Transfusion medicine

PJ-01

Effects of potassium adsorption filters on the removal of ammonia from blood products

A strategy to avoid the unnecessary administration of ammonia derived from blood products

Hiroshi Fujita[1], Yoko Shiotani[2], Yuko Takada[2], and Shigeko Nishimura[1]

[1] Department of Transfusion Medicine, Tokyo Metropolitan Bokutoh Hospital,
[2] Clinical Laboratory, Tokyo Metropolitan Bokutoh Hospital

Background

- While ammonia in the plasma usually does not pass through the blood brain barrier (BBB), ammonia may affect the brain, as a neurotoxin, in patients with traumatic brain injury (TBI). Excess intake of ammonia should be restricted in conditions involving BBB breakdown, such as TBI.
- Although blood product washing is the only method for removal of ammonia, washing of fresh frozen plasma (FFP) and albumin products is not possible. The potassium adsorption filter (PAF) can remove not only potassium, but also ammonia from red blood cell (RBC) solution.
- Therefore, we examined the effects of the PAF on the removal of ammonia from a range of blood products.

Materials and Methods (1) Blood products

- Blood products, such as RBC solution, FFP and PC (Japan Red Cross Society, Tokyo, Japan), used in the experiments were expired in our hospital. In Japan, RBC solution, FFP and PC should be used within 21 days, 1 year, and 4 days, respectively.
- Albumin products used in the experiments were purchased from Japan Blood Product Organization (Tokyo, Japan), and were within the expired date.

Materials and Methods (2) use of PAF

- After priming the PAF (KPF-1, Kawasumi Laboratories Incorporated, Oita, Japan) with saline (Terumo Corporation, Tokyo, Japan), saline was removed from the PAF before use. Two units of RBC solution and FFP were divided into two bags (1 unit of RBC:140mL, 1 unit of FFP: 120mL). Ten units of PC were divided into two bags (5 units of PC: 100mL) and 250ml of 5 % albumin products were divided into two empty bags (1bag: 125mL). One unit of RBC solution (140mL), 1 unit of FFP (120mL), 5 units of PC (100mL), 5% albumin solution bag (125mL) and 20% albumin (50 mL/one bottle) were filtered, with a flow rate of approximately 20 ml/minute, and collected into empty bags.
- Levels of potassium and ammonia concentration in the different blood products collected into the empty bags were measured by Biomedical Laboratories Company (Tokyo, Japan) and compared with the respective concentrations in the blood products before passage through the PAF. We calculated potassium or ammonia removal percentage (% removal) as follows:
- % removal= (mean concentration of potassium or ammonia in the blood products before use of PAF – values of potassium or ammonia in the blood products through PAF) ÷ mean value of potassium or ammonia in the blood products before use of PAF x 100 (%)

Materials and Methods (3) Effects of dilution by saline on the blood products through PAF

- In the experiments using RBC solutions, haemoglobin concentration and haematocrit were measured in order to determine the effect of dilution due to residual saline.
- Prothrombin time percentage (%PT), activated partial thromboplastin time (aPTT) and fibrinogen levels in the FFP were measured in order to determine the influences on clotting activity.
- Platelet numbers in the PC were measured in order to determine the effects of saline dilution.
- Potassium levels were not measured in the albumin products, because albumin products do not contain potassium. In order to determine the effects of dilution of saline, albumin concentrations were measured in the albumin products.

Table 1. Removing effects of potassium adsorption filter on the ammonia concentration in irradiated red blood cell solution

	Potassium (mEq/L)	Ammonia (μg/dL)
RBC #1 day19 A0 Rh (+) N=4	Prior 54.0 (1.0) →After 1.7 (0.0)* % removal: 96.9 (0.1)	Prior 273.5 (33.5) →After 46.3 (2.3)* % removal: 83.1 (0.8)#
RBC #2 day19 A0 Rh (+) N=4	Prior 62.8 (2.8) →After 2.7 (0.0)* % removal: 95.8 (0.1)	Prior 165.3 (5.1) →After 20.5 (1.6)* % removal: 87.6 (1.0)#
RBC #4 day24 A0 Rh (+) N=4	Prior 60.0 (0.4) →After 2.9 (0.0)* % removal: 95.2 (0.1)	Prior 249.0 (12.3) →After 46.3 (2.3)* % removal: 82.6 (1.6)#
RBC #4 day28 O Rh (+) N=4	Prior 59.5 (0.2) →After 1.3 (0.0)* % removal: 97.6 (0.1)	Prior 378.3 (50.0) →After 89.3 (50.0)* % removal: 76.3 (1.0)#
RBC #3 day24 O Rh(+) N=4	Prior 57.2 (0.2) →After 2.5 (0.0)* % removal: 94.8 (0.2)	Prior 227.5 (13.9) →After 38.5 (1.4)* % removal: 83.1 (0.6)#

Data represent the means with the standard errors in parentheses.
*: p < 0.05 vs. values from the sample through PAF, #: p < 0.05 vs. % removal of potassium

Table 2. Removing effects of potassium adsorption filter on the ammonia concentration in fresh frozen plasma and platelet concentrates

	Potassium (mEq/L)	Ammonia (μg/dL)
FFP #1 day367 O Rh (+) N=4	Prior 3.3 (0.0) →After 1.4 (0.0)* % removal: 57.5 (0.1)	Prior 157.0 (7.0) →After 123.0 (1.8)* % removal: 21.6 (1.1)#
FFP #2 day367 AB Rh (+) N=4	Prior 3.0 (0.0) →After 1.0 (0.0)* % removal: 64.7 (1.0)	Prior 198.8 (4.7) →After 136.8 (1.0)* % removal: 31.3 (0.5)#
FFP #3 day368 AB Rh (+) N=4	Prior 3.0 (0.0) →After 1.3 (0.0)* % removal: 62.6 (5.6)	Prior 185.0 (6.4) →After 129.5 (1.3)* % removal: 30.8 (0.7)#
PC #1 day5 O Rh (+) N=4	Prior 3.8 (0.0) →After 1.9 (0.1)* % removal: 54.8 (1.1)	Prior 947.5 (38.5) →After 455.8 (25.0)* % removal: 52.9 (2.6)
PC #2 day5 O Rh(+) N=4	Prior 3.5 (0.0) →After 0.8 (0.0)* % removal: 76.1 (0.7)	Prior 518.5 (39.5) →After 218.0 (5.6)* % removal: 53.9 (4.6)

Data represent the means with the standard errors in parentheses.
*: p < 0.05 vs. values from the sample through PAF, #: p < 0.05 vs. % removal of potassium

Table 3. Removing effects of potassium adsorption filter on the ammonia concentration in 20% and 5% albumin products

	Ammonia prior to use of PAF (μg/dL)	Ammonia through PAF (μg/dL)	% removal
20% albumin products			
Lot #1	244.8 (5.2)	57.5 (2.9)*	76.5 (1.2)
Lot #2	274.3 (5.8)	22.3 (1.5)*	91.9 (0.6)
Lot #3	291.2 (7.1)	32.3 (1.9)*	88.9 (0.6)
Lot #4	288.0 (7.3)	30.0 (0.7)*	89.6 (0.2)
5 % albumin products			
Lot #1	79.8 (1.0)	38.5 (0.9)*	51.9 (1.1)
Lot #2	74.3 (2.2)	35.3 (0.9)*	52.4 (1.3)
Lot #3	68.8 (1.9)	32.0 (1.3)*	49.2 (3.3)
Lot #4	63.3 (1.0)	23.2 (1.8)*	63.1 (2.9)

Data represent the means with the standard errors in parentheses.
*: p < 0.05 vs. values from the sample through PAF

Table 4. Effects of dilution by saline on the blood products through PAF

	prior to use of PAF	through PAF
RBC #1		
Hb (g/dL)	19.0 (0.1)	18.8 (0.0)
Hct (%)	52.9 (0.0)	52.3 (0.2)
FFP #1		
%PT (%)	98.0 (1.7)	99.2 (0.5)
aPTT (sec)	44.5 (0.7)	44.8 (0.2)
Fibrinogen (mg/dL)	468 (0.0)	479 (0.0)
PC #1		
Platelet counts (x10⁴/μL)	157.5 (1.0)	156.8 (0.7)
20% Albumin product #1		
Albumin (g/dL)	20.9 (1.1)	19.1 (0.2)
5% Albumin product #1		
Albumin (g/dL)	4.9 (0.0)	4.9 (0.0)

Data represent the means with the standard errors in parentheses.

Conclusion

This study demonstrates the increased levels of ammonia in blood products containing albumin products and reports for the first time that the PAF is useful for the removal of ammonia from a range of blood products in order to avoid the unnecessary ammonia derived from blood products.

Results (1)

Red blood cell solution

- Potassium and ammonia levels were high in the expired RBC solution (day 24~26, K: 54~62.8 mEq/L, ammonia: 165.3~378.3 μg/dL). Both potassium and ammonia levels in the RBC solution decreased significantly post-filtration, as shown in Table 1 (% removal of potassium: 94.8~97.6%, % removal of ammonia: 76.3~87.6%). In the RBC solution, the ammonia removing effects of the PAF were lower compared to the potassium removing effects.

