

Comparison of the resuscitative effects between lactated Ringer's and lactated Ringer's/hydroxyethyl starch 6% (200/0.5) combination to serum lactate level in hemorrhagic shock in *Lepus nigricollis* rabbits

Faisal Muchtar, Syafri Kamsul Arif, Andi Husni Tanra, Arif Santoso, Made Wiryana, Agussalim Bukhari, Nurpudji Astuti Daud, Hisbullah, Warsinggih, Ilhamjaya Patellongi

Abstract

Objective: The ideal resuscitative strategy in hemorrhagic shock remains a matter of debate. The standard resuscitation fluid comprises of crystalloid and colloid, such as lactated Ringer's (LR) and hydroxyethyl starch (HES), respectively. Whether the administration of LR/HES combination can provide better microcirculatory improvements than LR alone is still unknown.

Design: Experimental analytic study comparing the effect between LR and LR/HES (200/0.5) 2:1 combination to serum lactate level in hemorrhagic shock in rabbit (*Lepus nigricollis*).

Setting: This study was conducted at Hasanuddin University Medical Research Center (HUMRC), Makassar, Indonesia, from July to October 2019.

Patients and participants: A total of 30 *Lepus nigricollis* rabbits were included in this study. Fifteen animals were allocated in each group.

Interventions: After the initiation of induced hemorrhagic shock, a hypotension period was maintained for 30 minutes using infusion of LR/HES 6% (200/0.5) 1:2 combination if mean arterial pressure (MAP) decreased more than 30% from baseline, or if there was continuous

bleeding if MAP was decreased less than 20% from baseline. The animals in LR group were then resuscitated with LR, while the animals in combination group were resuscitated with LR/HES 6% (200/0.5) 2:1 30 ml/kg/hour, then continued with 3 ml/kg/hour.

Measurements and results: Serum lactate level was measured at baseline, after the initiation of hemorrhagic shock, and after resuscitation. After hemorrhage was initiated, 19 animals had a <20% decrease in MAP and 11 animals had a ≥20% decrease in MAP. There was a significant decrease in serum lactate level in both LR and combination group with <20% decrease in MAP. LR group had a significant difference in serum lactate level from before and after resuscitation, whereas in combination group, only those with <20% decrease in MAP had a significant difference. There were no significant differences in the changes of serum lactate level between both groups.

Conclusions: Resuscitation in both groups may decrease serum lactate level in hemorrhagic shock, and resuscitation with LR/HES 6% (200/0.5) 2:1 combination provided better improvement than LR alone.

Key words: Colloid, crystalloid, hemorrhagic shock, lactate, resuscitation.

From Department of Anesthesiology, Intensive Care, and Pain Management, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Faisal Muchtar, Syafri Kamsul Arif, Andi Husni Tanra, Made Wiryana, Hisbullah), Department of Pulmonology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Arif Santoso), Department of Clinical Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Agussalim Bukhari, Nurpudji Astuti Daud), Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Warsinggih), and Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Ilhamjaya Patellongi).

Address for correspondence:

Faisal Muchtar
Jl. Racing Centre, BTN Gardenia Blok F.19, Makassar, South Sulawesi, Indonesia
Tel: +6281242449794
Email: faisal_kedok@yahoo.com

Introduction

Hemorrhagic shock is a form of hypovolemic shock in which severe blood loss leads to inadequate oxygen delivery at the cellular level. Hemorrhagic shock has an estimated mortality rate of 60,000 deaths per year in the United States and 1.9 million deaths per year worldwide. Rapid resuscitative measure is required to reduce the rate of mortality and morbidity in patients with hemorrhagic shock. (1)

The ideal resuscitative strategy in hemorrhagic shock remains a matter of debate, including the type of resuscitation fluid, blood pressure target, transfusion strategy, and coagulopathy prevention, as well as the resuscitation target to achieve microcirculatory improvements. Disruption in microcirculation leads to hypoperfusion, hypoxia, and anaerobic metabolism. As lactate represents cell metabolism and mitochondrial function, an increase in lactate level may be seen as soon as 30 minutes after shock. Therefore, lactate level can be used to assess microcirculatory integrity in hemorrhagic shock. (1,2)

