

Original Article**Blood autotransfusion outcomes compared with Ringer lactate infusion in dogs with hemorrhagic shock induced by controlled bleeding\****Mansour Safaei<sup>1</sup>, Hassan Mousavi Takami<sup>2</sup>***Abstract**

**BACKGROUND:** The most common cause of shock in the surgical or trauma patient is hemorrhage. Crystalloid solutions and blood transfusion are the mainstays of treatment of hemorrhagic shock. Considering the disadvantages of allogeneic blood transfusion, such as risk of transmission of infectious diseases, and access and maintenance limitations, treatment of shock with autologous blood seems to be a decent solution. Autologous blood accumulated in body cavities in traumatic bleeding (such as hemothorax), and bloodshed in operation field during open heart or vascular surgeries, and similar situations, can be utilized again. In this study, autotransfusion effects compared with crystalloid fluid in the treatment of hemorrhagic shock was investigated.

**METHODS:** After induction of hemorrhagic shock in dogs by Wiggers type controlled bleeding, treating them in a group with autologous blood and another group with Ringer lactate were performed, and the results of treatment were studied.

**RESULTS:** There was no mortality in both treatment approaches. Immediately after treatment, crystalloid positive effects such as renormalized vital signs and appropriate consciousness were more noticeable than autotransfusion, while twenty-four hours after, the desired effects of autologous blood were more pronounced like decreased metabolic acidosis and improvement of diuresis.

**CONCLUSIONS:** Crystalloid during the first hours after treatment of hemorrhagic shock may be better than autologous blood as preferred treatment, while autotransfusion showed its benefits some hours after. This finding can be used to develop better strategies for treatment of hemorrhagic shock.

**KEYWORDS:** Hemorrhagic Shock, Autotransfusion, Crystalloid.

J Res Med Sci 2011; 16(10): 1332-1339

**H**emorrhagic shock (HS) is considered as a condition in which vital organ perfusion decreased, leading to inadequate delivery of oxygen and nutrients essential for normal tissue and cellular function. Regardless of etiology, the primary physiological responses in shock have resulted from tissue hypoperfusion and decreased cellular energy. The most common cause of shock in surgical or trauma patient is loss of circulating blood volume due to bleeding.<sup>1</sup> In hemorrhagic shock, the body can compensate for the initial loss of blood volume primarily through the neuroendocrine response to maintain hemo-

dynamics. This represents the compensated phase of shock. With continued hypoperfusion, cellular death and injury are ongoing and the decompensation phase of shock ensues. Microcirculatory dysfunction, parenchymal tissue damage, and inflammatory cell activation can perpetuate hypoperfusion. Ischemia/reperfusion injury will often exacerbate the initial insult. These effects at the cellular level, if untreated, will lead to compromise of function at the organ system level, thus leading to the "vicious cycle" of shock.<sup>1</sup>

Crystalloid solutions and blood transfusion are the mainstays of pre-hospital and in-

\* This manuscript was extracted from a university research proposal, with research project number: 389349.

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hospital treatment of hemorrhagic shock.<sup>2</sup> Ringer lactate solution, the most available balanced salt solution, is advised by the American Surgeons Association as the primary resuscitation fluid therapy in the treatment of hemorrhagic shock.<sup>3</sup> A fixed volume resuscitation method of solely lactated Ringer solution (LR), equal to three times the shed blood volume, is used in this model to study endogenous mechanisms such as remote organ injury and systemic inflammation. This method of resuscitation is proven to be effective in evaluating the effects of HS and trauma.<sup>4</sup> It is safe and it equilibrates rapidly throughout the extracellular compartment, restoring the extracellular fluid deficit associated with blood loss.<sup>2</sup> Poor intravascular retention of balanced salt solutions supports intravascular volume transiently, but later can cause tissue edema formation with impaired oxygen perfusion.<sup>5</sup> Crystalloid resuscitation after hemorrhagic shock does not restore hemodynamics to baseline and is associated with a marked endothelial cell dysfunction. Blood-containing resuscitation regimens preserve and maintain hemodynamics and are associated with the least microvascular dysfunction. Therefore, regimens for resuscitation from hemorrhagic shock must contain blood.<sup>6</sup>

