-43, 2008.
45. Cunha AR and Lobo SM: What happens to the fluid balance during and after recovering from septic shock? Rev Bras Ter Intensiva 27: 10-17, 2015.
46. Lee J, de Louw E, Niemi M, Nelson R, Mark RG, Celi LA, Mukamal KJ and Danziger J: Association between fluid balance and survival in critically ill patients. J Intern Med 277: 468-477, 2015.
47. Barmparas G, Liou D, Lee D, Fierro N, Bloom M, Ley E, Salim A and Bukur M: Impact of positive fluid balance on critically ill surgical patients: a prospective observational study. J Crit Care 29: 936-941, 2014.
48. Boldt J: Hydroxyethyl starch: pharmaceutical and clinical profile. Minerva Anestesiol 65: 305-309, 1999.