The standard resuscitation fluid comprises of crystalloid and colloid, with lactated Ringer's (LR) and Hydroxyethyl starch (HES) being the most commonly fluid given in hemorrhagic shock, respectively. It has been reported that administration large amounts of crystalloid may worsen the patient's condition due to tissue edema and decreased clotting factors. Overly aggressive resuscitation with crystalloid may also cause an increase in cytokine level, particularly tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1), and interleukin-6 (IL-6). (3) Colloid is not adverse effects-free either, with kidney injury being frequently reported in patients resuscitated with colloid. (4) These findings gave rise to a proposition to use a combination of crystalloid and colloid to minimize the side effects of each fluid types. However, whether the administration of LR/HES combination can provide better microcirculatory improvements than LR alone is still unknown. (5) To the best of authors' knowledge, this issue has not been studied previously.

Materials and methods

Settings and design

This is an experimental analytic study, which compared the effect between LR and LR/HES (200/0.5) 2:1 combination to serum lactate level in hemorrhagic shock in rabbit (*Lepus nigricollis*). This study was conducted at Hasanuddin University Medical Research Center (HUMRC), Makassar, Indonesia, from July to October 2019.

Animals

Animals' inclusion criteria were male, 6- to 8-month-old *Lepus nigricollis* rabbit, weighed 1,500-2,000 grams, no anatomical abnormality, and has never been on experimental studies before. Drop-out criteria included the animal being sick or dead before the study began, difficulty in identifying the intended blood vessels for resection, hemostasis not achieved after spontaneous hemorrhage phase, inability to establish intravenous (IV) access, and inability to establish intraarterial (IA) access. Animals were acclimatized with the experimental environment (room temperature 28 ± 2 °C, humidity $50\pm 10\%$, 12 hours light/dark cycle, provision of water and food) for a period of 14 days before the experiments and was treated according to the "Ethical Principles and Guidelines for Scientific Experiments on Animals" (2005) by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. All experimental protocols employed in this study were approved by the Medical Research Ethics Committee of Hasanuddin University Makassar (1035/UN4.6.4.5.3.1/PP36/2019).

Study protocol

A total of 30 animals were used as subjects and were randomly allocated into 2 groups (LR group and LR/HES 6% [200/0.5] 2:1 combination group), with 15 animals in each group. The animals were fasted (except for water) for 12 hours before the start of experiment. Animals were first anesthetized with intramuscular (IM) ketamine 40 mg/kg and diazepam 5 mg/kg, evaluated for 10 minutes, and continued with administration of continuous IV ketamine 2 mg/kg until the animal was unresponsive to needle stimulus. Hemodynamic parameters (blood pressure, heart rate, and mean arterial pressure [MAP]) were monitored and recorded in 5-minute interval, starting from pre-induction until the end of experiment. Twenty-six gauge catheter in right femoral artery was connected with transducer to measure blood pressure. Another catheter in right jugular vein was connected with infusion. Baseline serum lactate level was measured using Nova Lactate Plus Meter (Nova Biomedical, Waltham, MA, USA) after drawing blood from the artery.

This experiment consisted of 4 phases. Phase I was initiation of induced hemorrhagic shock. Blood were slowly (approximately 30 minutes) drained from left jugular vein into a tube until a 20-30% decrease from baseline MAP was achieved. The amount of blood drained was collected and measured. To prevent clotting, IA catheter was infused with 0.9% NaCl and heparin 1 U/ml. Phase II was

hypotension period, where the MAP achieved during phase I was maintained for 30 minutes. Infusion of LR/HES 6% (200/0.5) 1:2 combination was given if MAP was decreased more than 30% from baseline, or if there was continuous bleeding if MAP was decreased less than 20% from baseline, and continued with complete hemostasis. The animals were then observed without any intervention. Phase III was resuscitation period (120 minutes) after complete hemostasis. The animals in LR group was administered LR 30 ml/kg/hour to achieve MAP 10% from baseline, then continued with 3 ml/kg/hour if target MAP was achieved. The animals in combination group were administered LR/HES 6% (200/0.5) 2:1 30 ml/kg/hour to achieve MAP 10% from baseline, then continued with 3 ml/kg/hour if target MAP was achieved. Phase IV was observation period (120 minutes), where the effect of treatments mentioned above was observed. At the end of Phase IV, serum lactate level was measured. The animals were then observed for distress signs, such as gasping, foamy mouth, or unstable hemodynamic despite resuscitative measures. If any distress sign was found, euthanasia was conducted with IV pentobarbital 100 mg/kg.