Although allogeneic blood transfusion can be life preserver, it has disadvantages like limited availability of blood and the storage time limits. With increased storage time, potassium, lactate and ammonia levels are increased, and 2-3 diphosphoglycerate level -which is essential for the release of oxygen-, is sufficient for just two weeks after storage, then its amount decreases. Also, allogeneic blood transfusion has risks of disease transmission and reactions during injection.<sup>7</sup> In addition, the immunosuppressive effects of allogeneic blood, particularly the increased risk of postoperative infection and tumor recurrence should be considered.<sup>8</sup> Allogeneic blood transfusion is considered as an independent risk factor for postinjury multiple organ failure.<sup>9</sup>

In such bleeding into the chest cavity (hemothorax) or abdomen, or bleeding in operat-

ing field during emergency or elective surgery such as vascular or open heart surgery, and in similar circumstances using autologous blood in the operative field can have some benefits. To achieve this purpose, various methods have emerged for reinfusion of autologous shed blood (autotransfusion). In the simplest method, blood shed is held in a chamber (with or without anticoagulant), then after passing through a fine filter to separate the pollution, the transfusion is done immediately. This method can be used for reinfusion of blood loss, such as massive hemothorax. A more complex method that is used in elective surgery includes collecting and washing the blood before the operation with saline, and concentrating it before re-injection.<sup>10</sup>

Models of bleeding can be divided into controlled and uncontrolled bleeding. Controlled bleeding can be Wiggers type, in which blood sampling is done to the extent that the blood pressure levels reach to a predestined level,<sup>11</sup> or the type with a fixed volume in which a fixed volume and predetermined blood volume (regardless of blood pressure response to the hemorrhage) is taken.<sup>12,13</sup>

## Methods

In this animal trial study, eight Persian breed dogs, two to three years old and weighting between thirteen to twenty kilograms with no underlying diseases were selected and randomly divided into two equal groups. During the study period, animals were kept in veterinary hospital under animal care by veterinarian and veterinary technicians. The investigation was in line with the guide for the care and use of laboratory animals.<sup>14</sup>

Under general anesthesia with intramuscular ketamine 20-40 mg/kg, (with regard to its effect on blood pressure), vascular catheters were inserted and fitted in the femoral artery and femoral vein of dog.<sup>15</sup> Arterial catheter was used to monitor mean arterial pressure and intravenous catheters were used for blood sampling, blood extraction, injecting serum and autotransfusion.

After intravenous heparin injection<sup>16</sup> (100 units per kilogram of animal weight), creating controlled bleeding - (Wiggers type)<sup>11</sup> - was created until mean arterial pressure of 45 mm Hg, and blood storage in special bags at temperature of 38 degrees Celsius for one and half hours.<sup>17</sup> During this period, if the mean arterial pressure was below 45 mm Hg, blood inside the bag was returned to the animal circulation, and in contrast if there was an increase in the mean arterial pressure above 45 mmHg, through catheter embedded more blood was taken from animal and entered the bag.

After ninety minutes, resuscitation of the first group of animals was done by autotransfusion of bags of storage blood, and in the second group through the infusion of Ringer lactate serum three times more than the extracted blood from their circulation.<sup>3</sup> An hour after resuscitation, animals blood samples were sent for arterial blood gas analysis and complete blood count (CBC) and kidney function tests (BUN & Cr).<sup>18</sup> The next day the tests were repeated again. Until this time the dogs did not receive any food and water, orally or by other methods. Monitoring of systolic pressure, diastolic pressure, mean arterial pressure, heart rate, urine output and level of consciousness based on clinical findings were performed during the study.

The term of "primary assessment" refers to examinations or tests were done an hour after the shock therapy, while "secondary assessment" means examinations or tests were conducted in twenty-four hours after treatment of shock.

Mental status evaluation was done by review of the animal motor response to motion stimuli, eye response and level of agitation and drowsiness. After removing the catheters of the vessels, the venotomy and arteriotomy locations on the femoral veins and arteries of animals were repaired with 6.0 Prolene sutures. Two weeks later, after the end of our study, possible complications of these dogs were examined and fortunately there were no adverse effects to worry about.

Because of small number of samples (according to the type of study which was animal trial), detailed clinical and paraclinical findings for quantitative and qualitative variables were analyzed by SPSS software (version 17), with application of Wilcoxon and Mann Whitney tests.