Fresh frozen plasma

- Ammonia levels were high in the FFP solution expired (day 367~369, ammonia: 157.0~198.8 μg/dL), while potassium levels were lower than the normal values (K: 3.0~3.3 mEq/L), as shown in Table 2. Potassium and ammonia levels in the FFP were decreased significantly post-filtration, as shown in Table 2 (% removal of potassium: 57.5~64.7%, % removal of ammonia: 21.6~31.8%). In the FFP, the ammonia removing effects of the PAF were lower than the potassium removing effects. Moreover, the potassium and ammonia removing effects of the PAF in the FFP were lower than the removing effects in the RBC solution.

Results (2)

Platelet concentrates

- Ammonia levels were extremely high in the expired PC (day 5, ammonia: 518.5~947.5 μg/dL), while potassium levels were almost within normal values (K: 3.5~3.8 mEq/L), as shown in Table 2. Both potassium and ammonia levels in the PC were significantly reduced by passing through PAF, as shown in Table 2 (% removal of potassium: 54.8~76.1%, % removal of ammonia: 52.9~53.9%). In the PC, the ammonia removing effects of the PAF were not lower than the potassium removing effects. However, the ammonia removing effects of the PAF in the PC were lower than those in the RBC solution and higher than those in the FFP.

Albumin product

- Ammonia levels were extremely high in the 20% albumin products (ammonia: 244.8~291.2 μg/dL). The high levels of ammonia were significantly reduced by passing through PAF, as shown in Table 3 (% removal of ammonia: 76.5~91.9%). The PAF was more effective at removing ammonia in the 20% albumin products than in the RBC solution, FFP, PC or 5% albumin products, as shown in Table 3.
- Ammonia levels were slightly high in the 5% albumin products (ammonia: 63.3~79.8 μg/dL). The ammonia levels were significantly reduced after passing through PAF, as shown in Table 3 (% removal of ammonia: 49.2~63.1%). The ammonia removing effects of PAF in the 5% albumin products were lower than those in the 20% albumin products solution, as shown in Table 3.

Discussion (1)

Blood transfusion and albumin infusion may increase the ammonia load in patients with hyperammonaemia

Whole blood transfusion: J Clin Invest 1958; 37: 990-8.

- Bessman et al. reported a twofold increase in ammonia concentration in cirrhotic patients receiving whole blood transfusion after four hours of transfusion.

Albumin product infusion: Med J Aust 1960; 20: 290-3.

- Intravenous albumin infusion is known to increase ammonia levels in patients with liver disease and in those without liver disease.
- Moreover, in the patients with liver disease, increased ammonia levels were sustained until 6 hours after albumin infusion.

Discussion (2)

Recent research has shown that slight increases in ammonia resulted in mild MRI changes in adults and neonates

Adults with liver diseases: Am J Neuroradiol 2011; 32:413-18.

- Recent research has shown that slight increases in ammonia resulted in mild MRI changes in adults.

Neonate: Pediatr Neurol 2014; 51: 553-6.

- In the neonate with hyperammonaemia, brain MRI revealed abnormal findings.

Discussion (3)

Research has shown that in patients with multiple trauma, plasma ammonia levels increased.

- Ammonia levels in trauma patients increased, and patients with high levels of ammonia revealed the bleeding tendency and poor outcome.
- Hagiwara et al. reported that the plasma ammonia cut-off level for survival was 77 μg/dL.
  - J Trauma 2009; 67: 115-20.
- Outcome of TBI may be affected by increased levels of plasma ammonia, as ammonia acts as a neurotoxin.
  - Nat Med 2013; 19: 1572-4.

Effects of potassium adsorption filters on the removal of ammonia from blood products

Funding

- This study was supported by a grant from Tokyo Metropolitan Government.

Disclosure of conflicts of interest

- The authors have no conflicts of interest to declare.

Transfusion medicine

PJ-02

Effects of different uses of potassium adsorption filters between saline-filled and saline-removed methods on the removal of potassium from red blood cell concentrates

A strategy to use of potassium adsorption filters for preterm infant transfused small-volume pack

Hiroshi Fujita[1], Yoko Shiotani[2], Yuko Takada[2], and Shigeko Nishimura[1]

[1] Department of Transfusion Medicine, Tokyo Metropolitan Bokutoh Hospital,
[2] Clinical Laboratory, Tokyo Metropolitan Bokutoh Hospital

Background

- Red blood cell (RBC) transfusion places preterm infants with non-oliguria at high risk of cardiac arrest due to hyperkalemia.
- Potassium adsorption filters (PAFs) can remove potassium from RBCs. The typical protocol for use of PAFs in Japan involves priming the filter with 200 mL of saline and filling the filter with saline.
- However, the resulting dead volume (approximately 80 mL) is unnecessary in preterm infants, because the blood is diluted with saline.
- As transfusion volumes are generally ~10 mL, small volume-separated packs (30~80 mL each) are prepared for preterm infants.
- However, we are unable to use PAFs in small volume-separated packs due to the dead volume.
- In this study, we examined the effects of saline-filled and saline-removed methods of PAFs on the removal of potassium from RBCs.

Methods and Materials (1)

- The blood products including RBCs (Japan Red Cross Society, Tokyo, Japan) used in the experiments in our hospital were expired. In Japan, RBCs should be used within 21days.
- After the PAFs (KPF-1, Kawasumi Laboratories Incorporated, Oita, Japan) were primed with saline (Terumo Corporation, Tokyo, Japan), they were filled with saline (method A) or the saline was removed (method B) before use of PAF. Two units of RBCs were divided into two bags (140 mL per unit). One unit of RBC solution (140 mL) was filtered with a flow rate of approximately 20 mL/minute and collected into empty bags. Potassium and ammonia concentrations in the RBC collected in the empty bags were measured by Biomedical Laboratories Company (Tokyo, Japan) and compared to those in the RBC pre-PAF.
- Percent removal (% removal) was defined as follows:
  - % removal = (mean potassium or ammonia value in the RBC before use of the PAF − potassium or ammonia values in the RBC after use of the PAF) ÷ mean potassium or ammonia values in the RBC before use of PAF x 100 (%)

Methods and Materials (2)

- Erythrocyte damage: In order to evaluate erythrocyte damage, lactate dehydrogenase (LD) and free hemoglobin were measured by Biomedical Laboratories Company (Tokyo, Japan). We compared the differences in erythrocyte damage following PAF using method B as well as a transfusion filter (Terumo Corporation, Tokyo, Japan).
- Preparation of small volume-separated packs: Four small volume-separated packs (BB-TQ009J, Terumo Corporation, Tokyo, Japan) were divided from 1 unit of RBC using a Terumo Sterile Connection Device-II (Terumo Corporation, Tokyo, Japan). Each pack contained approximately 35 mL of RBCs. RBCs in three small volume-separated packs were filtered at a flow rate of approximately 20 mL/minute and collected into empty bags using method B. One small volume-separated RBC was used as the pre-PAF control.

Results (1) The effects of saline-filled (A) and a saline-removed (B) potassium adsorption filter methods on potassium concentrations

- The standard saline-filled (A) method used in Japan for PAF resulted in the removal of 89.7-93.1% of potassium from the RBC, as shown in Table 1. In comparison, the saline-removed PAF method (B) also removed 95.1-96.8% of the potassium. The % removal of potassium by PAF using a method B method was significantly higher than that by method A, as shown in Table 1.
- Hemoglobin and hematocrit levels did not decrease in the RBC solution after using PAF method B (RBC #2 in Table 1; pre-hemoglobin: 20.8 ± 0.0 g/dL, pre-hematocrit: 58.1 ± 0.0%; post-hemoglobin: 20.8 ± 0.0 g/dL, pre-hematocrit: 58.4 ± 0.1%, N = 4), while hemoglobin and hematocrit levels decreased significantly in the RBC solution following PAF method A (RBC #2 in Table 1; (pre-hemoglobin: 19.1 ± 0.0 g/dL, pre-hematocrit: 54.3 ± 0.1%; post-hemoglobin: 17.2 ± 0.0 g/dL, pre-hematocrit: 49.6 ± 0.2%, $p < 0.05$, N = 4). The changes in hemoglobin and hematocrit levels in the other RBC solutions (#1 and #3) were the same as those in RBC #2 (data not shown).

Table 1 Effects of potassium adsorption filter methods A and B on potassium concentrations in red blood cell solutions.

	Irradiated RBC#1	Irradiated RBC #2	Irradiated RBC#3
Saline-filled method (method A, N = 4)	92.7 ± 0.1%	93.1 ± 0.0%	89.7 ± 0.1%
Saline-removed method (method B N = 4)	96.8 ± 0.0%*	95.8 ± 0.0%*	95.1 ± 0.0%*

RBC: red blood cell solution
The potassium concentration of RBC#1 (AB type, Rh D+, day 25), RBC#2 (AB type, Rh D+, day 24), RBC#3 (AB type, Rh D+, day 24) prior to passing through PAF was 54.0 ± 1.0 mEq/L, 62.8 ± 2.8 mEq/L, 60.0 ± 0.4 mEq/L, respectively
Data represent the means ± standard errors
*: $p < 0.05$ vs. values from the sample obtained using method A.