Data analysis

Statistical analysis was performed using the IBM® SPSS® Statistics version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). Data normality tests was performed using Shapiro-Wilks statistical test. Independent t-test was performed to compare the baseline parameters between the 2 groups, as well as the changes in MAP and serum lactate level after hemorrhage. The effect of fluid resuscitation to serum lactate level and serum lactate recovery to baseline after resuscitation was analysed using paired t-test. Mann Whitney U test was used to compare the effects between LR and LR/HES 6% (200/0.5) 2:1 combination to changes in serum lactate level. P value of <0.05 was considered as significant.

Results

Homogeneity between the groups

After data normality tests was performed using Shapiro-Wilks statistical test, independent t-test was then performed on the baseline parameters between the 2 groups (**Table 1**). There were no significant differences in animals' body weight, MAP, and serum lactate level between the 2 groups (p=0.946, p=0.404, p=0.484, respectively).

Changes in MAP and serum lactate level after

hemorrhage

The amount of blood from jugular vein ranged between 8-18 ml, with a mean of 12.70 ml and standard deviation (SD) of 2.48. Independent t-test showed that there was no significant difference in the amount of drained blood between LR and combination group (13.20±1.78 ml vs 12.20±3.01 ml, respectively), with p value of 0.277.

After hemorrhage was initiated, 19 animals had a <20% decrease in MAP and 11 animals had a ≥20% decrease in MAP. In LR group, 8 had a <20% decrease in MAP and 7 had a ≥20% decrease in MAP. In combination group, 11 had a <20% decrease in MAP and 4 had a ≥20% decrease in MAP. The changes in MAP and serum lactate level after hemorrhage is demonstrated in **Table 2**. There was no significant difference in the mean changes of serum lactate level after hemorrhage between the 2 groups in both <20% and ≥20% decrease in MAP (p=0.563, p=1.000, respectively).

Effect of LR resuscitation to serum lactate level after hemorrhagic shock

Paired t-test was performed to analyze serum lactate level between before and after LR resuscitation in hemorrhagic shock (**Table 3**). There was a significant decrease in serum lactate level in those with <20% decrease in MAP (6.95 mmol/l to 4.35 mmol/l, p=0.012), while there was no significant decrease in serum lactate level in those with ≥20% decrease in MAP (5.40 mmol/l to 3.80 mmol/l, p=0.176).

Serum lactate recovery after LR resuscitation

Paired t-test was performed to analyze serum lactate recovery to baseline after resuscitation (**Table 4**). Both of those with <20% and ≥20% decrease in MAP had a significant difference (p=0.012, p=0.028, respectively) in serum lactate level from before and after resuscitation (2.65 mmol/l to 4.35 mmol/l and 2.50 mmol/l to 5.40 mmol/l). Significant difference implied that the resuscitation did not restore the serum lactate level back to the baseline value.

Effect of LR/HES 6% (200/0.5) combination resuscitation to serum lactate level after hemorrhagic shock

Paired t-test was performed to analyze serum lactate level between before and after LR/HES 6% (200/0.5) combination resuscitation in hemorrhagic shock (**Table 3**). There was a significant decrease in serum lactate level in those with <20% decrease in MAP (6.80 mmol/l to 3.90 mmol/l, p=0.003),

while there was no significant decrease in serum lactate level in animals with $\geq 20\%$ decrease in MAP (4.15 mmol/l to 2.80 mmol/l, $p=0.109$).

Serum lactate recovery to baseline after resuscitation in combination group

Paired t-test was performed to analyze serum lactate recovery to baseline after resuscitation (**Table 4**). Those with $<20\%$ decrease in MAP had a significant difference ($p=0.003$) in serum lactate level from before and after resuscitation (2.80 mmol/l to 3.90 mmol/l), whereas the difference in those with $\geq 20\%$ decrease in MAP was not significant ($p=0.068$). Significant difference implied that the resuscitation did not restore the serum lactate level back to the baseline value, and vice versa.

