A DISSERTATION
FOR THE DEGREE OF MASTER

Evaluation of Hemolysis by Rapid Autologous Transfusion in Dogs

개에서 빠른 자가 수혈 속도에 따른 용혈의 정도 평가

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Abstract

In very emergent situations requiring urgent blood transfusion, rapid transfusion can cause clinicians to worry about side effects of blood transfusion or mechanical hemolysis.[1] At this time, the rate of blood transfusion is considered to be the least mechanical hemolysis. Several studies to determine the evaluation of hemolysis for transfusion made in vivo have limited the rate of transfusion, while in vitro, it was confirmed that hemolysis did not occur even after transfusion was conducted at the fastest possible rate (a rate over 8,000 mL/hr).[3 4 5] Therefore, it was necessary to study whether the same result was obtained even in rapid blood transfusion in vivo.

After inhalation anesthesia from six healthy dogs, 20% of blood was collected from the jugular vein aseptically. The autologous transfusion was performed to compare the degree of hemolysis before and after transfusion with three methods; 1. slow method : the typical transfusion rate (5−10 mL/kg/hr), 2. full method : full drop via gravity flow which is the rate of transfusion used in emergencies (measured, mean 42 mL/kg/hr), 3. bolus method : and the rate of massive transfusion through 60 mL syringe and 18 μm filter (1.5 mL/kg/min, mean 88.24 mL/kg/hr). There was a significant difference in free hemoglobin (p=.007) and hemolysis (p=.008) between the bolus method and slow or full method. This describes that when transfusions are performed in vivo, hemolysis occurs more at the bolus rate than slow or full rate.

In an emergency where blood transfusions are indicated at a rapid rate, transfusion at a rate of full drop via gravity flow will have a better prognosis.

Keywords : dogs, hemoglobin, hemolysis, rapid, transfusion, autologous

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Introduction

In emergency room, a number of anemic patients secondary to acute blood loss are encountered. In some of these situations, fluids such as crystalloids and colloids alone may solve the problem, but they cannot be used in other cases.[1 2] In these cases, red blood cells are also needed to correct oxygenation to improve anemia−related tissue hypoxia as well as perfusion and circulation.[1] However, rapid transfusion in emergency situation can causes concerns on potential side effects of hemolysis.[1]

To minimize hemolysis, dripping blood via gravitational flow is preferred, as most of infusion pumps are considered not safe for the administration of RBC and platelet transfusions.[1] However, previous reports indicated that hemolysis was not shown with the typical transfusion rate of 4 mL/kg/hr or 2 mL/kg/hr.[3 4] Furthermore, the reports on faster blood transfusions are not available to the authors' best knowledge.

In veterinary medicine, consensus has not been established as to how fast blood transfusion causes significant hemolysis.[5] However, recent study has concluded that several methods of rapid transfusion in vitro do not cause significant hemolysis.[5] Therefore, there were no studies conducted in vivo to see if this would produce the same results in vivo. In vitro, mechanical hemolysis was evaluated through the transfusion set using the maximum possible rate (a rate over 8,000 mL/hr).[5] It is difficult to know how much hemolysis will occur in vivo at such a high rate, so instead, reasonable rate in emergent situation defining massive transfusion rate 1.5 mL/kg/min (90 mL/kg/hr) was used. Two of the most commonly used methods in clinical setting, full drop via gravity flow and syringe with filter were used.

The purpose of this study is to find out whether rapid blood transfusion induces hemolysis in vivo and which blood transfusion method causes more hemolysis.

Materials and Methods

Subjects

Six apparently healthy beagles, 3 females and 3 males, all neutered were included.

The median age was 2 years (range 1−3 years) and body weights ranged from 9 to 13 kg (mean±SD, 10.63±1.60 kg). Approval for all procedures was obtained from Institutional Animal Care and Use Committee (SNU−200226−7).

Study design

1. Blood collection

To minimize movement and prevent postural mechanical effect on hemolysis, general anesthesia was used with medetomidine (10 μg/kg, IM, Domitor®, Orion Pharm, Finland) for premedication and alfaxalone (2 mg/kg, IV, Alfaxan®, Jurox Inc., Australia) for induction, and isoflurane (1.0 MAC, Ifran Liq®., Hana Pharm Co., Ltd, Korea) for maintenance. Blood was collected by gravity flow on a table 1 meter low from the jugular vein of each dog using standard aseptic venipuncture technique into 300 mL capacity blood collection bags (Green TF Bag−300, Doowon Meditec, Korea) containing 1 mL CPDA anticoagulant/ 9 mL collected.