Results (2) RBC erythrocyte damage after PAF methods A and B

- Erythrocyte damage, as assessed by levels of free hemoglobin and LD in the RBC, was higher for method B compared to method A, as shown in Table 2.
- However, erythrocyte damage in the RBC using method B was almost similar that of a transfusion filter usually used at transfusion, as shown in Table 3.

Table 2. Erythrocyte damage in the RBC following use of potassium adsorption filters using methods A and B.

	Irradiated RBC#1	Irradiated RBC#2	Irradiated RBC#3
Saline-filled method (A) method			
Pre-PAF			
Free hemoglobin (mg/dL)	12.3 ± 2.3	42.3 ± 0.8	10.0 ± 0.4
LD (IU/L)	42.3 ± 0.9	112.8 ± 1.7	48.0 ± 0.7
Post-PAF			
Free hemoglobin (mg/dL)	12.3 ± 0.3	44.2 ± 0.5	10.8 ± 0.5
LD (IU/L)	47.5 ± 4.1	125.8 ± 1.5*	47.0 ± 0.8
Saline-removed method (B) method			
Pre-PAF			
Free hemoglobin (mg/dL)	12.7 ± 1.1	67.3 ± 0.8	12.0 ± 0.4
LD (IU/L)	47.8 ± 0.75	193.5 ± 0.9	51.0 ± 0.0
Post-PAF			
Free hemoglobin (mg/dL)	17.5 ± 0.3*	82.0 ± 1.1*	14.0 ± 0.4*
LD (IU/L)	55.5 ± 0.3*	272.2 ± 5.4*	60.2 ± 2.6*

RBC: red blood cell solution. Data represent the means ± standard errors.
*: $p < 0.05$ vs. values from the sample prior to passing through the PAF.

Table 3. Erythrocyte damage in red blood cell solutions after use of transfusion and potassium (B method) filters

	Before filter	After filter
Transfusion filter		
Free hemoglobin (mg/dL)	12.0 ± 0.0	16.5 ± 0.6*
LD (IU/L)	58.0 ± 0.9	65.0 ± 0.4*
Potassium adsorption filter (B method)		
Free hemoglobin (mg/dL)	10.5 ± 0.3	13.8 ± 0.5*
LD (IU/L)	54.0 ± 1.4	61.3 ± 0.8*

The results are representive of 3 independent experiments.Data represent the means ± standard errors.
*: $p < 0.05$ vs. values from the sample prior to passing through transfusion filter or PAF

Effects of saline-removed PAF method B on potassium concentrations in small volume-separated RBC packs

Two units of RBC were firstly divided into two bags.

- One unit of RBC was divided into three small volume-separated packs, each containing 35 mL of RBCs.
- The % removal of potassium in the three small volume-separated packs was 96.0 ± 0.1%, 95.9 ± 0.1%, and 95.7 ± 0.1%, respectively.

Discussion (1) Number of small volume-pack

Previous paper: *Arch Dis Child Fetal Neonatal Ed* 2004, 89: F182-F183.

- Gupta reported that eight and four packs per infant offered optimal cost and safety for those with birth weights < 1000 g and >1000 g, respectively.

Recent paper: *Arch Dis Child* 2012, 97:A84.

- Because neonatal transfusion practice has changed due to restrictive transfusion guidelines, micro-sampling methods, and the practice of delayed cord clamping, separate recommendations were made for infants with birth weights <1000 g.
- Recently, a single unit of RBCs is divided into four small-volume packs in our hospital, each containing approximately 35 mL of RBCs.

Discussion (2) To avoid the potassium load

Previous paper: *Pediatric Anesthesia* 2013, 23:1021-1026.

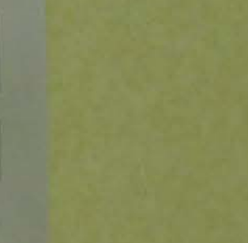
- To reduce potassium load during RBC transfusion, washed RBC have been used.
- However, few hospitals in Japan have the ability to wash RBCs.

In this study: PAFs may be a beneficial bedside tool for neonates with non-oliguric hyperkalemia.

- In Japan, after priming PAFs with 200 mL of saline, the saline is filled before use of the PAF. The resulting, dead volume (approximately 80 mL of saline) is unnecessary for preterm infants because the blood is diluted with saline.
- By removing the saline from the priming, neonates with non-oliguric hyperkalemia may receive small volume-separated packs passed through PAFs.
- We compared the effectiveness of PAFs for removing potassium between saline-filled and saline-removed methods. The saline-removed PAF method was more effective for removing potassium compared to the saline-filled method, as shown in Table 1.
- The % removal of potassium using the saline-filled method was likely lower was due to dilution by the saline.

Discussion (3) Safety of PAF

- The erythrocyte damage in the RBC passing through PAF using the saline-removed method might similar to that of the saline-filled method because the damage using the saline-removed method was similar to that of transfusion filters.
- PAFs consist of two filters: one filter similar to transfusion filters, and a resin filter that adsorbs potassium.
- Therefore, we believe the saline-filled PAF method to be safe.
- Moreover, RBC in the small volume-separated pack could be passed through PAF, and the potassium concentrations were removed enough.



Conclusion

- **PAF using a saline-removal method was more effective than the standard saline-filled method.**
- **The saline-removed method might useful for RBC transfusion of small volume-separated packs.**

Effects of different uses of potassium adsorption filters on saline-filled and saline-removed methods for the removal of potassium from red blood cell solutions

Funding

- This study was supported by a grant from Tokyo Metropolitan Government.

Disclosure of conflicts of interest

- The authors have no conflicts of interest to declare.

Transfusion medicine

PJ-03

Increased plasma Cathepsin S and Trombospondin-1 in patients with acute ST-elevation myocardial infarction

Rahel Befekadu[1], Kjeld Christiansen[2], Anders Larsson[3], Magnus Grenegård[4]

[1]Department of Laboratory Medicine, Section for Transfusion medicine, Örebro University Hospital, Örebro, Sweden, [2]Department of Cardiology, Örebro University Hospital, Örebro, Sweden, [3]Department of Medical Sciences, Academic Hospital, Uppsala, Sweden, [4]Department of Biomedicine, School of Medical Sciences, Örebro University, Örebro, Sweden.

Background and Objective

The role of cathepsins in the pathological progression of atherosclerotic lesions in ischemic heart disease has been described in detail more than a decade ago.

Cathepsin S (Cat-S) is one of the 11 family members, which are lysosomal proteases that participate in numerous physiological systems. The expression and activity of these proteins are changed during various inflammatory diseases, including rheumatoid arthritis, atherosclerosis.

The aim was to examine changes in plasma cathepsin S (Cat-S), trombospondin-1 (TSP-1) and platelet function in patients with ST-elevation myocardial infarction (STEMI) before and after percutaneous coronary intervention (PCI)

Materials and methods

Patients were divided into two groups depending on the degree of coronary vessel occlusion: those with closed (n = 90) and open culprit vessel (n = 40).

Cat-S and TSP-1 were analyzed from peripheral venous blood samples drawn before, 1-3 days after and 3 months after PCI using enzyme-linked immunosorbent assay.

Result

In STEMI patients, plasma Cat-S and TSP-1 levels were initially high but declined rapidly over a period of 1-3 days. Although troponin-I were higher (P<0.01) in patients with closed culprit lesion, there were no differences in Cat-S and TSP-1 levels between the two patient groups (blood samples obtained before and shortly after PCI intervention).

However, in blood samples obtained 3 months after PCI, plasma Cat-S (but not TSP-1) was significantly higher (P<0.001) in patient with closed culprit lesion. There were no differences in demographic data, cardiovascular risk factors, between the two patient groups (Table I and II)

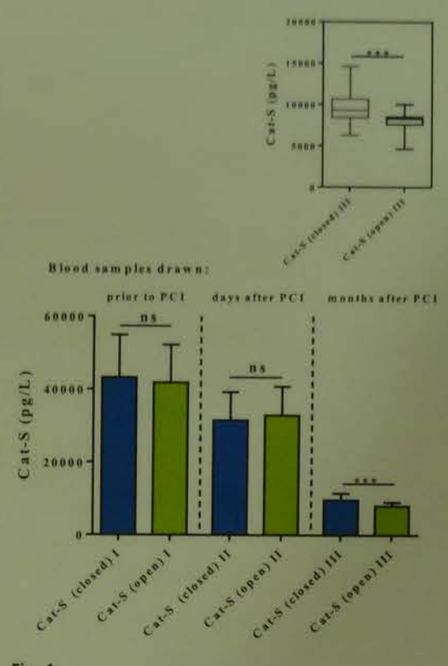


Fig. 1.
Plasma level Cat-S in STEMI patients, 1-3 days and three month after PCI

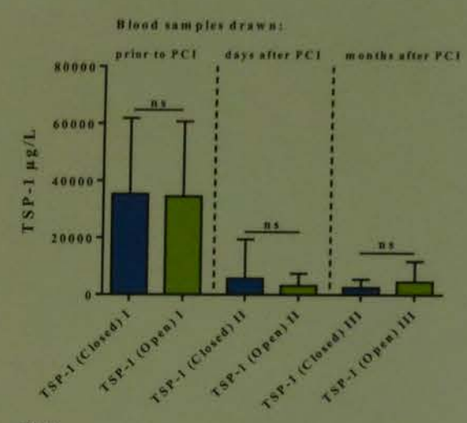


Fig 2.
Plasma level TSP-1 in STEMI patients

Table 1.
Demographic and clinical features of the patients at admission (data are presented as mean values ± standard deviation).

