

Original Article

Essential Composition of Blood Product: What is Different in a Unit of Red Cell Products ?

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Abstract:

Background: Blood products are considered as “drug” but there are variations in the production process. So different types of red cell products have different characters. **Objective:** To compare the composition of red blood cell products from different whole blood volume and preparation methods and provide information for doctor to use products properly. **Materials and Methods:** We measured volume, total hemoglobin, number of white cells in 77-200 units of each type of red cell products from 450 and 350 whole blood collection, red cells separated by platelet rich plasma and buffy coat methods and compared between them. **Results:** Total hemoglobin was 63 g for 450 mL and 45 g for 350 mL whole blood collection ($p < 0.0001$). Red cells separated by platelet rich plasma methods contain average 63 g total hemoglobin and white cell about 3×10^9 cells in both packed red cells (PRC) (270 mL volume) and red cell in additive solution (AS RC) (volume 350 ml). The leukocyte poor red cells produced by buffy coat method contain only 47 g total hemoglobin and white cells 0.8×10^9 cells (volume of 262 mL). Leuko-depleted filtration of AS RC and LPB lower white cell to 0.09×10^6 cells, 0.05×10^6 cells/unit while containing 63 g and 47 g of hemoglobin, respectively ($p < 0.0001$). **Conclusion:** There is significant less total hemoglobin in 350 mL whole blood collection than 450 mL whole blood collection. Red cells from PRP method had significantly higher Hb (63 vs 47 g) and white cells (3.0 vs 0.8×10^9 cells) than red cells from buffy coat production. Leuko-depleted filtration of red cells can lower white cell to less than 1×10^6 cells but with different amount of Hb in the final product.

Keywords : ● Red cell products ● Quality control ● Product variability

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นิพนธ์ต้นฉบับ

องค์ประกอบที่สำคัญในผลิตภัณฑ์โลหิต: ผลิตภัณฑ์เม็ดเลือดแดงแต่ละยูนิต มีอะไรที่แตกต่างกัน ?

อุษณีย์ ศิริบุญฤทธิ์ ปาริชาติ เพิ่มพิกุล วราภรณ์ มีสมรรถ สายฝน กล้าเด็ด และ ประพันธ์ กันภัย

ภาควิชาเวชศาสตร์การธนาคารเลือด คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

บทนำ ส่วนประกอบของเลือดเปรียบเสมือน “ยา” ที่ใช้ในการรักษา แต่การผลิตส่วนประกอบของเลือดมีความหลากหลายทำให้ได้ผลิตภัณฑ์เม็ดเลือดแดงชนิดต่างๆ ที่มีคุณสมบัติแตกต่างกัน **วัตถุประสงค์** เพื่อศึกษาเปรียบเทียบองค์ประกอบในผลิตภัณฑ์เม็ดเลือดแดงที่ได้จากปริมาณเลือดบริจาคและวิธีการเตรียมที่แตกต่างกัน เพื่อให้ข้อมูลแก่แพทย์ในการเลือกใช้อย่างเหมาะสม **วัสดุและวิธีการ** ทำการวัดและเปรียบเทียบ ปริมาตร ปริมาณ hemoglobin (Hb) และจำนวนเม็ดเลือดขาวในผลิตภัณฑ์เม็ดเลือดแดงชนิดต่างๆ **ผลการศึกษา** Whole blood (WB) จากเลือด 450 มล และ 350 มล มีปริมาณ Hb 63 กรัม และ 45 กรัม ($p < 0.0001$) Packed red cells (PRC) และ Red cell in additive solution (AS RC) ที่ผลิตจากเลือด 450 มล มีปริมาณ Hb และเม็ดเลือดขาวไม่แตกต่างกัน (Hb 63 กรัม เม็ดเลือดขาว 3×10^9 cells) แต่มีปริมาตรต่อหนึ่งยูนิตที่แตกต่างกัน (PRC 270 มล, AS RC 350 มล) Leukocyte poor blood (LPB) มีปริมาณ Hb 47 กรัม และเม็ดเลือดขาว 0.8×10^9 cells ซึ่งน้อยกว่า PRC และ AS RC ($p < 0.0001$) AS RC และ LPB ที่ผ่านการกรองเม็ดเลือดขาวออกจะเหลือเม็ดเลือดขาว 0.09×10^6 และ 0.05×10^6 cell โดยมีปริมาณ Hb 63 กรัม และ 47 กรัม ตามลำดับ ($p < 0.0001$) **สรุป** เลือดบริจาค 450 มล และ 350 มล มีปริมาณ Hb ที่แตกต่างกัน ผลิตภัณฑ์เม็ดเลือดแดงที่เตรียมโดยวิธี platelet rich plasma (PRP) กล่าวคือ PRC และ AS RC มีปริมาณ Hb และ เม็ดเลือดขาวสูงกว่า LPB ซึ่งเตรียมโดยวิธี buffy coat อย่างมีนัยสำคัญ การกรองเม็ดเลือดขาวออกสามารถลดปริมาณเม็ดเลือดขาวได้เหลือน้อยกว่า 1×10^6 cells โดยไม่มีการเสียเม็ดเลือดแดงเพิ่มเติม