Comparison of the effects between LR and LR/HES 6% (200/0.5) 2:1 combination fluid to changes in serum lactate level

Mann-Whitney U test was performed to analyze the difference the effects between LR and combination fluid in resuscitating hemorrhagic shock (**Table 5**). There were no significant differences in the changes of serum lactate level between the 2 groups ($p=0.247$, $p=0.705$, respectively).

Discussion

Rapid resuscitation is one of the chains of survival in patients with hemorrhagic shock, with the aim to restore the patient's intravascular volume and oxygen-carrying capacity before the shock becomes irreversible. (1) Utilization of serum lactate level to guide resuscitation can reduce mortality and morbidity in patients with shock. Given its strong relationship to the occurrence of inadequate tissue oxygenation and with morbidity and mortality, serum lactate levels could represent a useful goal of initial resuscitation in shock. Previous studies have indicated that the relationship between the presence of tissue hypoxia and increased lactate levels in humans was similar to animals. (6)

Serum lactate level in rabbits has a relatively wide range. Hupfeld, et al reported a serum lactate level between 1-22 mmol/l in both healthy and ill rabbits. (7) Therefore, random serum lactate examination is not a significant marker for a particular condition. However, changes in serum lactate level trend may be used as a potential marker to evaluate the successfulness of resuscitation. The animals in this study had homogeneous characteristics (**Table 1**), thus not presenting as a confounding factor or bias that might affect the study's results.

There were significant changes in circulatory parameters after the shock was initiated. These find-

ings demonstrated the occurrence of blood vessel endothelial disruption in shock. The increase in serum lactate level evinced the presence of anaerobic metabolism due to tissue hypoxia. (8,9) As previously mentioned, serum lactate level may represent tissue hypoperfusion associated with signs of organ dysfunction in critically ill condition. Typically, the more severe the shock is, the more significant the circulatory parameters value would be. Interestingly, in this study, the serum lactate level was found to be higher in the group with a decrease in MAP of $<20\%$, rather than in MAP of $\geq 20\%$.

In this study, LR alone provided a significant effect in reducing serum lactate level within 2 hours of resuscitation in the group with $<20\%$ decrease in MAP, as showed in **Tables 3** and **4**. This finding concurred with a study by Regnier, et al (10) and Alan, et al, (11) where it was stated that lactate clearance within 2 hours was an important outcome predictor in both traumatic and severe sepsis patient. Another study reported that a 10% decrease in serum lactate level in 6th and 24th hour was correlated with a reduced acute physiology and chronic health evaluation (APACHE) score during 72-hour follow-up. (12) However, it must be emphasized that lactate clearance may be affected by multiple factors, such as the presence of other type of shock, hypothermia, anemia, and administration of catecholamine. (13) In the group with $\geq 20\%$ decrease in MAP, the changes of serum lactate level was not statistically significant. This may be due to inadequate resuscitation and/or more time needed for inter-compartment fluid shifting to reach equilibrium state after hemorrhagic shock. It was also probable that resuscitation with fluid alone was not enough to provide significant improvement in serum lactate level. (14)

Resuscitation with HES was expected to provide better effect in hemorrhagic shock. Colloid administration aimed to reduce the amount of fluid administered with the intention of decreasing the side effects associated with fluid overload. (15) Torres, et al reported that colloid administration was associated with better reduction in lactate level compared to LR. (16) In this study, resuscitation with combination fluid has similar effects to that of LR alone, where serum lactate level had a significant decrease in the group with $<20\%$ decrease in MAP, but not in the group with $\geq 20\%$ decrease in MAP. The animals in combination group had a better recovery in serum lactate level from before to after resuscitation (in the group with $\geq 20\%$ decrease in MAP). On the other hand, LR group does not show an improvement in serum lactate level in the group

with either $<20\%$ nor $\geq 20\%$ decrease in MAP. Moreover, the decrease in serum lactate level in combination group with $<20\%$ decrease in MAP was more significant than that of LR group ($p=0.003$, $p=0.012$, respectively). This suggested that combination group may provide better serum lactate profile than LR group. However, statistical analysis showed that there was no significant difference on the serum lactate level changes between the 2 groups, as seen on **Table 5**.

