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# Correction of blood coagulation dysfunction and anemia by supplementation of red blood cell suspension, fresh frozen plasma, and apheresis platelet: Results of in vitro hemodilution experiments

Ling Li, BS Med Tech [a,1], Jiangcun Yang, PhD [a,*,1], Yang Sun, PhD [a], Qianli Dang, PhD [c], Cuixiang Xu, PhD [d], Ping Chen, BS Med Tech [a], Ting Ma, BS Med Tech [a], Jiangkang Ren, MS [b,**]

[a] Department of Transfusion Medicine, Shaanxi Provincial People's Hospital, Xi'an 710068, China
[b] Department of Laboratory, Shaanxi Provincial People's Hospital, Xi'an 710068, China
[c] Department of Dermatology, Shaanxi Provincial People's Hospital, Xi'an 710068, China
[d] Shaanxi Provincial Center for Clinical Laboratory, Shaanxi Provincial People's Hospital, Xi'an 710068, China

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ABSTRACT

*Purpose:* This study aimed to determine the optimal composition and timing for the administration of blood supplements during in vivo blood transfusion with red blood cells suspension (pRBC), fresh frozen plasma (FFP), and apheresis platelet (PLT) administered for the correction of anemia and coagulation dysfunction caused by in vitro hemodilution.

*Materials and methods:* We collected blood samples from 24 healthy volunteers and prepared various dilutions of whole blood with normal saline: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The diluted blood samples were then supplemented with blood components at various proportions and then analyzed to determine the values of the routine blood indices, coagulation indices, and thromboelastogram measures.

*Results:* At hemodilutions of 40%, 50%, and 60%, the hemoglobin, coagulation indices, and platelet number and function reached critical levels, necessitating supplementation with pRBC, FFP, and PLT, respectively. When hemodilution was 90%, the supplementation required was approximately 1:1.3:0.9 of pRBC/FFP/PLT.

*Conclusion:* The use of pRBC, FFP, and PLT in appropriate proportions can correct the blood coagulation dysfunction and anemia caused by in vitro hemodilution, and these proportions can be used as guidelines for in vivo massive transfusion.



## 1. Introduction

The timely administration of blood transfusion in appropriate amounts is critical to the rescue of patients with acute, life-threatening conditions accompanied by massive blood loss. However, massive blood transfusion often leads to mortality due to the associated complications such as dysfunctional blood coagulation, acidosis, and hypothermia. The optimal proportion of the various blood components transfused, namely, red blood cells (RBC), fresh frozen plasma (FFP), and platelet (PLT), and the protocol adopted for the transfusion need to be clearly defined to achieve hemostasis and prevent fatalities. Fresh frozen plasma infusion in early stages of transfusion has been shown to reduce mortality in patients receiving massive transfusion, and the recommended proportion of FFP/PLT/RBC is 1:1:1 [1–7]. However, according to our clinical experiences and observations, surgeons from different areas of China maintain different reasoned opinions about the optimal proportion of blood components and timing of their administration; moreover, there is a general dismissal of the importance of platelet proportion in the blood. To evaluate the differences in the practices adopted in the country and abroad and determine the optimal timing and proportion for the infusion of blood supplements, we chose the in vitro experimental model, considering the findings of De Souza et al [8] on in vivo and in vitro models of hemodilution. We sought to conduct in vitro experiments to determine the changes in the routine blood indices, coagulation indices, and the thrombelastogram measures induced by the administration of the blood supplements RBC, FFP, and apheresis PLT, at various proportions, for the restoration of the hemoglobin levels and correction of the coagulation dysfunction after hemodilution. Both national and international guidelines for massive transfusions and surgical blood transfusion should be followed to determine the control ranges of the levels of hemoglobin, blood coagulation indices, and PLTs [9–14,7,15].

* Correspondence to: J. Yang, Department of Transfusion Medicine, Shaanxi Provincial People's Hospital, 256 Youyi West Road, Xi'an 710068, China. Tel.: +86 29 85253261 3073; fax: +86 29 85236987.
** Correspondence to: J. Ren, Department of Laboratory, Shaanxi Provincial People's Hospital, 256 Youyi West Road, Xi'an 710068, China. Tel.: +86 29 85253261 3073; fax: +86 29 85236987.
*E-mail addresses:* yjc65@sina.com (J. Yang), renjiankan123@163.com (J. Ren).
[1] These authors contributed equally to this paper and should be considered as co-first authors.