คำสำคัญ : ● Red cell products ● Quality control ● Product variability

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2561;28:25-33.

Introduction

In Thailand, there are several red cell products available for transfusion. There is whole blood that came from 450 mL bleed from a blood donor who weighs ≥ 50 kg. While the blood supply is not enough for hospital need, this is why we have to collect 350 mL blood from a donor who weighs between 45-50 kg. We cannot expect the same amount of hemoglobin from the different volume of blood donation. The problem about having two sizes of blood unit can be overcome if the doctors can understand the difference and know exactly which one they prescribe. Blood bank staff call the smaller whole blood unit “a small single unit”. But to find a unit for patient’s request, “a unit” can be any unit and blood bank staff may pick up any available small unit while a doctor who prescribes red cell may expect for the standard unit. This is the first problem, but it is not all, we have more factors and variations that doctor may not know about red cell products. There are various type of red cell concentrates, produced by different methods. The packed red cells produced by centrifugation of whole blood and removed the majority of supernatant plasma. Red cells in additive solution produced by centrifugation of whole blood and removed almost all plasma then added 100 mL of additive solution for red cell preservation. Leukocyte poor blood produced by using special top-bottom bag system and separated red cells from buffy coat and plasma with hard spin, so there is less white cells content in red cell product. Red cells can be further reduced white cells by passing through the leuko-depleted filter. A unit of red cells is not simple anymore, there are several production factors that influence the outcome. The objective of this study is to provide essential information including volume, hematocrit, total hemoglobin, residual white cells and a plasma volume of red cell products produced by different collection volume and separation methods for doctor’s prescription and compare between them. Blood product is concerned as drug¹, this information will be useful for clinician just like drug

information and can be used for benchmarking with another blood bank.

Materials and Methods

This study was approved by Siriraj IRB number 175/2554. We calculated sample sizes from pilot QC data to achieve reliable 95%CI using nQuery 6.01.

$$N = \frac{Z\alpha/2^2\delta^2}{d^2}$$

$\alpha = 0.05$

$Z\alpha_{/2} = 1.96$

δ = standard deviation

d = acceptable error = 5

The calculated sample size was 200 each for whole blood (450 mL and 350 mL collection), 100 each for PRC (from 450 and 350 mL collection), 150 for AS RC, 110 for LPB, 77 for AS RC and 79 for LPB-LD. We retrieved data from blood product QC laboratory during January to December 2011.

We collected whole blood 350 and 450 mL from qualified donor with the minimum acceptance hemoglobin of 12.5 g/dL.²

The blood bags that we used were

1. Double bag 350 mL (Teruflex, Terumo Corp, Tokyo, Japan) for heavy spin method
2. Triple bag system for platelet rich plasma method
 - a. Teruflex (Terumo Corp, Tokyo, Japan and Triple Blood-packTM unit)
 - b. Triple Blood-Pack (Fenwal Inc, Marico, USA)
3. Quadruple bag (top-bottom system) for buffy coat separation method
 - a. Teruflex Quadruple blood bag using T-ACE semiautomated presser (Terumo Corp, Tokyo, Japan)
 - b. OptipacTM using Optipress semiautomated presser (Fenwal Inc, Marico, USA)

Separation of blood components was done in “closed system” within 8 hours of blood collection by 3 different validated methods: the heavy spin method, platelet rich

plasma (PRP) method, and buffy coat (BC) methods.³ The centrifugation was done using calibrated refrigerated blood bank centrifuge (Sorvall™ RC3BP plus; Thermo Scientific, Waltham, MA)

These three production methods were shown in Figure 1. The light spin of PRP preparation method separate PRP from PRC. In this step, we can produce AS RC if the additive solution was added to red cells. All blood products were stored in qualified condition with 24 hours temperature monitoring (Labguard 2, Bruz cedex, France).