2. Transfusion techniques

Each dog was autologously transfused, using 3 different transfusion techniques via a single 22 G cephalic catheter from 1 meter high. The first method (slow, control) was transfused via gravity flow using a standard transfusion line (20 drops/mL) with built-in 200 μm filter (Doowon Meditec, Korea). Delivery rate was adjusted by regulating drops/min manually at standard transfusion rate (5-10 mL/kg/hr). At this rate, the transfusion time did not exceed 4 hours. The second method (full) was transfused via gravity flow using a standard transfusion line (20 drop/mL) with built-in 200 μm filter (Doowon Meditec, Korea) at full drop. The third method (bolus) was transfused using a syringe infusion pump with blood delivered through 60 mL syringe with an 18 μm aggregate filter (Hemo-nate, Utah Medical Products Inc., Midvale, Utah 84047, USA). Delivery rate was the same speed as massive transfusion, 1.5 mL/kg/min (90 mL/kg/hr). The filter in bolus method was changed every 60 mL, because that is the maximum volume that they are able to filter effectively.[6] Rate of transfusion time was recorded (mL/hr) for each trial. Sterility was performed thoroughly throughout the procedure.

3. Sampling, measurement and evaluation

Blood samples (0.75 mL) were collected immediately before and after completion of the transfusions. Samples were collected by jugular venipuncture using standard aseptic technique with 21 G needle and 3 mL syringe gently. EDTA (1.5 mL tube) for CBC analysis using a hematology analyzer (ADVIA 120 Hematology System, Bayer, Tarrytown, NY) and remaining sample was transferred to a microcentrifuge tube to check gross hemolysis.[7,8] Visually, the plasma color was checked using a color-based hemolysis grading system for each sample.[8]

To minimize variability, the same investigator (HJH) performed all of the simulated transfusions and attempts were made to use an equivalent force for each transfusion.

Total hemoglobin, hematocrit and plasma free hemoglobin before and after autotransfusion were used to calculate percent hemolysis (% hemolysis), which is measurable with ADVIA 120 Hematology System (Bayer, Tarrytown, NY).[7]

$$\% \text{ hemolysis} = (100-\text{HCT}) \times (\text{plasma fHb [g/dL]} / \text{tHb [g/dL]})$$ [5]

Statistical analysis

Data were analyzed using statistical software (SPSS statistical program, IBM SPSS Statistics 25, IBM Corporation, NY, USA). Basic descriptive statistics, including mean and standard deviation, were calculated for the difference between free hemoglobin of before and after transfusion and the difference between hemolysis percentage of before and after transfusion. Kruskal–Wallis, an analysis used by three or more groups to see differences in the means of dependent variables, was used to determine the differences in variables depending on the group. Bonferroni was used for post–hoc test.

Results

There was a difference in the time taken for blood transfusion according to the transfusion method. In slow method, in which the drop was controlled manually and injected to transfusion at typical transfusion rate of 5−10 mL/kg/hr, it took an average of 3.20±0.40 hours, and the average transfusion rate was 6.34±0.77 mL/kg/hr (5.33−7.40 mL/kg/hr). In full method using full drop via gravity flow, the average transfusion time took 20.64±4.61 minutes, and the average transfusion rate was 59.28±9.47 mL/kg/hr (38.95−66.67 mL/kg/hr). In bolus method, to reach the massive transfusion rate (1.5 mL/kg/min), 60 mL syringe and 18 μm filter with a syringe pump set were used, it took an average of 13.72±0.97 minutes and the average transfusion rate was 88.24±5.93 mL/kg/hr (1.47 mL/kg/min).

The differences in free hemoglobin and hemolysis among three methods were summarized in Table 1. The comparison of differences in free hemoglobin among slow, full, and bolus method was represented graphically in Figure 1 and 2. There were significant differences in plasma fHb (p=.007) or % hemolysis (p=.008) among methods. Both free hemoglobin and hemolysis were occurred more in the bolus method than in the slow and full method.