## Results

According to observations of primary assessment, the dogs who were treated with autotransfusion had higher heart rate [mean of 143 beats per minute (BPM)] than the group treated with Ringer lactate (mean of 117 BPM) ( $p < .04$ ). As well, in the primary assessment, the level of consciousness in dogs of autotransfusion group, were lower than in the other group ( $p < .02$ ) [Table 1]. Inversely, examinations of secondary assessment showed higher levels of consciousness in dogs of autotransfusion group ( $p < .03$ ), and lower heart rate (mean 89 BPM) in them ( $p < .01$ ), compared to those in Ringer lactate group (mean 110 BPM), and there was significant bradycardia, in accordance with results ( $p < .03$ ), as well. In the secondary assessment, the level of consciousness of autotransfusion group significantly improved compared to the primary assessment ( $p < .03$ , Table 1). However, there was no significant difference in the level of consciousness of Ringer lactate group. Likewise a significant reduction in tachycardia (mean of 143 BPM vs. 89 BPM) within twenty-four hours occurred in autotransfusion group ( $p < .03$ ) while the Ringer lactate group did not so (mean 117 BPM vs. 110 BPM).

Neither of these two groups (Ringer lactate group and autotransfusion group) showed any statistically significant difference in urinary output. However, despite the Ringer lactate group, in which urine output in the primary and secondary assessment showed no significant difference, and just two dog had normal diuresis after 24 hours, in the autotransfusion group improvement of diuresis occurred within twenty-four hours ( $p < .03$ ), and all dogs of this group had normal urine output at this time (Table 1).

**Table 1.** Comparison of different variables between the two groups

	Group Statistics				
	Groups	Number of Dogs	Mean	Std. Deviation	P value
Base Excess of Extracellular Fluid (mmol/L) - One Hour After	Autotransfusion	4	-12.275	2.67	p = 0.44
	Crystalloid	4	-13.075	4.10	
Base Excess of Extracellular Fluid (mmol/L) - 24 hours later	Autotransfusion	4	-3.450	1.56	p = 0.28
	Crystalloid	4	-3.825	2.08	
Blood Urea Nitrogen (mg/dL) - One Hour After	Autotransfusion	4	18.25	2.63	p <.03
	Crystalloid	4	32.75	11.23	
Blood Urea Nitrogen (mg/dL) - 24 Hours Later	Autotransfusion	4	34.00	9.01	P = 0.2
	Crystalloid	4	42.50	13.52	
Creatinine (mg/dL)- One Hour After	Autotransfusion	4	1.005	.141	p = 0.11
	Crystalloid	4	.875	.12	
Creatinine (mg/dL) - 24 Hours Later	Autotransfusion	4	.900	.14	p <.05
	Crystalloid	4	.775	.09	
Hemoglobin (g/dL)- One Hour After	Autotransfusion	4	15.525	.42	-
	Crystalloid	4	10.775	.63	
Hemoglobin (g/dL)- 24 Hours Later	Autotransfusion	4	18.075	5.08	-
	Crystalloid	4	11.000	3.29	
Heart rate (Beat Per Minute) - One Hour After	Autotransfusion	4	142.75	19.38	p <.04
	Crystalloid	4	117.00	15.36	
Heart rate (Beat Per Minute) - 24 Hours Later	Autotransfusion	4	89.00	2.58	p <.01
	Crystalloid	4	110.00	16.24	
Mean Arterial Pressure (mmHg) - One Hour After	Autotransfusion	4	93.25	16.17	p = 0.19
	Crystalloid	4	84.50	15.41	
Mean Arterial Pressure (mmHg) - 24 Hours Later	Autotransfusion	4	92.00	6.48	p <.02
	Crystalloid	4	80.75	6.29	
O2 Saturation (%) - One Hour After	Autotransfusion	4	93.833	1.79	p = 0.55
	Crystalloid	4	95.900	1.29	
O2 Saturation (%) - 24 Hours Later	Autotransfusion	4	76.475	5.75	p <.05
	Crystalloid	4	83.650	4.72	
Systolic Blood Pressure (mmHg) - One Hour After	Autotransfusion	4	132.50	22.54	p = 0.19
	Crystalloid	4	118.75	20.56	
Systolic Blood Pressure (mmHg) - 24 Hours Later	Autotransfusion	4	131.25	14.93	p = 0.07
	Crystalloid	4	113.75	13.15	
Diastolic Blood Pressure (mmHg) - One Hour After	Autotransfusion	4	73.75	13.76	p = 0.23
	Crystalloid	4	67.50	13.22	
Diastolic Blood Pressure (mmHg) - 24 Hours Later	Autotransfusion	4	72.50	5.00	p <.03
	Crystalloid	4	63.75	4.78	
Crystalloid Administrated (mL)	Autotransfusion	4	.00	.00	-
	Crystalloid	4	1687.50	309.23	
Transfused Blood (mL)	Autotransfusion	4	700.00	216.02	-
	Crystalloid	4	.00	.000	
Mental State ** - One Hour After	Autotransfusion	4	4.50	1.29	p <.02
	Crystalloid	4	1.75	.95	
Mental State ** - 24 Hours Later	Autotransfusion	4	1	.00	p <.03
	Crystalloid	4	2.25	.95	
Urinary Output ^^ - One Hour After	Autotransfusion	4	.50	.57	p = 0.25
	Crystalloid	4	.75	.50	
Urinary Output ^^ - 24 Hours Later	Autotransfusion	4	2	.00	p = 0.052
	Crystalloid	4	1.25	.95	
Weight (kg)	Autotransfusion	4	17.25	1.25	p = 0.44
	Crystalloid	4	16.75	2.98	