Number of subjects closed blood vessel	90
Age, years	71[46-96]
Sex M/F	68/22
BMI	27.3[16.0-38,3]
Smoker %	62
Previous MI %	12
Diabetes mellitus %	17
Hyperlipidemia %	51
PCI(h)	0.11[90]
PPT	224
WBC	13.5
CK	82.8

BMI body mass index, MI myocardial infarction, PCI percutaneous coronary intervention, PPT platelet particle concentration, WBC white blood cell count, CK creatinine kinase.

Table 2.
Demographic and clinical features of the patients at admission (data are presented as mean ± standard deviation

Number of subjects open blood vessel	40
Age, years	68[43-93]
Sex M/F	25/15
BMI	27.3[20.0-45.3]
Smoker %	60
Previous MI %	20
Diabetes mellitus %	20
Hyperlipidemia %	40
PCI(h)	0.15[40]
PPT	228
WBC	10.1
CK	83

BMI body mass index, MI myocardial infarction, PCI percutaneous coronary intervention, PPT platelet particle concentration, WBC white blood cell count, CK creatinine kinase.

References

1. Li X, Liu Z, Cheng Z, Cheng X. Cysteinyl cathepsins: multifunctional enzymes in cardiovascular disease. Chonnam Med J. 2012;48(2):77-85.
2. Reiser J, Adair B, Reinheckel T. Specialized roles for cysteine cathepsins in health and disease. J Clin Invest. 2010;120(10):3421-31.
3. Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B, et al. Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochim Biophys Acta. 2012;1824(1):68-88.
4. Leung LL. Role of thrombospondin in platelet aggregation. J Clin Invest. 1984;74(5):1764-72.

Acknowledgments
This study was supported by Örebro University Hospital Research Foundation and AFA Insurance.

Conclusion

Cat-S and TSP-1 levels are high during acute STEMI and this may contribute to new knowledge related to foregoing plaque rupture.

Elevated levels of Cat-S months after acute MI probably reflect the severity of the heart disease and may be important for prognosis.

Future studies with large patient groups may reveal the causality of Cat-S in myocardial infarction.

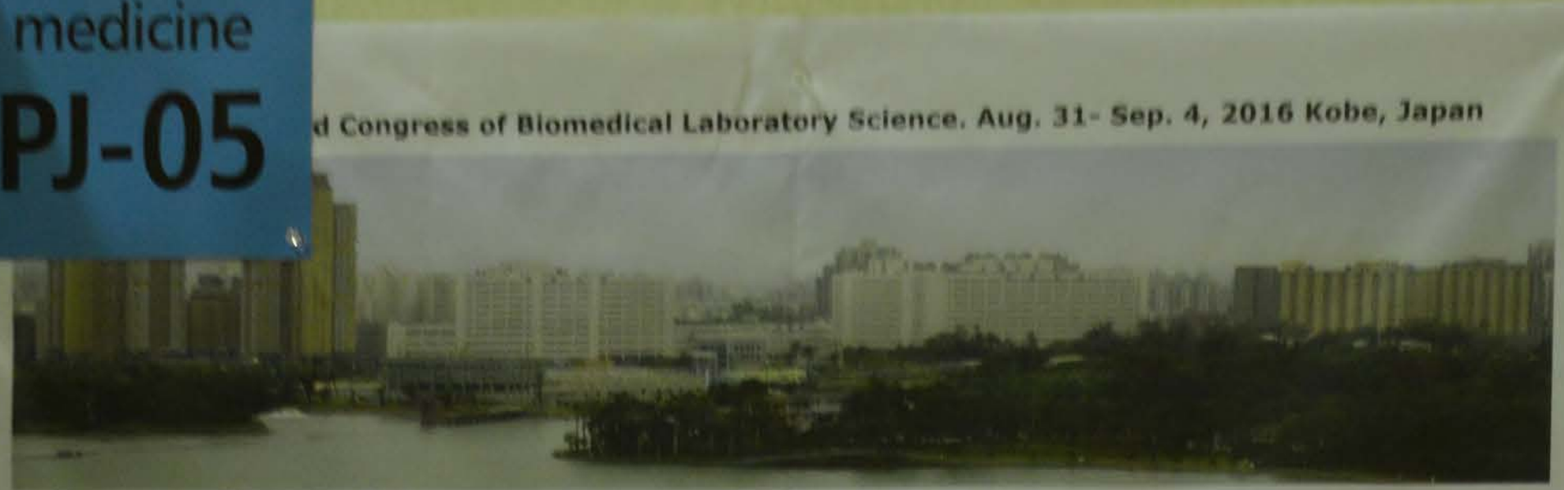
Contact - rahel.befekadu@regionorebrolan.se

ÖREBRO UNIVERSITY

Region Örebro County
Örebro University Hospital

Transfusion medicine

PJ-05



d Congress of Biomedical Laboratory Science. Aug. 31- Sep. 4, 2016 Kobe, Japan

Shortened waiting time of blood transfusion for out-patients by application QCC technique

Ko-Ming Hou , Chig-Yi Hsu, Hung-Mei Lee, Huey-Ling You, Hock-Liew Eng

Department of Laboratory Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of medicine, Taiwan.

Background:

Transfusion therapy is an important part of modern medical practice. For outpatients, the physical discomfort and prolonged waiting preparation time for transfusion therapy might cause the patients fall into a bad mood that might result in an increase of medical dispute. For continuous improvement of patient care and under the premise of safety in blood transfusion, with application QCC technique, we have tried to shorten the waiting time of blood transfusion for outpatients and aiming to achieve within two hours, beginning from recipient specimen collection for matching till receiving the blood units at the nurse station of outpatient transfusion.

Methods:

All related data of blood transfusion from all outpatients receiving blood transfusion from March 2014 to June 2014, were first collected. Using the QCC method, with the participation of nursing department and other related personels, the collected data were analyzed in accordance to pre-testing , testing and post-testing phases , including the time taken for blood specimen collection and transportation to blood bank for matching, time for testing process in blood bank, waiting time in transfusion room after receiving the blood. Different kinds of blood products were analyzed separately (Figure 1) to find out all possible factors those might cause prolonged waiting time. In the following one year (from July 2014 to June 2015), strategies of improvement were implemented and related countermeasures were then executed, aimed to shorten the waiting time for outpatient transfusion within 2 hours, 80% for non-filtered blood components (WB, PRBc, SFP, FFP, Cryo and, PLT), and 60% for filtered blood products (LPR, LPP).

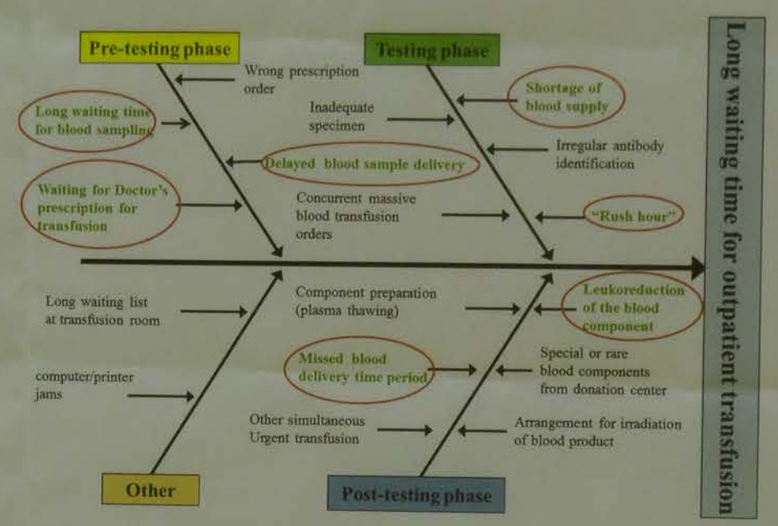


Figure 1: cause analysis - Fishbone Diagram.

Results:

As shown in Table 1, a total of seven main possible causes of prolonged waiting time were found after the initial data analysis. After thorough interdepartmental discussion, strategies of improvement were implemented and related countermeasures were then executed (Table 1).

Table 1: strategies and countermeasures are executed

	Responsible site	Causes	Strategies and countermeasures executed
Pre-testing phase	Blood sampling counter	Long waiting time for blood sample collection and transportation	Outpatients transfusion orders are processed immediately on receiving as cases from Emergency Department.
	Outpatient department	Prescription order decision after OPD inspection and the result of blood tests	As for oncology out-patients, blood transfusion order prescribed beforehand at the last OPD. Blood sampling for cross-matching can be sampled at the same time for other follow-up blood tests before the present OPD.
Testing phase	Blood bank	Extra-bench for outpatients transfusion	1.First priority processing of orders for outpatient as those from emergency department.
			2.Additional to on-duty, there are on-call staffs standby for large amount of specimens
		Shortage of blood supply	1.Always keep enough shelf storage of most common antigen negative blood units from Blood Donation Center.
			2.LIS/HIS computer programming to notify patients with specific allo- antibodies a week before their appointed visiting time at OPD and the matched units can be prepared and cultivated from Blood Donation Center.
Post-testing phase	Blood bank	Long processing time for Leukoreduction and thawing of plasma	Increase the shelf storage of Leukoreduced blood components and pre-thawed plasma
		Missing the time period of blood delivery to OPD transfusion room	Increased time monitor and earlier schedule for blood delivery as needed

Waiting time after execution of related countermeasures:

Pre-testing phase (from blood specimens collection till receiving in blood bank) :

For non-filtered blood products --- The average time was shortened from 77 minutes from to 27 minutes. For filtered blood products --- The average time was shortened from 79 minutes to 30 minutes.