The special leuko-reduction process is optional for some products by connecting red cell bag with leuko-reduction filters (Pall BPF 4, Pall Corporation, USA) using the sterile connecting device (T-SCD; Terumo Corp, Tokyo, Japan and CompoDock; FreseniusHemoCare, Bad-Homburg, Germany).

The measurement in this study

We determined the volume of blood product by weighed the product and converted to volume based on specific gravity 1.053 for whole blood, 1.09 for PRC, 1.06 for AS RC and 1.03 for plasma.⁴

The measurement including hemoglobin, white cells, and platelet count were done using automated cell counter Sysmex XS-800i, Kobe, Japan. except for white cells in the leuko-depleted product was manually counted using Nageotte chamber.⁵

Results

Table 1 showed total volume, hemoglobin, plasma volume and white cells content in one unit of whole blood from 450 mL and 350 mL blood collection. Table 2 showed data of PRC from 450 mL and 350 mL blood collection. WB and PRC from 450 mL collection contained average 63 g of hemoglobin while 350 mL collection contained about 45 g of hemoglobin. All the composition of PRC and WB from 350 mL collection were about 70-80% of the same parameter from 450 mL collection, which is significantly difference. Table 3 showed detail composition of red cells from 450 mL whole blood collection, the PRC and AS RC contained significant more hemoglobin (63-64 g) and significant more white cells ($3.0\text{-}3.2 \times 10^9$ cells) than leukocyte poor red cells (Hb 47 g and white cells 0.8×10^9 cells) produced from buffy

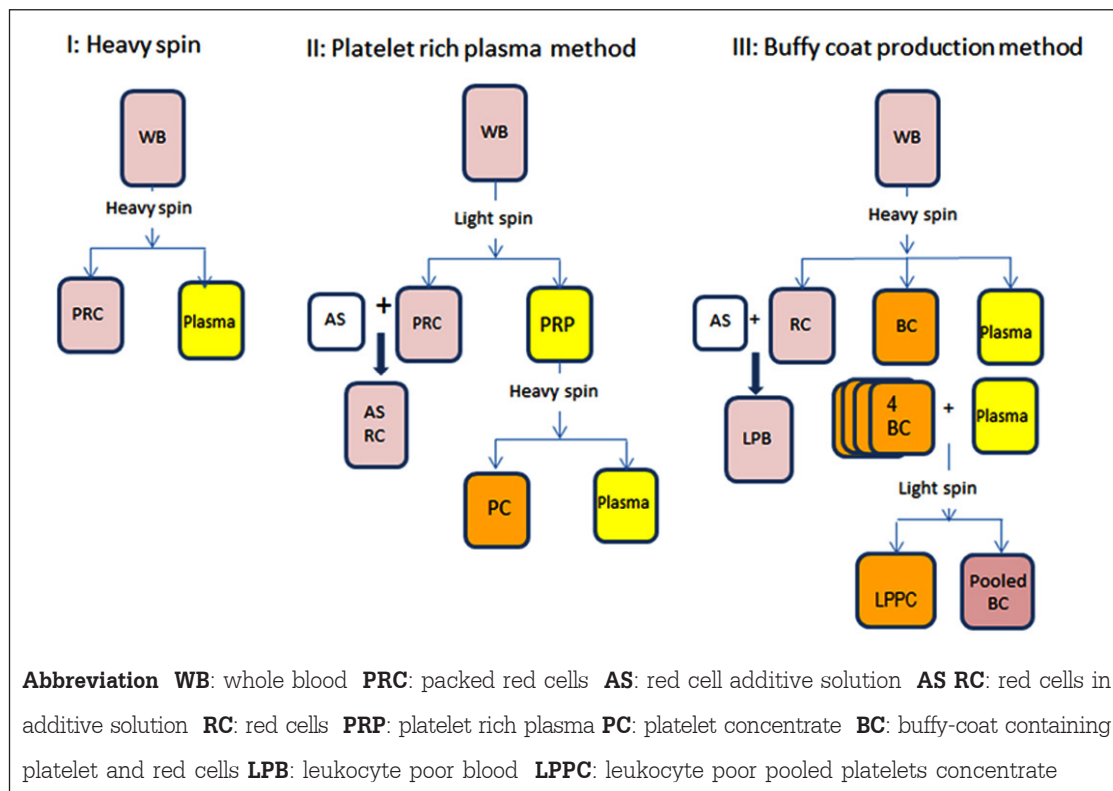


Figure 1 Blood component preparation methods

Table 1 Composition of whole blood from 350 mL and 450 mL blood collection (n = 200)