\*\* Mental State was defined in 6 levels; 1 as totally conscious, and 6 as coma.

^^ Urinary output was defined in 3 categories; 0: anuria, 1: oliguria and 2: normal diuresis.

In primary assessment of mean arterial pressure, systolic pressure and diastolic pressure, there was no statistical significant difference between the two groups (mean of 93 mmHg for autotransfusion group and mean of 84.5 mmHg for the other group); while mean arterial pressure was higher in the secondary assessment of autotransfusion group (mean of 92 mmHg for autotransfusion group and mean of 81 mmHg for the other,  $p < .02$ ). Without a significant difference in systolic pressure between the two groups, diastolic pressure of autotransfusion group was significantly higher than that of the other group. In the gap between primary and secondary assessment, mean arterial pressure, systolic pressure and diastolic pressure in both groups did not significantly change.

Our dogs showed no significant difference in hemoglobin (Hb) evaluation between the two assessments. Hb rose from 15.5 and 10.8 g/dL to 18 and 11 g/dL for autotransfusion and Ringer lactate groups, accordingly. While the creatinine of autotransfusion group between the two assessments were almost the same, Ringer lactate decreased mean blood creatinine levels from .87 to .77 mg/dL after twenty-four hours (Table 1).

Arterial blood gas (ABG) analysis between the two groups showed no significant differences in metabolic acidosis. Severity of metabolic acidosis after twenty-four hours declined in autotransfusion group significantly  $p < .04$  and mean base excess of extracellular fluid was dropped from -12.3 to -3.4 mmol/L, while in Ringer lactate group metabolic acidosis reduction rate was not significant.

During the primary assessment of ABG, six dogs (three dogs from each group) showed severe metabolic acidosis (less than -10 mmol/L base deficit), and the remaining two dogs (one dog from each group) had moderate acidosis (-6 to -9 mmol/L base deficit). In the secondary assessment, two dogs (one dog from each group) had no acidosis and three other dogs of autotransfusion group showed mild acidosis (-3 to -5 mmol/L base deficit); while two dogs of the other group had mild acidosis, and moder-

ate acidosis remained stable in another dog of Ringer lactate group. In the primary assessment mean base deficit was -12.7 mmol/L (-12.3 mmol/L for autotransfusion group and -13.1 mmol/L in Ringer lactate group), where as in the secondary assessment it was -3.6 mmol/L (-3.4 mmol/L for autotransfusion group and -3.8 mmol/L in Ringer lactate group, Table 1).

## Discussion

This study attempted to investigate outcomes of resuscitation of hemorrhagic shock with autotransfusion compared to Ringer lactate. In this study, despite drop in mean arterial pressure to 45 mm Hg, which led to severe metabolic acidosis in six dogs, there was no mortality in both groups; this can mention positive role and effectiveness of both ways in the emergency treatment of hemorrhagic shock. Tissue hypoperfusion from precapillary vasoconstriction leads to anaerobic metabolism and acidosis.<sup>18</sup> Severe metabolic acidosis is related greatly with multi-organ failure and death.<sup>19</sup>