Table 1. Mean of differences between groups slow, full, and bolus of fHb and HL

Measurements	Methods	Mean	SD	X^2	p−value
⊿ fHb	slow	−.222	.1048	9.901	.007
	full	−.182	.0806		
	bolus	.043	.1058		
	total	−.120	.1511		
⊿ HL (%)	slow	−.582	.5090	9.609	.008
	full	−.578	.3220		
	bolus	.317	.3896		
	total	−.281	.5818		

*p<0.05, **p<0.01, fHb : free hemoglobin, HL (%) : hemolysis

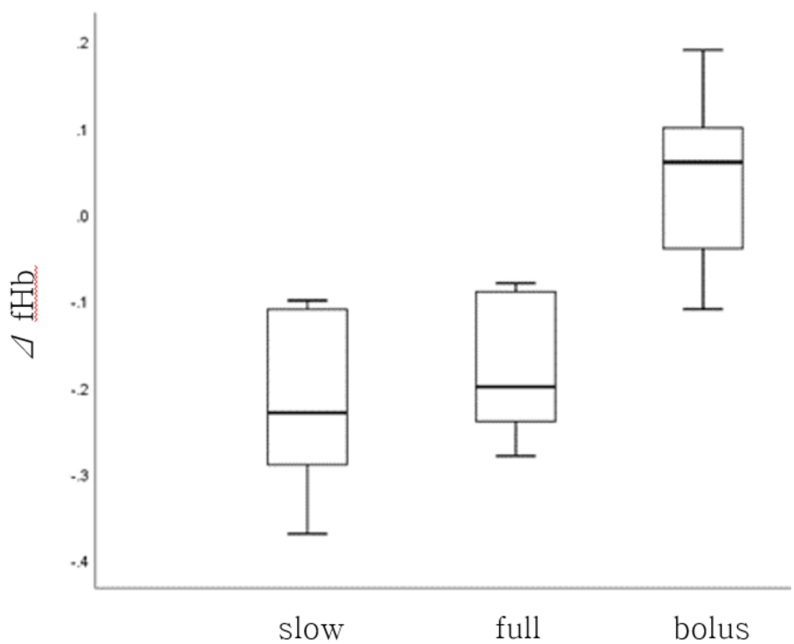

Figure 1. The comparison of differences in free hemoglobin among three methods (slow, full, bolus)

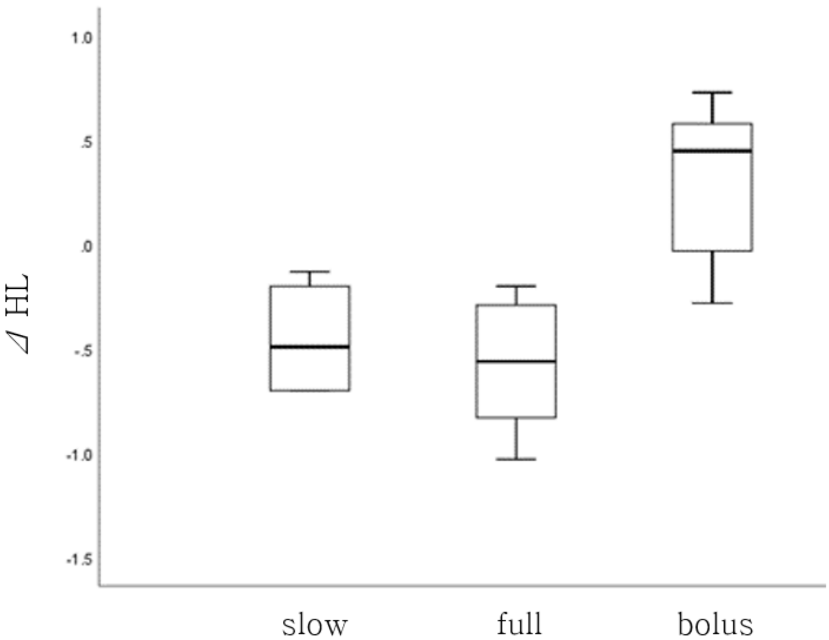

Figure 2. The comparison of differences in hemolysis among three methods (slow, full, bolus)

However, even in the bolus method where hemolysis occurred relatively much, gross hemolysis was not remarkable in all 6 cases after centrifuged.