Therefore, it can be concluded that resuscitation with both groups may decrease serum lactate level in hemorrhagic shock, and resuscitation with LR/HES 6% (200/0.5) 2:1 combination provided better improvement than LR alone. It must be noted that this is an experimental study with animal

model, hence more definitive studies in humans is necessary to confirm these findings. Future research that evaluate the benefit-risk of crystalloid and colloid combination are also warranted.

Declaration of competing interest

No potential conflict of interest relevant to this article was reported.

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Acknowledgments

None.

Table 1. Baseline parameters of the 2 groups

Variable	Group		p value*
	LR (n=15)	Combination (n=15)	
Body weight (gram)	1676.67±111.59	1680.00±153.30	0.946
Mean arterial pressure (mmHg)	63.77±7.46	65.89±6.20	0.404
Serum lactate level (mmol/l)	2.45±0.92	2.68±0.88	0.484

Legend: LR=lactated Ringer's; *=independent t-test.

Table 2. Changes in MAP and serum lactate level after hemorrhage

Shock condition	Variable	Mean changes (%)		
		LR (n=15)	Combination (n=15)	p value*
<20% decrease in MAP (n=19)	Serum lactate level (increase)	72.43	57.45	0.563
≥20% decrease in MAP (n=11)	Serum lactate level (increase)	44.44	38.50	1.000

Legend: MAP=mean arterial pressure; LR=lactated Ringer's; *=independent t-test.

Table 3. The effect of resuscitation with LR and combination to serum lactate level

Shock condition	Variable	LR group (n=15)			p value*	Combination group (n=15)			p value*
		Changes (mmol/l)				Changes (mmol/l)			
		Before	After	Delta		Before	After	Delta	
<20% decrease in MAP (n=19)	Serum lactate level	6.95	4.35	2.60	0.012	6.80	3.90	2.90	0.003
≥20% decrease in MAP (n=11)	Serum lactate level	5.40	3.80	1.60	0.176	4.15	2.80	1.35	0.109

Legend: LR=lactated Ringer's; MAP=mean arterial pressure; *=paired t-test.

Table 4. Serum lactate recovery to baseline after resuscitation in LR and combination group

Shock condition	Variable	LR group (n=15)			p value*	Combination group (n=15)			p value*
		Changes (mmol/l)				Changes (mmol/l)			
		Before	After	Delta		Before	After	Delta	
<20% decrease in MAP (n=19)	Serum lactate level	2.65	4.35	1.70	0.012	2.80	3.90	1.10	0.003
≥20% decrease in MAP (n=11)	Serum lactate level	2.50	5.40	2.90	0.028	1.95	2.80	0.85	0.068

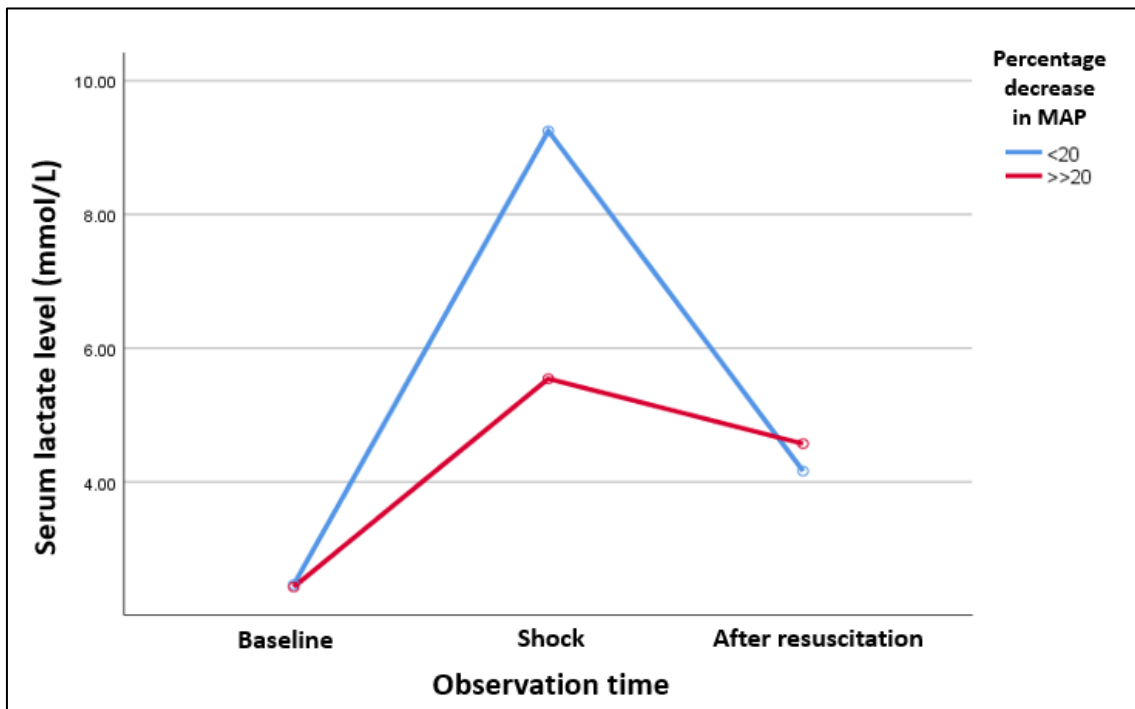
Legend: LR=lactated Ringer's; MAP=mean arterial pressure; *=paired t-test.