However, crystalloids are not the best plasma expanders; no more than one-fifth of unexcreted fluid should remain within the plasma volume after equilibration, and, for this reason, a large volume is necessary.<sup>20</sup> These data are in agreement with good hemodynamic performance and consequently, good systemic oxygenation immediately after crystalloid administration, but after 120 minutes this was no longer maintained.<sup>5</sup> The main disadvantage of using crystalloid solutions is their rapid movement from the intravascular to the extravascular space, leading to three hours or more time requirement for replacement, and resulting in tissue edema.<sup>21</sup>

The poor intravascular retention of balanced salt solutions supports intravascular volume transiently but later can cause tissue edema with impaired oxygen perfusion.<sup>5</sup> Resuscitation from hemorrhagic shock results in progressive vasoconstriction in all intestinal arterioles irrespective of the resuscitation regimen. Crystalloid resuscitation after hemorrhagic shock does not restore hemodynamics

to baseline and is associated with a marked endothelial cell dysfunction. Blood-containing resuscitation regimens preserve and maintain hemodynamics and are associated with the least microvascular dysfunction.<sup>6</sup> Masoumi et al. showed that applying different dosages of nitroglycerin (50 µg/min, 100 µg/min and 150 µg/min) had no different effect on maintenance of hemodynamic stability and the need for blood transfusion after coronary artery bypass graft surgery.<sup>22</sup>

Stukanov et al. presented the results of treatment of 60 patients with 2<sup>nd</sup> degree hemorrhagic shock at pre-hospital and hospital stages by different variations of infusion therapy. They established that balanced infusion therapy in patients with 2<sup>nd</sup> degree hemorrhagic shock helps to stabilize the hemodynamic system and improve the general condition. The balanced infusion therapy did not lead to unfavorable changes in acid-alkaline and electrolyte balance.<sup>23</sup> Moss et al. compared the use of normal saline and blood in shock resuscitation. They were found to be equally efficacious in returning cardiovascular parameters to preshock levels. The only difference was that the saline group was more acidotic at the conclusion of resuscitation.<sup>24, 25</sup> Zollman et al. compared shed blood to shed blood plus Ringer's lactate and found no difference in survival.<sup>15, 25</sup> It was observed by Rhee et al. that lactated Ringer's solution exacerbated neutrophil superoxide burst activity and increased neutrophil adherence.<sup>26</sup> Also, it has been shown that aggressive crystalloid resuscitation was followed by increased cytokine activation including IL-1, IL-6, and TNF.<sup>27</sup> Deb et al. demonstrated that both hetastarch and Ringer lactate result in increased cell apoptosis via Bax protein<sup>28</sup>, and that resuscitation with Ringer lactate increases immediate apoptosis during hemorrhagic shock fluid resuscitation.<sup>29</sup> In dogs submitted to pressure-guided hemorrhagic shock and fixed-volume resuscitation, the smaller intravascular volume expansion by 6% hydroxyethyl starch-hypertonic saline solutions provided worse recovery of systemic oxygenation and gastric perfusion compared

with LR.<sup>30</sup> In another study by Deb and colleagues, rats were subjected to a controlled 40% hemorrhage followed by resuscitation by three times the volume of shed blood with a variety of resuscitation fluids. Resuscitation lasted for one hour, then followed by removal of the liver and small intestine for analysis. Lactated Ringer's resuscitation generated a marked and immediate increase in apoptosis compared to non-resuscitated rats, resuscitated with either hypertonic saline or whole blood.<sup>31</sup> There are occasional studies suggesting no upregulation of the immune response to trauma after Ringer lactate resuscitation.<sup>32</sup>

Prehospital autotransfusion of life-threatening hemothorax is simple, safe and easy to perform.<sup>33</sup> The preliminary results of one study by P Barriot et al. suggest that autotransfusion should be developed in the pre-hospital setting (and in the emergency room of the trauma center) since it represents the only hope of survival for patients with life-threatening hemothorax.<sup>33</sup>

As we can see in the findings of our study, one hour after treatment with crystalloid, heart rate and level of consciousness were much more appropriate than in treatment with autotransfusion method. Twenty-four hours after, in the secondary assessment, heart rate and level of consciousness in dogs under autotransfusion were better than dogs that have received only crystalloid. Improvement of diuresis, significant reduce in the severity of metabolic acidosis, and increased mean arterial pressure and diastolic pressure were of the other benefits of treatment of hemorrhagic shock by autotransfusion method, which were more prominent twenty-four hours after the therapy.