Testing phase (Processing time at blood bank)

For non- filtered blood products --- The average time was increased from 58 minutes from to 70 minutes. For filtered blood products: The average time was increased from 83 minutes to 89 minutes.

Post-testing phase (From blood bank to transfusion) :

For non- filtered blood product --- The average time was shortened from 15 minutes from to 12 minutes. For filtered blood products: The average time was shortened from 32 minutes to 30 minute, (Figure 2, 3 and 4)

Aim achievement: percentage of overall waiting time within 2 hours:

For non-filtered blood products : 47.7 before and 71.8 after QCC
For filtered blood product: 18.1 before and 38.5% after QCC (Figure 4)

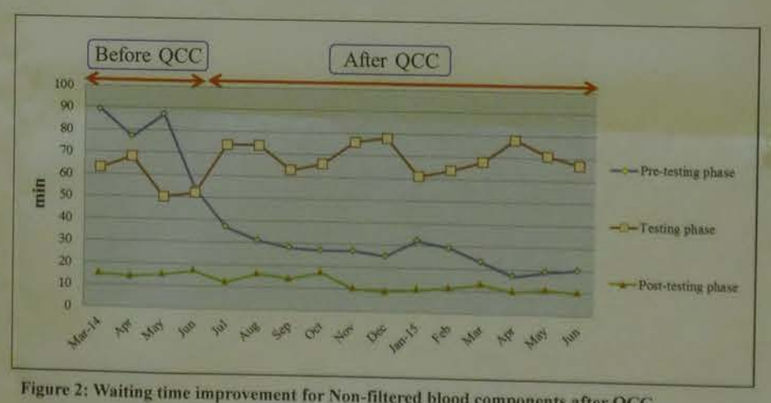


Figure 2: Waiting time improvement for Non-filtered blood components after QCC

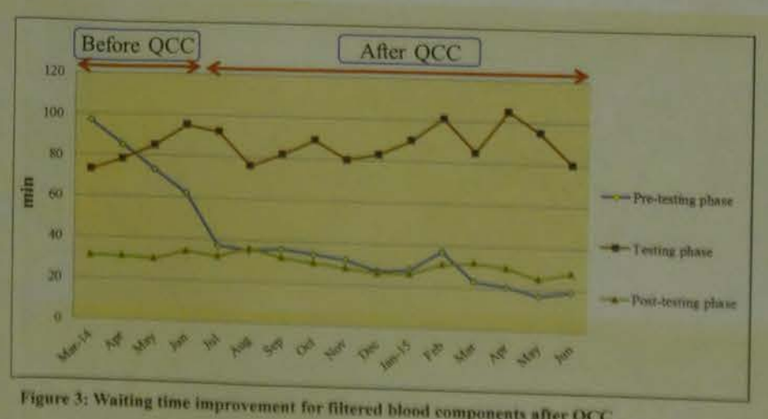


Figure 3: Waiting time improvement for filtered blood components after QCC

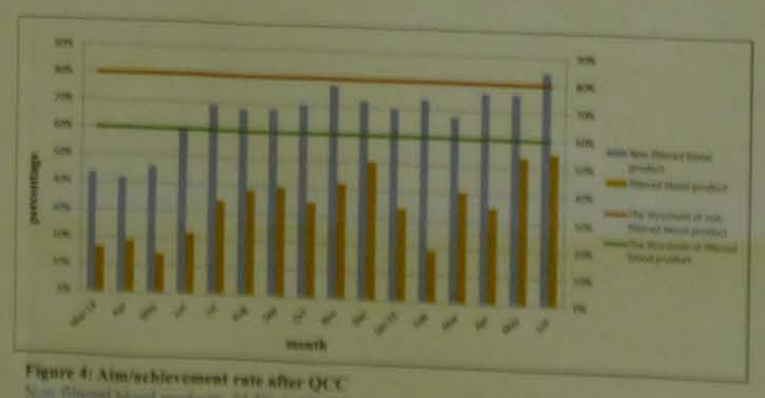


Figure 4: Aim/achievement rate after QCC
Non-filtered blood products: 74.6% (with an increase rate of 56.5%)
Filtered blood products: 48.7% (with an increase rate: 112.7%)

Conclusion:

QCC technique is really useful and can effectively shorten the pre-transfusion waiting time of outpatient patients. With inter-department cooperation and thorough discussion, the underlying causative factors of prolonged waiting time were explored and some strategies and countermeasures can be implemented to shorten the waiting time, greatly improve the quality of outpatient care in the field of blood transfusion.

C-2

Transfusion medicine

PJ-07

Efforts to test for specific infections (HBV, HCV, HIV) before-and-after blood transfusions

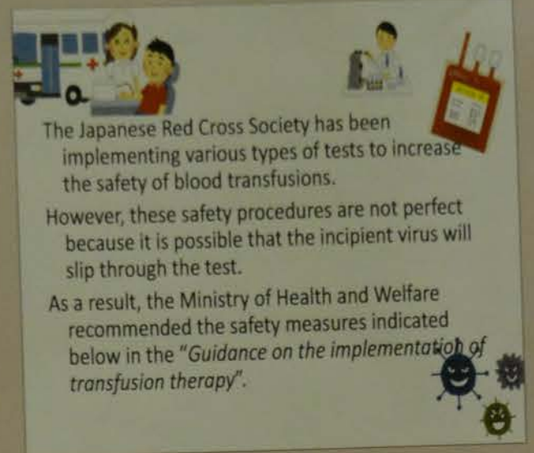
Mami Kikuchi[1], Naoko Saita[2], Mie Kasawaki[2], Suzuyo Katakawa[2], Mamoru Inoue[2], Yutaka Kobayashi[2]

1 Tokushima red cross hospital, Japan
2 Kyoto 2nd red cross hospital, Japan

INTRODUCTION

The functions of blood are indispensable to maintaining life.

Since blood functions can't be completely substituted in the present, it is in many cases impossible to administer medical treatment without transfusion therapy.

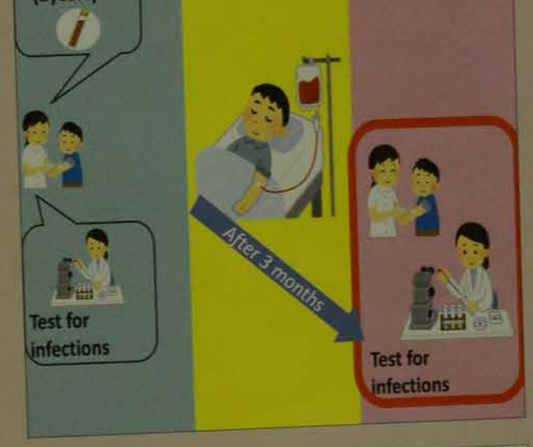


The Japanese Red Cross Society has been implementing various types of tests to increase the safety of blood transfusions.

However, these safety procedures are not perfect because it is possible that the incipient virus will slip through the test.

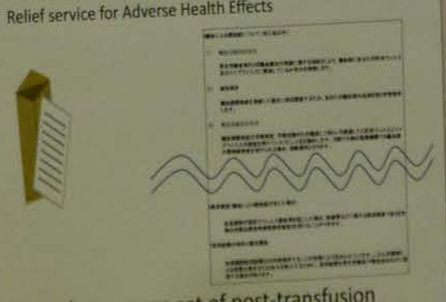
As a result, the Ministry of Health and Welfare recommended the safety measures indicated below in the "*Guidance on the implementation of transfusion therapy*".

"Guidance on the implementation of transfusion therapy"

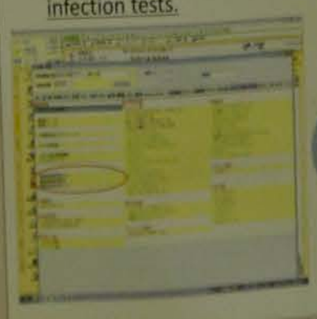


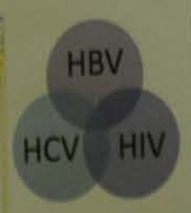
Our first approach

- Added new information to our consent forms.
  i. store serum
  ii. test for infections
  iii. Relief service for Adverse Health Effects



- Created a program set of post-transfusion infection tests.





Our second approach

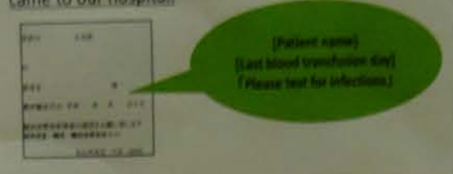
- Begin keeping the records from blood transfusions.