Parameter	WB (450 mL)	WB (350 mL)	p value
Volume (mL)	516 ± 10	402 ± 10	p < 0.0001
Red cell volume (mL)	193 ± 17	140 ± 10	p < 0.0001
Hematocrit (%)	37 ± 3	35 ± 2	p < 0.0001
Total hemoglobin (g)	63 ± 6	45 ± 3	p < 0.0001
White cell (x10 ⁹) per unit	3.1 ± 0.8	2.5 ± 0.6	p < 0.0001
Plasma (mL)	260 ± 18	213 ± 11	p < 0.0001

Table 2 Composition of packed red cells produced from 450 mL and 350 mL whole blood (n = 100)

Parameter	PRC (450 mL)	PRC (350 mL)	p value
Volume (mL)	270 ± 24	198 ± 13	p < 0.0001
Red cell volume (mL)	187 ± 15	138 ± 11	p < 0.0001
Hematocrit (%)	69 ± 4	70 ± 5	p < 0.0001
Total hemoglobin (g)	63 ± 5	45 ± 3	p < 0.0001
White cell (x10 ⁹) per unit	3.0 ± 0.8	2.5 ± 0.6	p < 0.0001
Plasma (mL)	67 ± 12	48 ± 9	p < 0.0001

Table 3 Composition of red cell products

Parameter	PRP method		BC method	p value
	PRC (450 mL)	AS RC*	LPB*	
Volume (mL)	270 ± 24	353 ± 28	262 ± 19	
Red cell volume (mL)	187 ± 15	193 ± 19	146 ± 16	p < 0.0001
Hematocrit (%)	69 ± 4	55 ± 3	55 ± 3	
Total hemoglobin (g)	63 ± 5	64 ± 6	47 ± 6	p < 0.0001
White cell (x10 ⁹) per unit	3.0 ± 0.8	3.2 ± 0.9	0.8 ± 0.4	p < 0.0001
Plasma (mL)	67 ± 12	48 ± 13	13 ± 5	

Note : * = 100 mL additive solution was added to red cell during production

n = 100 for PRC (450 mL), 150 for AS RC, 110 for LPB

= Hct of PRC and Hct of AS RC is significant difference (p < 0.0001)

coat production methods. The total volume of packed red cells was closed to leukocyte poor red cells but was much less than AS RC.

Leuko-depleted filtration of red cells can effectively lower total number of the white cell from 10⁹ to less than 1x10⁶ cells. In this study, AS RC-LD contained significant more Hb (57 g) than the LPB-LD (47 g) as shown in Table 4. The total amount of white cells in both AS RC-LD and LPB-LD were far less than the target level of 1x10⁶ cells even though they are significant difference.

Discussion

Our study aimed to present amount of each composition in red cell blood products which there are several variations in collection and preparation. The most concerned composition is the amount of red cells or hemoglobin in each unit which doctor prescribes to get a therapeutic effect. The other concerns are impurities such as remaining white cells and plasma which can cause the side effect of transfusion. Our blood center collects two different sizes of whole blood (350 mL and 450 mL).⁶ The volume of 350 mL blood

Table 4 Composition of leuko-depleted red cell products

Parameter	AS RC-LD*	LPB-LD*	p value
Volume (mL)	341 ± 29	247 ± 17	p < 0.0001
Red cell volume (mL)	194 ± 19	140 ± 13	p < 0.0001
Hematocrit (%)	57 ± 4	56 ± 2	p = 0.422
Total hemoglobin (g)	63 ± 5	47 ± 5	p < 0.0001
White cell (x 10 ⁶) per unit	0.09 ± 0.06	0.05 ± 0.02	p < 0.0001

Note: * = 100 mL additive solution was added to red cell during production

Table 5 Hemoglobin increment in male and female patients

BW (kg)	Male			Female		
	TBV (mL)	A	B	TBV (mL)	A	B
50	3,750	1.68	1.20	3,250	1.93	1.38
60	4,500	1.40	1.00	3,900	1.62	1.15
70	5,250	1.20	0.86	4,550	1.38	0.99
80	6,000	1.05	0.75	5,200	1.21	0.87

BW = body weight; TBV = total blood volume

A = hemoglobin increased (g/dL) after transfusion of blood product with 63 gm of hemoglobin