It seems that here, due to the high volume of crystalloid infusion, at first extracellular fluid (ECF) deficit was compensated quickly and the hemodynamic disturbance was more effectively treated, but when more time passed, because of fluid extravasation and tissue edema formation and maybe because of some other disadvantages of Ringer lactate (which were described above), its influence was diminished.

Similarly, on the opposite side, autotransfusion by persistent maintaining of ECF fluid, more effectively has been able to improve hemorrhagic shock adverse effects.

### Conclusion

According to the findings of our study, crystalloid at the first hours after treatment of hemorrhagic shock may be better than autologous blood as preferred treatment, while autotransfusion showed its benefits some hours after. This finding may be used to develop better strategies for treatment of hemorrhagic shock. It seems in emergencies, in earlier phase of hemorrhagic shock, crystalloids remain still the preferred choice of treatment, while con-

sidering the benefits of autologous blood, its use along with crystalloid, especially for elective cases such as critically ill or major surgical patients and sometimes emergency patients such as severe hemothorax, appears logical is recommended.

### Acknowledgements

The authors acknowledge the Isfahan veterinary hospital staffs, veterinary technicians and Dr. Shahriar Adibi (veterinarian). Also the authors are thankful to Isfahan University of Medical Sciences for support of this study. This manuscript was extracted from a university research proposal, with research project number: 389349.

### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

Both authors have carried out the study, participated in the design of the study and coordinated and carried out all the experiments and participated in manuscript preparation. Both authors have read and approved the content of the final manuscript.

### References

1. Schwartz SI, Brunicki FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, et al. Schwartz's principles of surgery. 9<sup>th</sup> ed. New York: McGraw-Hill; 2010. p. 91-9.
2. Krausz MM. Initial resuscitation of hemorrhagic shock. *World Journal of Emergency Surgery* 2006; 1: 14.
3. American College of Surgeons, Alexander RH, Proctor HJ. Advanced trauma life support course for physicians: student manual. Washington, DC: American College of Surgeons; 1993. p. 87-106.
4. Kohut LK, Darwiche SS, Brumfield JM, Frank AM, Billiar TR. Fixed volume or fixed pressure: a murine model of hemorrhagic shock. *J Vis Exp*. 2011 Jun 6; (52). pii: 2068. doi: 10.3791/2068.
5. Cerqueira Braz JR, Nascimento DP, Filho OP, Gobbo Braz L, Vane LA, Galvao Vianna PT, et al. The early systemic and gastrointestinal oxygenation effects of hemorrhagic shock resuscitation with hypertonic saline and hypertonic saline 6% dextran-70: a comparative study in dogs. *Anesth Analg* 2004; 99: 536-46.
6. Zakaria eR, Spain DA, Harris PD, Garrison RN. Resuscitation regimens for hemorrhagic shock must contain blood. *Shock* 2002; 18(6): 567-73.
7. Tremblay LN, Rizoli SB, Brenneman FD. Advances in fluid resuscitation of hemorrhagic shock. *Canadian journal of surgery* 2001; 44(3): 172-9.
8. Vamvakas EC. Transfusion-associated cancer recurrence and postoperative infection: meta-analysis of randomized, controlled clinical trials. *Transfusion* 1996; 36(2): 175-86.
9. Moore FA, Moore EE, Sauaia A. Blood transfusion. An independent risk factor for postinjury multiple organ failure. *Arch Surg* 1997; 132(6): 620-4.
10. Popovsky MA, Devine PA, Taswell HF. Intraoperative autologous transfusion. *Mayo Clin Proc* 1985; 60(2): 125-34.
11. Wiggers CJ. Physiology of shock. New York: Commonwealth Fund, 1950.
12. Wiggers CJ, Werle JM. Exploration of a method for standardizing hemorrhagic shock. *Proc Soc Exp Biol Med* 1942; 49: 604-6.
13. Foex BA, Kirkman E, Little RA. Injury (nociceptive afferent nerve stimulation) modifies the hemodynamic and metabolic responses to hemorrhage in immature swine. *Crit Care Med* 2004; 32(3): 740-6.