Date of transfusion	Patient ID	Patient name	Date of testing before transfusion	Date of storing serum	Date of testing after transfusion
2012.05.01	111111	A	2012.05.01	2012.05.01	
2012.05.02	222222	B	2012.04.30	2012.05.02	
2012.05.03	333333	C	2012.04.01	2012.04.01	2012.08.30
2012.05.03	444444	D	2012.05.03	2012.05.03	
2012.05.03	555555	E	2012.04.25	2012.04.25	

Problems & Examination

- Most patients didn't receive the tests.
- We guessed that we need to tell directly their doctors about the necessity of the infection tests.

We started to distribute a notice about test infections after blood transfusions to their doctors when they came to our hospital.



When do they come to our hospital?

We checked the next appointment of each patient.

It was so hard work...

To lessen work

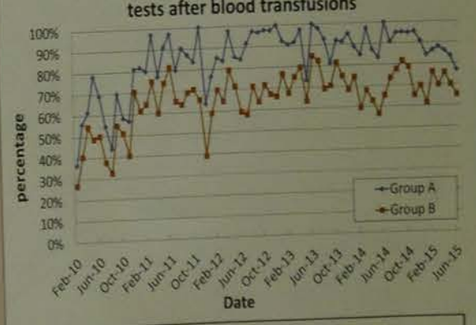
- We used a DWH* to make a list of outpatients.
- We searched for patient IDs that received blood transfusions on the list using a spreadsheet.

Patient ID	Doctor
123456	X
234567	Y
111111	Y
345678	Z
456678	Z
222222	X
765432	X
333333	Y
876543	Z

Patients that received transfusion

*DWH(Data Ware House):System that store data of electronic medical records.
We can use DWH to distill specific information from huge amount of data.

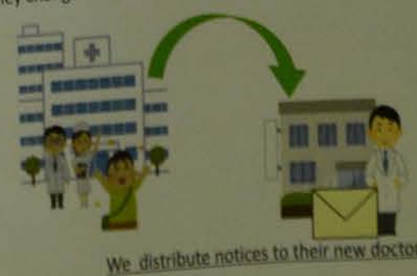
Percentage of patients that received infections tests after blood transfusions



- Group A : Patients that received infections tests (they came to our hospital after acute-phase treatment)
- Group B : Patients that received infections tests (they came to our hospital or were transferred to other hospital after acute-phase treatment)

We thought that we should check them after being transferred to other hospital, and the percentage of patients who receive the tests should increase.

If they change hospitals within three months



We distribute notices to their new doctors

We hope more patients will receive infections tests within six months.

There are two benefits in finding infections by blood transfusions as soon as possible.

- We can use a "Relief service for Adverse Health Effects" to treat and support the patients.
- We can prevent the spread off problems by eliminating virally-contaminated fresh frozen plasma(FFP) in the inventory.

*FFP components are held in inventory for period of six months and supplied to medical institutions following the removal of any blood that was suspected of being infected during this period.

plasma Cathepsin S and lin-1 in patients with acut on myocardial infarction

Christiansen[2], Anders Larsson[3], Magnus Grenegård[4]
e, Section for Transfusion medicine, Örebro University Hospital, Örebro, Sweden, pital, Örebro, Sweden, [3]Department of Medical Sciences, Academic Hospital, Uppsala, cine, School of Medical Sciences, Örebro University, Örebro, Sweden.

on of
been
s,
ume-
ivity of
natory
erosis.
in S
ion in
TEMI)
on (PCI)
g on
th closed
enous
3 months
ssay.

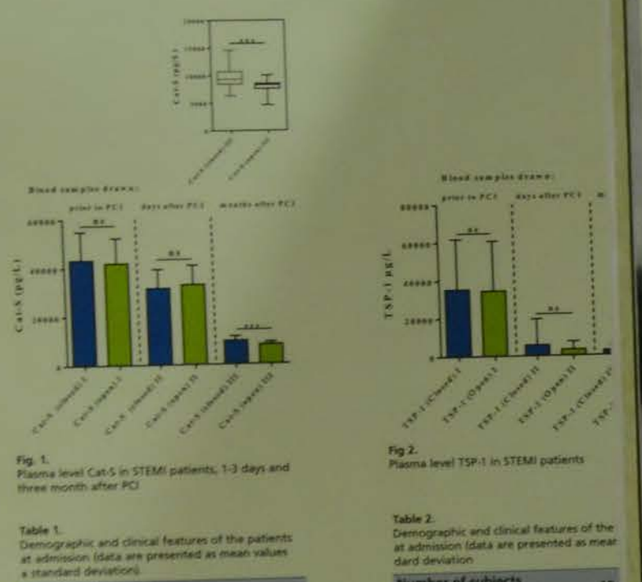


Fig. 1.
Plasma level Cat-S in STEMI patients, 1-3 days and three month after PCI

Fig 2.
Plasma level TSP-1 in STEMI patients

Table 1.
Demographic and clinical features of the patients at admission (data are presented as mean values a standard deviation).

Number of subjects closed blood vessel	90
Age, years	71[46-96]
Sex M/F	68/22
BMI	27.3[16.0-38.3]
Smoker %	62
Previous MI %	12
Diabetes mellitus %	17
Hyperlipidemia %	51
PCI(h)	0.11[90]
PPT	224
WBC	13.5
CK	82.8

Table 2.
Demographic and clinical features of the at admission (data are presented as mear dard deviation

Number of subjects open blood vessel	40
Age, years	68[43-1
Sex M/F	25/1
BMI	27.3[20.0
Smoker %	60
Previous MI %	20
Diabetes mellitus %	20
Hyperlipidemia %	40
PCI(h)	0.15[4
PPT	228
WBC	10.1
CK	83

Conclusion

Cat-S and TSP-1 levels are high during acute STEMI and this n
contribute to new knowledge related to foregoing plaque ru
Elevated levels of Cat-S months after acute MI probably refle
severity of the heart disease and may be important for progr
Future studies with large patient groups may reveal the caus
of Cat-S in myocardial infarction.

efekadu@regionorebrolan.se

Region Örebro County
Örebro University Hos

Transfusion medicine

PJ-08

Genotyping of Mi^a Blood Type in Eastern Taiwan

Yi-Lun Chen[1], Chu-Fen Chang[1], Chun-Hung Cho[2]
Department of Laboratory Medicine, Mennonite Christian Hospital[1]
Department of Hematology & oncology, Mennonite Christian Hospital[2]

BACKGROUND :

In Taiwan, Miltenberger type III (Mi.III; GP.Mur) is the most important blood type other than ABO blood types. GP.Mur has the highest frequency in Southeast Asia. In Taiwanese population, the occurrence is 2-7%. Anti-"Mia" is one of the most frequent red cell alloantibodies (1%) and can react with GP.Mur and other types of Miltenberger antigens. Anti-"Mi^a" could cause acute intravenous hemolytic transfusion reaction and is related to hydrops fetalis and hemolytic disease of the fetus and newborn (HDFN). As there is no commercial antiserum for detection of Mia related antigens, molecular methods could aid in typing of the GP.Mur.

METHODS :

Polyclonal anti-"Mi^a" serum was used to screen red cell samples with Mia related antigens. Whole blood samples of the positive individuals were collected for DNA extraction using QIAamp DNA Mini Kit (QIAgen GmbH, Germany). Genotyping was carried out using RBC-ReadyGene MNS kit (inno-train Diagnostik GmbH, Germany).

RESULTS :

72 samples produced positive reaction with anti-"Mi^a" serum. Genomic DNA samples of these individuals were extracted and tested with genotyping kit. All the 72 samples have GP.Mur gene. 29 samples with serological typing all have concordant results of genotyping of M, S, and s genes. By confirmation with genotyping, 8 (72.72%) in 11 positive samples typed with anti-N are false positive due to GP.Mur.

Serology	S	s	M	N	Mi^a
Genotyping	S	s	M	N	GP.Mur
A1	0	4+	2+	3+	2+s
	-	+	+	+	+
A2	0	4+	3+	4+	2+s
	-	+	+	-	+
A3	0	4+	3+	3+	2+s
	-	+	+	-	+
A6	0	4+	4+	4+	2+s
	-	+	+	-	+
A7	0	4+	3+	3+	2+s
	-	+	+	-	+
A8	0	4+	2+s	-	2+s
	-	+	+	-	+
A9	0	4+	2+s	3+	2+s
	-	+	+	+	+
A10	0	W+	+	+	+
	-	+	+	-	+
105	0	4+	1+w	4+	1+s
	-	+	+	+	+
117	0	4+	3+	4+	1+s
	-	+	+	-	+
121	0	4+	3+	2+	2+
	-	+	+	-	+

Table 1. Typing results of 11 anti-N reacting samples. The area marked in green dashed line indicates discrepancies.

CONCLUSION :

Results of serological typing and genotyping of GP.Mur are concordant, indicating molecular typing could assist in identification of blood types with limited source of antiserum. Furthermore, molecular typing could be used to confirm serological results, and samples with weak antigens, and to type patients who received transfusion recently. Most importantly, genotyping may confirm serological typing and discover possible false reaction like the cases of anti-N reacting with GP.Mur.