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Previous studies have investigated the effects of in vivo and in vitro hemodilution on blood coagulation or on the changes in the blood coagulation dysfunction resulting from the dilution of blood by different media [8,16–20]. However, studies on interventions for dilution-induced dysfunction in blood coagulation are limited and address only intervention with rFVIIa [16,17]. However, blood coagulation is complex and involves several components; therefore, analysis of only rFVIIa in coagulation dysfunctions may be insufficient. During the administration of massive transfusion in emergent situations, RBC, FFP, PLT, and other blood components are primarily infused; however, no in vitro studies have been conducted on the correction of coagulation dysfunction and reduction in hemoglobin levels by analyzing the blood composition after hemodilution.

We aimed to determine the optimal composition and timing for the administration of blood supplements during in vivo blood transfusion with RBC suspension, FFP, and apheresis PLT administered for the correction of anemia and coagulation dysfunction caused by in vitro hemodilution.

## 2. Materials and methods

### 2.1. Study protocol

The dilutions were prepared from blood samples of volunteers selected from among personnel and interns working at the laboratory. Twenty-four (14 male) subjects were included (age, 19-50 years). Subjects were excluded if they had anemia, coagulation disorders, hepatorenal diseases, or menstrual periods and if they had received anticoagulant drugs. The study protocol was approved by the hospital ethics committee. All the subjects provided written informed consent for participation in the study.

### 2.2. Sources of blood supplements

The RBC suspensions, FFP, and apheresis PLTs were provided by the Shaanxi province blood center. The RBC suspension and FFP were prepared as per the recommended standards for the preparation of blood products, and the apheresis PLTs were collected by Haemonetics MCS + cell separators (Haemonetics Corp, Braintree, USA). The blood unit was computed as follows: 1 U RBC suspension was prepared from 200 mL whole blood, at a volume of 140 to 172 mL; 1 U FFP was derived from 200 mL of whole blood, at a volume of 100 mL; and 1 bag of apheresis PLT suspension was considered as a 10-U PLT curative dose and had a volume of 150 to 250 mL. The blood compositions used for the experiment were approved by the hospital ethics committee and filed for the records.

### 2.3. Blood sampling and assays

Through a vein puncture at the antecubital fossa, venous blood samples (26 mL for each subject) were collected using a 21-gauge butterfly needle into a 20-mL polypropylene syringe, to prevent activation of clotting by contact with glass. The same experienced examiner collected all the samples. To minimize the effects of venous endothelial damage caused by the use of a tourniquet; the first aspirate of 2 mL of blood was discarded.

Blood samples for both thromboelastography (TEG) analysis and the standard blood and coagulation tests were collected in the same sitting. Routine blood analysis was performed to measure the RBC count, hematocrit (Hct), hemoglobin content (Hb), and PLT count by using Coulter LH 750, a fully automatic hemacytometer, with Coulter 5C Cell control (abnormal I; abnormal II; normal). The routine coagulation indices prothrombin time (PT), activated partial thromboplastin time (APTT), international standardization ratio (INR), thrombin time (TT), and plasma fibrinogen level (FIB) were measured using the fully automatic coagulation analyzer Sysmex CA-7000 system, with the controls Dade Ci-Trol 1,