Table 5. The comparison of the effects between LR and LR/HES 6% (200/0.5) 2:1 combination to change serum lactate level

Shock condition	Variable	Changes (mmol/l)		p value*
		LR	Combination	
<20% decrease in MAP (n=19)	Serum lactate level (decrease)	2.60	2.90	0.247
≥20% decrease in MAP (n=11)	Serum lactate level (decrease)	1.60	1.35	0.705

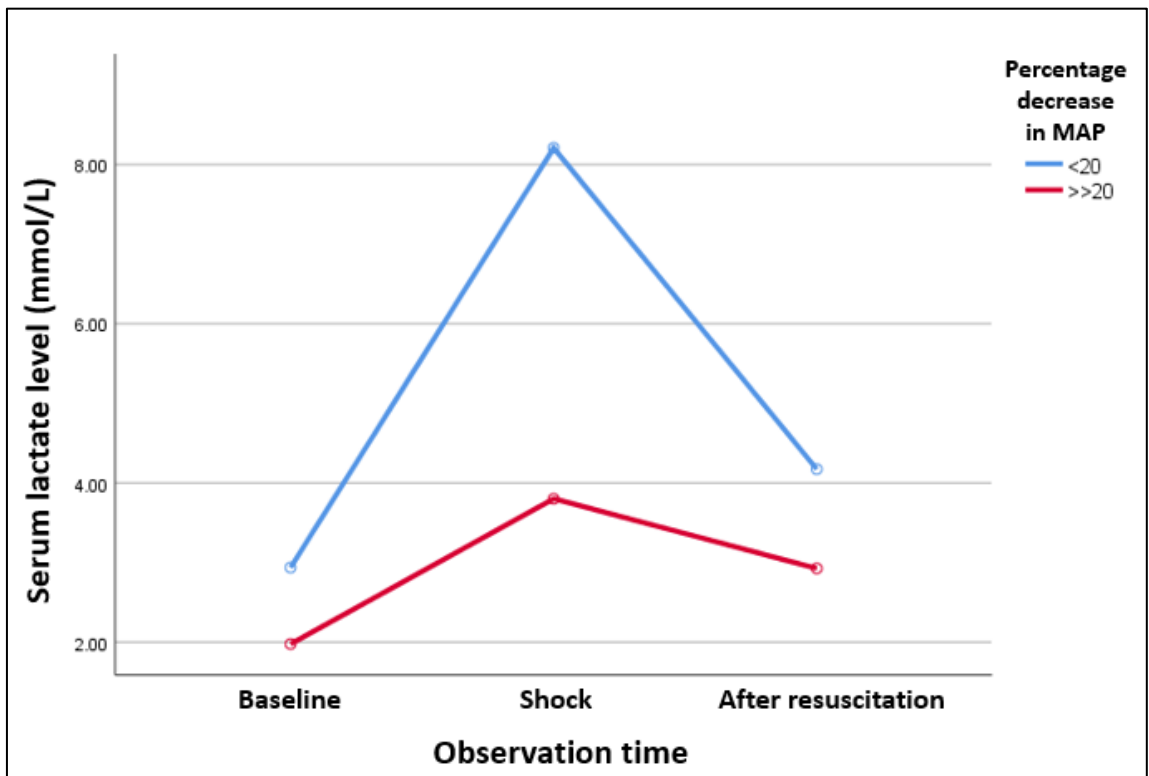
Legend: LR=lactated Ringer's; HES=hydroxyethyl starch; MAP=mean arterial pressure; *=Mann-Whitney U test.