Reaction-No.	1	2	3	4	5	6	7
Internal control (size in bp)	434	434	434	434	434	434	1070
PCR Product (size in bp)	270	270	105	104	152	150	640
Specificity	[illegible]	N	S	[illegible]	Vw	Mg	[illegible]

Figure 1. Electrophoresis result of a M, s, and GP.Mur positive sample.

Mennonite Christian Hospital

PJ-11

AB0 Blood Group

D-2 D-2

Transfusion medicine PJ-10

Transfusion medicine PJ-11

The 32nd World Congress of Biomedical Laboratory Science

PREVALENCE OF THE ANTIBODIES OF THE NEW HISTO-BLOOD SYSTEM - FORS SYSTEM

Fernando Mendes[1], Carlos Jesus[1]; Camilla Hesse[2]; Clara Rocha[3]; Nádia Osório[1]; Ana Valado[1]; Armando Caseiro[1]; António Gabriel[1]
Paulo Teixeira[1] ;Lola Svensson[3], Wafa Abu Siba[4], Cristina Pereira[5], Jorge Tomaz[5]

[1]Biomedical Science Department of ESTeSC– Coimbra Health School, Polytechnic Institute of Coimbra, Coimbra, Portugal; [2]PhD, Biomedical Scientist, Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden; [3]Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Departmere Complementary Sciences, Coimbra, Coimbra, Portugal; , [4]Medical Laboratory Sciences Department – Al-Quds University, Abu Dis, Palestina, [5]Blood Bank Service – Coimbra Hospital and Universitary Center (CHUC), Coimbra, Portugal,

AIM

Define strategies for search anti-Forssman antibody and study the frequency of anti-Forssman antibody in a Portuguese blood donor population.

RESULTS

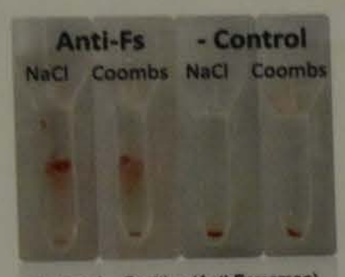


Figure 1 – Positive (Anti-Forssman) and negative (Apae plasma) control of Sheep RBC.

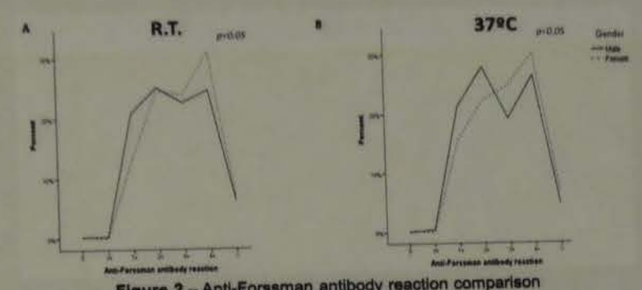


Figure 2 – Anti-Forssman antibody reaction comparison between genders (male – normal line; female – dashed line), at room temperature (A) and 37 °C (B). *P-value* <0,05.

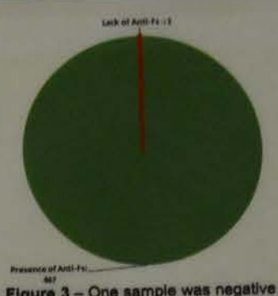
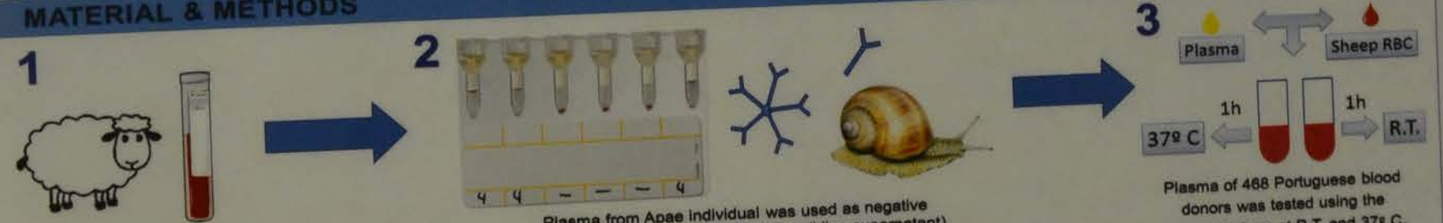


Figure 3 – One sample was negative for the screening of Anti-Fs (red) and 467 sample were positive (green).

DISCUSSION AND CONCLUSION

This results are very important to the investigation of this new blood system. We can conclude that the sheep RBC can be used for the screening of human anti-Forssman (Fig. 1). Anti-Forssman Ab exists in a higher concentration in females than in males (Fig. 2) The frequency of anti-Fs antibody is undoubtedly very high in this blood donor population. **In 468 Portuguese samples tested we found one without the presence of the anti-Forssman antibody** (Fig. 3). In the literature is referred that only three English families express Forssamn Antigen, without the anti-Fs, and it might be possible that **we found a Portuguese** person **without this antibody**. Our results support the low prevalence of this phenotype, and high prevalence of the antibody. As future perspectives we pretend to study the RBC of the donor that lacks the anti-Fs Ab and him family in order to clarify if they are FORS positive and which impact these Ab can have in medicine transfusion.

MATERIAL & METHODS



Sheep erythrocytes were washed 6 times and suspended at 0,8%.

Plasma from Apae individual was used as negative control and anti-Fs (M1/22.25.8HL cell line supernatant) and lectin *Helix* Pomatia was used as positive control.

Plasma of 468 Portuguese blood donors was tested using the tube technique at R.T. and 37º C.

INTRODUCTION

Many living beings express specific antigens (Ag) on RBC, such as AB0 and Forssman, but this expression is different between them. Forssman Ag has been seen as an animal Ag since early 1900s, but some publications claim an involvement in human tumors. Fs-antigen may also act as receptors for certain pathogens, emerging naturally antibodies. Anti-Fs Ab maybe a possible barrier for transfusions and trans-plantation.

In 1987, three unrelated English families were reported with an ABO subgroup called A_{pae}.[1]

The A_{pae} positive family members showed a divergent antibody and lectin reaction pattern of the RBC. The *lectin Helix Pomatia* (strong reaction), polyclonal Ab anti-A (weak response) and monoclonal Ab anti-A (no reaction), suggesting a controversy.[1]

Svensson L. *et al* (Institute of Biomedicine, GU and Dept. of Laboratory medicine, LU) found evidences that resulted in the conclusion that the subgroup A_{pae} should be abolished and instead a new histo-blood group: the FORS blood group system (accepted by ISBT 2012 as blood group system 31) was established.[2]

In this frequency study of human anti-Forssman Ab. KODE™ technology was used. KODE™ technology is a method that can modify various biosurfaces with bioactive products, within a couple of hours using a simple protocol, without affecting cell viability and functionality.[3]

REFERENCES

1. Stamps R, Sokoll RJ, Leach M, et al. A new variant of blood group A: Apae. *Transfusion* 1987;27(4):315-318
2. Svensson L, Hult AK, Stamps R, et al. Forssman expression on human erythrocytes: biochemical and genetic evidence of a new histo-blood group system. *Blood*. 2013;121(8):1459–68.
3. Frame T, Carroll T, Korchagina E, Bovin N, Henry S. Synthetic glycolipid modification of red blood cell membranes. Transfusion. 2007;47(5):876–82.

Transfusion medicine

PJ-11

PJ-11

AB0 Blood Group, Secretor Status effect fungal Infections in Diabetes Mellitus

Cláudia Faria[1*]; Fabiana Ribeiro[1*], Amélia Pereira[1], Ana Borges[1], Ana Menezes[1], Ana Valado[1], Armando Caseiro[1], Nádia Osório[1], Clara Rocha[2,4], Júlio Loureiro[3], Maria Larcher[3],Maria Loureiro[3], Regina Rigueiro[3], António Gabriel[3] and Fernando Mendes[1,3,4]

*Both authors had the same contribution for the paper.

1. Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Department Biomedical Laboratory Sciences, Coimbra, Portugal; 2. Clinical Pathology Laboratory, Hospital District of Figueira da Foz E.P.E, Figueira da Foz, Portugal; 3. Polytechnic Institute of Coimbra, ESTESC – Coimbra Health School, Department Complementary Sciences, Coimbra, Portugal; 4. Institute for Systems Engineering and Computers at

Introduction

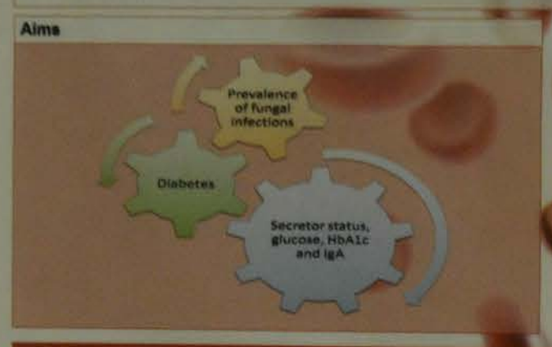
Diabetes Mellitus (DM) is a common disease and the incidence is increasing. DM is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. This varies from autoimmune destruction of pancreas β-cells with consequent insulin deficiency (Diabetes type 1), to abnormalities that result in resistance to insulin action and an inadequate compensatory insulin secretor response (Diabetes type 2) [1,2].