**Table 1**
Changes in blood the values of indices after in vitro dilution of whole blood with physiological saline

| Parameter | No. | Undiluted blood | 9:1 | 8:2 | 7:3 | 6:4 | 5:5 | 4:6 | 3:7 | 2:8 | 1:9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RBC | 24 | 4.16 ± 0.16 | 3.84 ± 0.12[a] | 3.37 ± 0.14 | 2.90 ± 0.12 | 2.47 ± 0.20 | 2.05 ± 0.15 | 1.67 ± 0.22 | 1.23 ± 0.05 | 0.82 ± 0.04 | 0.40 ± 0.02 |
| Hb | 24 | 129.93 ± 7.2 | 119.09 ± 6.46[b] | 104.91 ± 6.93[a] | 89.64 ± 5.20 | 77.06 ± 5.64[c] | 63.63 ± 5.41 | 50.81 ± 4.23 | 37.69 ± 2.73 | 24.69 ± 1.85 | 11.94 ± 0.85 |
| PLT | 24 | 165.53 ± 40.87 | 140.45 ± 25.72[a] | 128.55 ± 26.39 | 107.27 ± 25.8 | 108.88 ± 23.01 | 90.63 ± 22.74 | 75.56 ± 17.34[c] | 54.5 ± 14.08 | 36.19 ± 8.46 | 16.69 ± 4.72 |
| Hct | 24 | 0.38 ± 0.02 | 0.35 ± 0.02[b] | 0.31 ± 0.02 | 0.27 ± 0.01[a] | 0.23 ± 0.02[c] | 0.19 ± 0.01 | 0.16 ± 0.02 | 0.12 ± 0.01 | 0.08 ± 0.01 | 0.04 ± 0.00 |
| PT | 24 | 11.23 ± 0.67 | 11.87 ± 0.62[b] | 12.59 ± 0.8[a] | 13.74 ± 0.94 | 14.73 ± 1.29 | 16.94 ± 1.74 | 20.12 ± 1.70[c] | 26.11 ± 3.36 | 44.02 ± 13.67 | 47.9 ± 0.00 |
| PTR | 24 | 0.93 ± 0.06 | 0.98 ± 0.05[b] | 1.05 ± 0.07[a] | 1.14 ± 0.08 | 1.22 ± 0.10 | 1.41 ± 0.13 | 1.67 ± 0.12 | 2.16 ± 0.23 | 3.60 ± 0.98 | 3.66 ± 0.00 |
| INR | 24 | 0.93 ± 0.06 | 0.98 ± 0.05[b] | 1.04 ± 0.06[a] | 1.14 ± 0.07 | 1.22 ± 0.11 | 1.4 ± 0.16 | 1.66 ± 0.22[c] | 2.16 ± 0.50 | 4.11 ± 2.55 | 3.67 ± 0.00 |
| APTT | 24 | 26.17 ± 4.66 | 29.50 ± 9.38 | 36.15 ± 6.66[b] | 45.63 ± 10.61[a] | 54.34 ± 11.99[c] | 73.77 ± 16.58 | 103.64 ± 15.55 | 121.15 ± 1.06 | ND | ND |
| FIB | 24 | 2.39 ± 0.46 | 1.97 ± 0.33[b] | 1.64 ± 0.29[a] | 1.27 ± 0.24 | 1.09 ± 0.21 | 0.93 ± 0.29[c] | 0.65 ± 0.12 | 0.43 ± 0.07 | 0.30 ± 0.04 | ND |
| TT | 24 | 16.77 ± 1.03 | 18.35 ± 5.08 | 17.17 ± 1.46 | 18.67 ± 3.99 | 18.28 ± 1.86[b] | 19.33 ± 2.19 | 20.77 ± 2.33[a] | 23.63 ± 2.06 | 28.71 ± 2.47 | 38.77 ± 3.65 |
| R | 6 | 7.40 ± 1.42 | 6.70 ± 1.38 | 6.20 ± 1.50 | 6.36 ± 1.40 | 6.57 ± 1.80 | 8.57 ± 2.55 | 8.63 ± 2.29 | 9.72 ± 1.97[b,c] | 11.92 ± 1.78[a] | 20.93 ± 4.35 |
| K | 6 | 2.36 ± 0.5 | 2.66 ± 0.59 | 2.80 ± 0.68 | 3.12 ± 1.20 | 2.83 ± 0.62 | 4.19 ± 1.94[b] | 4.53 ± 0.85[a] | 6.15 ± 2.02 | 10.86 ± 3.06 | ND |
| Angle | 6 | 64.79 ± 4.83 | 64.26 ± 3.40 | 63.64 ± 2.91 | 62.78 ± 4.97 | 63.04 ± 4.23 | 56.54 ± 6.58[b] | 55.6 ± 4.53 | 54.64 ± 5.5 | 48.49 ± 3.22[a] | 38.28 ± 1.81 |
| MA | 6 | 61.103.07 | 53.78 ± 4.53[b] | 49.48 ± 5.36 | 49.72 ± 6.77 | 51.19 ± 4.63 | 45.68 ± 6.14[a] | 39.72 ± 4.30[c] | 35.21 ± 7.14 | 23.31 ± 4.19 | 10.52 ± 2.96 |
| CI | 6 | 1.60 ± −1.16 | 1.17 ± −1.09 | 0.86 ± −1.72 | 2.09 ± −2.02 | 3.71 ± −2.26 | 3.18 ± −3.11[b] | 1.96 ± −5.09[a] | 2.3 ± −6.05 | 2.76 ± −7.99 | ND |

ND indicates no data (outside the range of the test equipment).
[a] $P < .001$, start from this dilution, compare the test result thenceforth and the original detect value $P < .001$.
[b] $P < .05$, start from this dilution, compare the test result thenceforth and the original detect value $P < .05$.
[c] Critical value of the parameter necessitating supplementation with blood components and corresponding dilution ratio of blood.