Figure 1. The changes in serum lactate level in LR group, before and after LR resuscitation in hemorrhagic shock



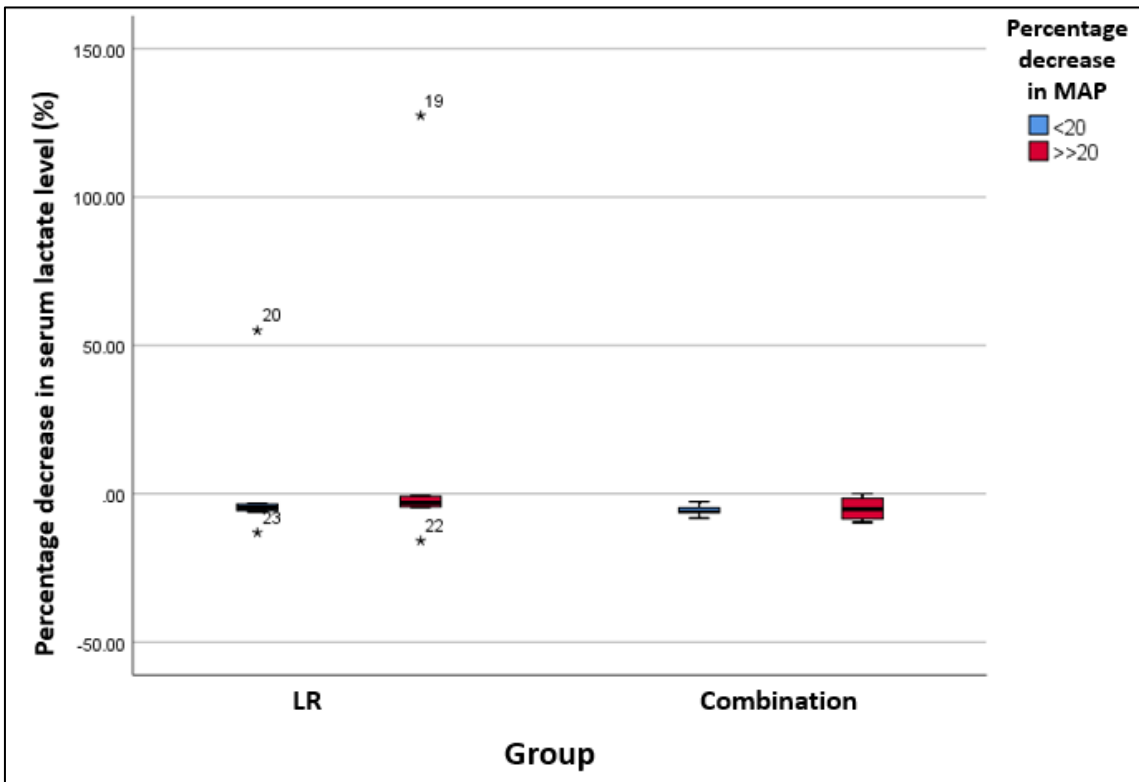
Legend: LR=lactated Ringer's; MAP=mean arterial pressure.

Figure 2. The changes in serum lactate level in combination group, before and after LR/HES 6% (200/0.5) resuscitation in hemorrhagic shock



Legend: LR=lactated Ringer's; HES=hydroxyethyl starch; MAP=mean arterial pressure.

Figure 3. Comparison of the changes in serum lactate level after resuscitation with LR and LR/HES 6% (200/0.5) 2:1



Legend: LR=lactated Ringer's; HES=hydroxyethyl starch; MAP=mean arterial pressure.

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