B = hemoglobin increased (g/dL) after transfusion of blood product with 45 gm of hemoglobin

collection is about 77% of 450 mL collection lead to proportional different of volume, hemoglobin, white cells and plasma content between products. The doctor should be aware of this fact to prevent misunderstanding as well as wrong expectation from a smaller unit. The smaller red cell unit is still useful for the pediatric patient who needs a smaller amount of red cells but it is not appropriate to use smaller red cell unit to transfuse several units for an adult patient because the increment of hemoglobin will be far below doctor's expectation. Estimated transfusion outcome is not quite simple because the blood volume is depended on sex and body weight. From Table 5, the rough estimation about an increase of hemoglobin 1 gram from transfusion of one unit of blood is not always correct⁷.

The second part of the result is comparison amount of red cells, white cells, and plasma in each unit of red cell products produced by a different protocol including PRC, AS RC, LPB, and LDB. The doctor may percept volume of product as an indicator for mount of red cells, but this is not true. The total hemoglobin from 450 ml

whole blood collection is about 63 g and PRC, AS RC produced by PRP method, contains about 60 g of Hb but had a different volume of plasma and total volume. PRC was produced by removal majority of plasma. In AS RC almost all plasma was removed before immediately adding 100 mL of red cell additive solution (AS) to red cells. This is why PRC hematocrit is about 70-80% while AS RC is about 55-65% and overall AS RC had mean plasma content less than PRC (Table 3). When we transfused red cells, the recipient of different but compatible ABO type, red cell product with less plasma volume is the better choice so the product of choice will be AS RC. For the patient who cannot tolerate high volume or patient who should restrict intake volume, PRC is preferred because of lower final volume. In neonate who requires red cells exchange transfusion, the packed red cells are the product of choice because these patients cannot tolerate large volume of additive solution.⁸

Buffy coat removal red cells or LPB is quite popular in Thailand especially for the hematologic patient who

require chronic transfusion. Majority of white cells were removed from red cell products to lower the febrile non-hemolytic transfusion reaction. Buffy coat removal is considered leuko-reduction in Thailand while in European countries, the buffy coat production method is basic production method because of using semi-automated presser and superior quality of platelets⁹ and further leuko-reduction will be achieved by leuko-depleted filtration. The setting of automated presser at our institute for BC production aim to get a low number of white cells in red cell products. Our data showed the mean total Hb of LPB was about 47 g per unit while data from other studies report meaning Hb about 51-55 g per unit.^{10,11} In figure 2, LPB has less hemoglobin content than PRC and AS RC even though this unit produced from 450 mL whole blood collection because of red cell loss in buffy coat. We try to adjust

the condition of BC production to reduce red cell loss in the buffy coat but had to trade off with higher residual leukocyte in final red cell products. However, the amount of hemoglobin in final red cell product is more than minimum 43 g stated by international standard.¹² The average amount of white cell in LPB is about 0.8×10^9 cells which is less than EU standard (1.2×10^9 cells).¹² Lower of the white cell to less than 5×10^8 cells per unit which is the target for prevention of FNHTR¹³ can be achieved in only 36% of LPB. Theoretically, LPB is not effective to prevent FNHTR so the doctor should prescribe leuko-depleted blood instead. Another important information is Hb content in a unit of LPB is far less than AS RC unit. The doctor should realize this fact and should expect only 45 g of hemoglobin from each unit of LPB.