Discussion

The data presented here suggest that, in marked contrast to previously reported finding in vitro, the iatrogenic hemolysis could be happened during various rapid transfusion techniques in vivo. The previous study was subjected to the following administration five techniques: gravity-driven (control), an infusion pump at maximal rate, application of a pressure bag, manual compression, and syringe bolus.[5] The results did not show significant increases in % hemolysis or plasma fHb noted among any of the trial methods, compared to the control.[5] Based on this previous in vitro study, the present study was prepared in anticipation of the same results in vivo. However, the results showed that relatively higher hemolysis occurs when transfused at massive transfusion rate (bolus) than transfusion with gravity flow at full opening (full) which was a different result from the in vitro experiment.

In another previous in vivo study, blood was transfused to dogs with volumetric peristaltic infusion pump, syringe infusion pump with 18 μm filter, and gravity flow.[4] Comparison of osmotic fragility, red cell count, and free hemoglobin before and after transfusion showed no significant difference. However, the transfusion rate was just 2 mL/kg/hr, which was actually very slow compared to the transfusion rate required in emergencies.[4] It is thought that it was a similar study that applied blood transfusions in vivo to the present study, but the result was presumed different because of the rate. Full and bolus methods was respectively 9.3 and 14 times faster than slow method, typical maximal transfusion rate. Bolus method was 1.5 times so faster than full method that the forceful injection to reach over this fast rate could lead to hemolysis.[9]

Although hemolysis was presented in the calculated value, hemolysis was not observed visually even bolus method. According to the previous study, the degree of hemolysis by visual evaluation of the supernatant was evident when hemolysis was more than 1%.[8] When comparing the three transfusion methods, the bolus method showed relatively higher hemolysis, but when visually confirmed, there was no significant difference. This is presumably because the difference in % hemolysis is less than 1%. However, in vitro study, the difference between hemolysis before and after transfusion was within 1% in all five transfusion methods, which was not different from this in vivo study.[5] The reason why 1% is important in hemolysis is that it is possible to transfuse blood when the hemolysis is within 1% in the blood transfusion in human medicine, which is not yet established in veterinary medicine, but it could be applied.[5 8]

The reason why hemolysis is important in blood transfusion is free hemoglobin. Generally, free hemoglobin is removed by complexing with haptoglobin, but if severe hemolysis, haptoglobin clearance of plasma fHb is overwhelmed. Excessive hemoglobin leads to scavenging of nitric oxide. Depletion of this essential endogenous vasodilator causes microvascular vasoconstriction, hypertension, and damage to organ perfusion and contribute to multiple organ dysfunction syndrome.[9−12] Therefore, efforts to reduce iatrogenic hemolysis are required.

In this study, it is tried to reduce iatrogenic hemolysis. First of all, autologous transfusion was used to exclude the immunological transfusion reaction. One of side effects that clinicians worry about in the blood transfusion are transfusion reactions. Transfusion reactions can be divided into two categories: immunological and non−immunological. Free hemoglobin is particularly worrisome in non−immunological reactions when transfused rapidly.[1] Following hemolysis, increased plasma free hemoglobin is related to organ damage, especially renal injury.[11]

The effect on hemolysis in blood transfusion is considered to be pump type, age of transfused blood unit, the presence of in−line filters, transfusion rate, handling of blood and transfusion gauge.[6 9 13−15] Typically, use of infusion pumps to deliver red blood cell transfusions has been shown to cause damage and decrease survival of transfused red blood cells.[13] Many clinicians prefer gravity flow and standard blood filter set (170−260 μm) for canine blood product transfusions. Also, a human neonatal syringe filter set with a 150 μm filter or a syringe pump with HemoNate (18 μm) filter can be used for cats and small dogs.[2 3] In particular, this method is used in autotransfusion.[6] Blood salvaged intraoperatively or after intracavitary hemorrhage is reinfused intravenously after careful filtering without the need to crossmatching.[6] Therefore, full and bolus methods were applied in this study as commonly used methods in emergency situation.

During storage, red blood cells undergo a series of physiologic, biochemical, and structural alterations leading to decreased viability and aggregation and increasing the risk for oxidative damage.[1] Several of the detrimental effects seen in stored RBCs, such as hemolysis and microparticle accumulation, are associated with an increased risk of adverse reactions following the transfusion.[1]

Longer duration of RBC storage was associated with development of coagulation failure and thromboembolic disease.[16] For this reason, in this study, whole blood were collected from the dog and transfused immediately to prevent RBCs from reducing membrane integrity during storage.[1]

In order to try to minimize blood handing, the transfusion process and blood collection process were carried out by only one investigator (HJH) to prevent differences in hemolysis caused by handling by several people. The blood transfusion line through which the blood passes, the gauge of the venous catheter, and the venipuncture to measure the degree of hemolysis were kept in practice in general clinical practice.