14. Institute of Laboratory Animal Resources (U.S.). Guide for the care and use of laboratory animals. 7<sup>th</sup> ed. Washington DC: National Academies Press; 1996.
15. Zollman W, Culpepper RD, Turner MD, Hardy JA, Hardy JD. Hemorrhagic shock in dogs. Comparison of treatment with shed blood alone versus shed blood plus Ringer's lactate: intravascular pressures, cardiac output, oxygen consumption, arteriovenous oxygen differences, extracellular fluid PO<sub>2</sub>, electrolyte changes, and survival rates. *Am J Surg* 1976; 131(3): 298-305.
16. Reul GJ, Jr., Solis RT, Greenberg SD, Mattox KL, Whisnand HH. Experience with autotransfusion in the surgical management of trauma. *Surgery* 1974; 76(4): 546-55.
17. McDonald MC, Izumi M, Cuzzocrea S, Thiernemann C. A novel, potent and selective inhibitor of the activity of inducible nitric oxide synthase (GW274150) reduces the organ injury in hemorrhagic shock. *J Physiol Pharmacol* 2002; 53(4 Pt 1): 555-69.
18. Marzi I. Hemorrhagic shock: update in pathophysiology and therapy. *Acta Anaesthesiol Scand* 1997; 111( Suppl): 42-4.
19. Davis JW, Parks SN, Kaups KL, Gladen HE, O'Donnell-Nicol S. Admission base deficit predicts transfusion requirements and risk of complications. *J Trauma*. 1996; 41(5):769-74.
20. Svensen C, Hahn RG. Volume kinetics of Ringer solution, dextran 70, and hypertonic saline in male volunteers. *Anesthesiology* 1997; 87(2): 204-12.
21. Davies MJ. Crystalloid or colloid: does it matter? *J Clin Anesth* 1989; 1(6): 464-71.
22. Masoumi GH, Hidarpour E, Sadeghpour Tabae A, Ziayefard M, Azarasa A, Abneshahidi A, Javid Anbardan S, et al. Evaluating hemodynamic outcomes of different dosages of intravenous nitroglycerin after coronary artery bypass graft surgery. *J Res Med Sci* 2011; 16(7): 910-5.
23. Stukanov MM, Lukach VN, Girsh AO, Iudakova TN, Gorin PV, Chugulev IA, et al. Comparative study of infusion therapy options in patients with hemorrhagic shock. *Anesteziol Reanimatol*. 2011; 2: 27-30.
24. Moss, GS, Proctor HJ, Homer LD, Herman CM, Litt BD. A comparison of asanguineous fluids and whole blood in the treatment of hemorrhagic shock. *Surg Gynecol Obstet* 1974; 129: 1247.
25. Strodel WE, Callahan M, Weintraub WH, Coran AG. The effect of various resuscitative regimens on hemorrhagic shock in puppies. *J Pediatr Surg* 1977; 12(6): 809-19.
26. Rhee P, Burris D, Kaufmann C, Pikoulis M, Austin B, Ling G, et al. Lactated Ringer's solution resuscitation causes neutrophil activation after hemorrhagic shock. *J Trauma* 1998; 44(2): 313-9.
27. Hierholzer C, Harbrecht B, Menezes JM, Kane J, MacMicking J, Nathan CF, et al. Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. *J Exp Med* 1998; 187(6): 917-28.
28. Deb S, Sun L, Martin B, Talens E, Burris D, Kaufmann C, et al. 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. *J Trauma* 2000; 49(1): 47-53.
29. Deb S, Martin B, Sun L, Ruff P, Burris D, Rich N, et al. Resuscitation with lactated Ringer's solution in rats with hemorrhagic shock induces immediate apoptosis. *J Trauma* 1999; 46(4): 582-8.
30. Barros JM, do Nascimento P Jr, Marinello JL, Braz LG, Carvalho LR, Vane LA, et al. The effects of 6% hydroxyethyl starch-hypertonic saline in resuscitation of dogs with hemorrhagic shock. *Anesth Analg*. 2011; 112(2): 395-404.
31. Khan MA, Garner JP. Hartmann's solution in haemorrhagic shock - now and the future. *J R Army Med Corps* 2007; 153(2): 81-5.
32. Oelschlager BK, Caragnano C, Carpenter J, Baker CC. Effect of resuscitation with hydroxyethyl starch and lactated Ringers on macrophage activity after hemorrhagic shock and sepsis. *Shock* 1994; 2(2): 141-4.
33. Barriot P, Riou B, Viars P. Prehospital autotransfusion in life-threatening hemothorax. *Chest* 1988; 93(3): 522-6.