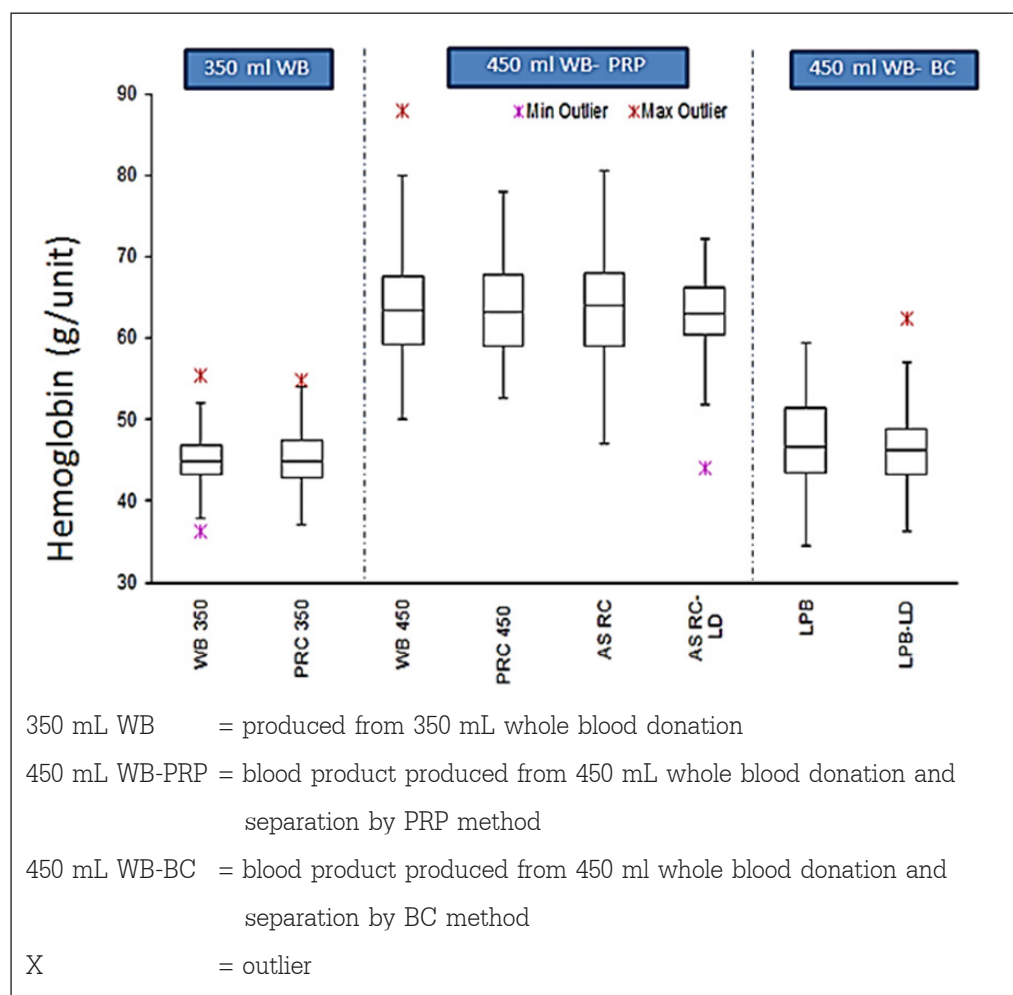


Figure 2 Comparison of total hemoglobin in different red cell products

The leuko-depleted filtration can effectively reduce white cell to lower than 1.0×10^6 cells per unit which can prevent FNHTR, HLA alloimmunization, platelet refractoriness^{14,15} and CMV infection.¹⁶ Our results showed that AS RC-LD contained significant higher Hb and plasma volume than LPB-LD. The amount of residual white cells is also significantly different ($p < 0.0001$) but all total number of the white cells are far below 1×10^6 . Leuko-depleted filtration is effective enough to reduce the number of the white cell to less than 1×10^6 cells without prior buffy coat removal. Thus, if we transfuse 3 units of AS RC-LD ($63 \times 3 = 189$ g) compare with 3 units of LPB-LD ($47 \times 3 = 141$ g) we transfuse 48 g less hemoglobin (Figure 2). Thalassemia patient who was transfused 3 units of LPB-LD every 3 weeks for a year may get about 800 g less hemoglobin than AS RC-LD which equal to 13 units of PRC. From these data, we recommend blood center to select PRC or AS RC for leukoreduction filtration instead of LPB.

Compare with the national and international standard, our red cell blood products comply with these standards.¹² All the blood product detail data is useful for the clinician to calculate and prescribe more reasonable dose of red cells, iron chelation for Thalassemia, predict more accurate patient's response. The Thalassemia International Federation recommended that clinician should prescribe red cells dose for Thalassemia patient base on product hematocrit and target increase of Hb¹⁷ so the information for both red cells concentration and the total volume of red cells should be provided for clinician for better care of the patient. While whole blood should be transfused to only an ABO identical recipient, other red cell products such as PRC, AS RC can be transfused to ABO-compatible recipient. When we transfuse non-ABO identical red cell, in this condition, LPB which contain minimal plasma is better than AS RC and PRC.

Conclusion

Our data showed the average value of red cells, white cells and plasma content of each "unit" from different collection volume and preparation methods. This set of data should be communicated and available to the doctor who prescribes blood product so they can practice rationale use of blood in the same way as the rational use of drug. Data on the composition of blood products can also be used for comparison between production methods and production center.

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