The third method, bolus, is to adjust to rate to the rate of the massive transfusion. In cases of severe hemorrhage, patients may require large volumes or rapid infusions of blood products to achieve hemodynamic stabilization. This resuscitation strategy is termed massive transfusion. Massive transfusion is defined as receiving one blood volume, 90 mL/kg in the dog, or more of blood products within 24 hours, or transfusion of 50% of one blood volume within 3 hours, or 150% of one blood volume regardless of time, or 1.5 mL/kg/min of blood products over 20 minutes.[2] Because syringing to expedite RBC transfusions cause clinically significant hemolysis, the present study was applied within massive transfusion rate required in an emergency situation.[9]

In human medicine, it is reported that the effects of catheters gauge on hemolysis during transfusions were not significant, while it is also reported that rapid transfusion through 23 G or smaller needles can cause hemolysis.[9 14 15 17] Therefore, 22 G catheter was used in this study. However, the previous studies were human studies, so it is a limitation that there is no report in veterinary medicine.

As the rate used in each transfusion were different, it is difficult to distinguish whether hemolysis was performed at a high rate or by using a filter and syringe pump. Even if the transfusion set and gravity flow alone are faster, hemolysis is less than expected. In the case of bleeding in the third space that requires faster transfusion, autotransfusion using a filter and syringe is indicate. It should be remembered that hemolysis may occur at this time, and that hypocalcemia or kidney injury may need to be monitored for visual hemolysis or hematuria.

Conclusion

There are many situations in which emergency blood transfusions are required in the emergency room. Even when rapid blood transfusions are required, worries that mechanical hemolysis may occur, sometimes hesitating to speed up the transfusion beforehand. Unless concerned about overhydration due to rapid transfusion or side effects of immune transfusion reaction, the results show not hesitating to open transfusion set as a full drop. It is also concluded that the method with syringe pump using a syringe with filter to reach the rate of massive transfusion was hemolytic compared to the slow and full method, but not enough to worry about kidney damage by increasing free hemoglobin too much.

Further research will be needed because it is difficult to tell whether it is because of the faster transfusion rate or different methods of transfusion.

References

1. Deborah C. Silverstein, Kate Hopper. Fluid therapy. *Small animal critical care medicine*. 2nd ed. St. Louis, MO: Elsevier Saunders. 2015: 321–341.

2. Kenneth J. Drobatz, Kate Hopper, Elizabeth Rozanski, and Deborah C. Silverstein. Fluid and blood product therapy. *Textbook of small animal emergency medicine* 1st ed. Hoboken, NJ: Wiley Blackwell. 2019: 1115–1125, 1151–1160

3. Brandon W. Heikes and Craig G. Ruaux. Effect of syringe and aggregate filter administration on survival of transfused autologous fresh feline red blood cells. *J Vet Emerg Crit Care*. 2014; 24 (2): 162–167

4. Ruth I McDevitt, Craig G. Ruaux, and Wendy I. Baltzer. Influence of transfusion technique on survival of autologous red blood cells in the dog. *J Vet Emerg Crit Care.* 2011; 21(3): 209–216

5. Jessica M. Weeks, Miryam M. Reems. In vitro iatrogenic hemolysis of canine packed red blood cells during various rapid transfusion techniques. *J Vet Emerg Crit Care*. 2021; 31: 25–31

6. Duane A. Robinson, Kristina Keifer, Rachel Bassett, and Jane Quandt. Autotransfusion in dogs using a 2–syringe technique. *J Vet Emerg Crit Care.* 2016; 26 (6): 766–774

7. Jolanta Kunicka, Micheal Malin, David Zelmanovic, Marc Katzenberg, William Canfield, Phyllis Shapiro. Automated quantitation of hemoglobin–based blood substitutes in whole blood samples. *Am J Clin Pathol.* 2001; 116: 913–919

8. Brittany Jaeger, Miryam Reems. Visual inspection of stored canine blood for hemolysis compared with measured plasma–free hemoglobin to assess suitability for transfusion. *Can Vet J.* 2018; 59: 1171–1174

9. Willem Lambertus De Villiers, Adriaan Albertus Murray,

Andrew Ian Levin. Expediting red blood cell transfusions by syringing causes significant hemolysis. *Transfusion.* 2017; 57 (11): 2747–2751

10. Rui R F Ferreira, Augusto J F de Matos. In vitro hemolysis of stored units of canine packed red blood cells. *J Vet Emerg Crit Care.* 2018; 28 (6): 512–517

11. Iris C Vermeulen Windsant. Hemolysis is associated with acute kidney injury during major aortic surgery. *Kidney Int.* 2010; 77 (10): 913–920

12. Lin Lyu, Cun Long, Feilong Hei, Bingyang Ji, Jinping Liu, Kun Yu, Liang Chen, Jing xin Yao, Qiang Hu, Jinxiao Hu. Plasma free hemoglobin is a preditor of acute renal failure during adult venous–arterial extracorporeal membrane oxygenation support. *J Cardiothorac Vasc Anesth*. 2016; 30 (4): 891–895

13. Ana Maria Miranda Martins Wilsson. Infusion pumps and red blood cell damage in transfusion therapy: an integrative revision of the academic literature. *Rev Lat Am Enfermagem.* 2016; 24 : e2763

14. Wilcox GJ, Barnes A, Modanlou H. Does transfusion using a syringe infusion pump, and small–gauge needle cause hemolysis? *Transfusion.* 1981; 21: 750–751

15. Miller MA, SCHlueter AJ. Transfusions via hand–held syringes and small–gauge needles as risk factors for hyperkalemia. *Transfusion.* 2004; 44: 373–381

16. L Hann. Effect of duration of packed red blood cell storage on morbidity and mortality in dogs after transfusion: 3,095 cases (2001–2010). *J Vet Intern Med.* 2014; 28 (6): 1830–1837

17. Thomas J. Gniadek, Shelley Pulkrabek, Stephanie Kinney, Nicole D. Zantek, and Claudia S. Cohn. Mechanical hemolysis in pediatric patients associated with rapid transfusion and one–way valve. *Transfusion.* 2018; 58: 1228–1233

Abstract

응급실에서는 급성 실혈에 의해 반드시 긴급한 수혈이 필요한 상황들을 직면하게 된다. 하지만 매우 응급한 상황일지라도 빠르게 수혈을 하는 것은 임상 수의사에게 수혈 부작용이나 물리적인 용혈에 대한 걱정을 야기시키게 된다. 이때 어떤 수혈 속도가 가장 물리적 용혈이 덜 되는지에 고려하게 된다. 생체 내에서 이루어진 수혈에 대한 용혈의 정도를 파악하는 여러 연구들은 수혈 속도에 제한을 두고 있었고, 생체 외에서 이루어진 실험에서는 가능한 한 가장 빠른 속도 (8,000 mL/hr)로 수혈을 진행한 후에도 용혈이 되지 않음을 확인하였다. 따라서, 생체 내의 빠른 수혈에서도 같은 결과를 보이게 되는지에 대한 연구가 필요하였다.

실험 방법으로는 여섯 마리의 개에서 흡입 마취 후 경정맥에서 20%의 혈액을 채혈한 뒤, 자가 수혈을 이용하여 3 가지 속도으로 전혈 수혈을 진행하였다. 일반적인 빈혈 환자의 최대 수혈 속도 (5-10 mL/kg/hr)와 응급 상황일 때 사용하는 수혈의 속도인 중력 full drop (본 실험에서는 평균 42 mL/kg/hr 로 측정됨)과 syringe 와 filter 를 통한 대량 수혈에 상응하는 속도 (1.5 mL/kg/min, 90 mL/kg/hr)로 수혈하여 수혈 전후의 용혈의 정도를 비교하였다. 일반수혈속도, 중력 full drop 과 대량 수혈 속도 수혈의 free hemoglobin(p=.007)과 hemolysis(p=.008)의 수혈 전후 차이에 유의적인 차이가 있었다.

이것은 생체 내로 수혈을 할 때, 일반적인 수혈 속도나 중력 full drop 속도에 비해 대량 수혈 속도에서 용혈이 일어남을 보여준다.

 응급 수혈이 지시되는 상황에서 매우 빠른 속도로 수혈을 진행하게 된다면 대량 수혈의 속도로 수혈을 진행할 때에는 용혈이 일어날 수 있음을 알 수 있다. 따라서 용혈에 의한 부작용이 걱정되는 환자에서 중력 full drop 정도의 속도로 수혈을 진행한다면 환자의 예후에 더 좋은 영향을 미칠 것이다.