These patients can have a high morbidity and mortality with consequent quality of life decrease. Uncontrolled diabetes predisposes to a variety of superficial and systemic infections [3,4]. One of the possible causes of this increased prevalence of infections in DM patients is that some microorganisms become more virulent in high glucose environment. Another possible mechanism is the increased adherence of microorganisms to diabetic when compared with non-diabetic cells [5].

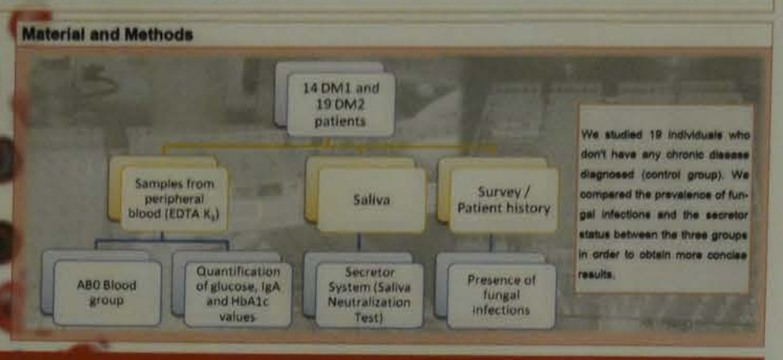
Several studies have shown an association between the genetically determined inability to secrete ABH antigen (Ag) and the susceptibility to certain infections. It is thought that non-secreting diabetic patients are more prone to the occurrence of fungal infections [6,7]. The presence of the Ag's in saliva may form a barrier against external pathogens in general and pollutants. Therefore, it is speculated that the expression of ABH Ag in saliva might interfere with the binding locals of the microorganisms to their receptors, and then intervene in the development and prevention of oral infectious diseases [8,9].

The adhesion of the microorganism to mucosal, not only depends of the secretor status, but can also depend of the Immunoglobulin A (IgA). The main biological function of IgA is to protect against invasion of microorganisms such as bacteria and viruses at mucosal surfaces, inhibiting the adhesion mechanism of these to epithelial cells [10]. Low levels of IgA can leads to atrophy on the secretory mucosa which can decreases the capacity of secretion of IgA that leads to alterations in the local immunity, resulting in a considerable increase in mucosal infections [11].

Aims



Material and Methods



Results

Table 1: Characterization of DM groups and control sample in with to the gender and age

Fungal Infection		Gender		Age (years)		
		Female	Male	Median	Minimum	Maximum
DM I	Without	50%	50%	42	30	59
	With	62.5%	37.5%	53	40	65
DM II	Without	50%	50%	65	39	86
	With	80%	20%	62	52	70
Control sample		78.9%	21.1%	80	30	90

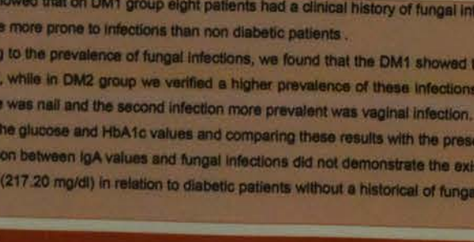
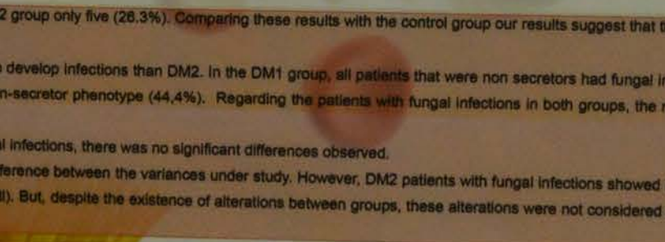


Table 2: Characterization of age of diagnosis and type of fungal infections in DM patients

Fungal Infection		Age of diagnosis (years)			Type of infection				
		Median	Minimum	Maximum	Nail	UTI	Skin	Vaginal	Eye
DM I	Without	28	2	52	0%	0%	0%	0%	0%
	With	17	8	50	50%	12.5%	12.5%	25%	0%
DM II	Without	15	2	34	0%	0%	0%	0%	0%
	With	10	4	44	60%	0%	0%	20%	20%



Discussion

Our results showed that on DM1 group eight patients had a clinical history of fungal infections (57.1%) and DM2 group only five (26.3%). Comparing these results with the control group our results suggest that these individuals are more prone to infections than non diabetic patients .

Still, attending to the prevalence of fungal infections, we found that the DM1 showed to be more susceptible to develop infections than DM2. In the DM1 group, all patients that were non secretors had fungal infections (28.6%), while in DM2 group we verified a higher prevalence of these infections in diabetics with the non-secretor phenotype (44,4%). Regarding the patients with fungal infections in both groups, the most prevalent type was nail and the second infection more prevalent was vaginal infection.

Relatively to the glucose and HbA1c values and comparing these results with the presence or absence of fungal infections, there was no significant differences observed.

The comparison between IgA values and fungal infections did not demonstrate the existence of a significant difference between the variances under study. However, DM2 patients with fungal infections showed lower IgA values (217.20 mg/dl) in relation to diabetic patients without a historical of fungal infections (305.82 mg/dl). But, despite the existence of alterations between groups, these alterations were not considered sta-

Conclusion

Diabetics are more susceptible to infections than healthy individuals. It's reported and we also confirmed in our study, that patients that are non-secretors can develop infections more easily than diabetics that are secretors. Therefore it is important to monitor and control these individuals in respect to glucose and IgA values for future prevention of fungal infections in order to contribute to the prevention of co-morbidities, despite not having significant results. These results may be due to our sample population, and for best results on these variables would be better to study a broader range of patients.

We believe that this type of research is important on diabetic patients, since DM is a high incident and prevalent disease. These findings can allow define profiles in DM patients more prone to fungal infections, and so, improve the quality of life and early preventive measures to be implemented.

Bibliography

1. Zimet, P., Winter, J. F., Charbonnier, B., Eschwège, E., Rosenwaks, P. J., Rosen, P. F., ... Sassolas, J. P. (2002). Prevalence and classification of diabetes mellitus. Diabetes Care, 25, 1903-1907. doi:10.2337/dc25-1903
2. Scott, R., & Hanif, P. D. (2010). Diagnosis and classification of diabetes mellitus in women pancreatic. World journal of ..., 7215-47. doi:10.5714/wjg.v16.i6.7215
3. Bruce, R. D., Ramaiyenkatha, L. K., & Bhavnani, ... (2010). Diabetes mellitus as a predisposing factor to oral candidosis. ... journal of the British Dental ..., 655-9. doi:10.1111/j.1464-5491.2009.01791.x
4. Akram, S. M., Chandrashekar, U., Bhowmick, K., & Kumar, ... (2011). Clinical profile of urinary tract infections in diabetic patients. The Australasian medical journal, 7(1), 28-32. doi:10.4066/AMJ.2014.1908
5. Tiwari, A., Dwivedi, I., Bhoot, R., & Dwivedi, ... (2011). Candida albicans isolates from diabetic patients and non-diabetic subjects: comparison of virulence factors and biofilm forming ability. The Australasian medical journal, 7(1), 28-32. doi:10.4066/AMJ.2014.1908
6. Shin, K.-S., Chung, S.-C., Kim, Y.-K., Lee, S.-H., & Kim, H.-W. (2005). The relationship between antifungal susceptibility and the biofilm formation ability of clinical isolates of Candida. Microbiology and immunology, 49, 519-528.
7. Au, P. Z., Blackwell, D. C., Mackenzie, D. A., Weir, D. M., Blair, P. K., Greening, G. S., ... Gerry, S. F. (1985). Diabetic patients' and normal subjects' antibody responses to Candida albicans antigens in saliva. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 59(1), 30-33. doi:10.1016/0278-2391(85)90140-4
8. Shin, K.-S., Chung, S.-C., Kim, Y.-K., Lee, S.-H., & Kim, H.-W. (2005). The relationship between antifungal susceptibility and the biofilm formation ability of clinical isolates of Candida. Microbiology and immunology, 49, 519-528.
9. Au, P. Z., Blackwell, D. C., Mackenzie, D. A., Weir, D. M., Blair, P. K., Greening, G. S., ... Gerry, S. F. (1985). Diabetic patients' and normal subjects' antibody responses to Candida albicans antigens in saliva. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 59(1), 30-33. doi:10.1016/0278-2391(85)90140-4
10. Bradwell, A., P. A., James, P., Watt, R., Edlin, A., Patrick, A., ... Clarke, S. (1984). Blood group, secretor status and oral candidosis in patients with diabetes mellitus. Journal of medical microbiology, 18(1), 43-8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6321871
11. Samaranayake, W., Lamey, J., Samaranayake, P., MacFarlane, W., Fisher, W., Memory, M., & MacGrath, D. (1985). The relationship between oral candidosis, secretor status and in-vitro adherence of Candida albicans to polystyrene. Microbiology (Reading, England), 148 (Pt 8, 2109-13. doi:10.1099/00221287-148-8-2109
12. Bel Imhof, R., Barsoumian, G., Reynolds, P., Mongrain, M. D., Rumble, G., & Pursell, J. (2002). Effect of salivary secretory IgA on the adhesion of Candida albicans to polystyrene. APMIS, 110(10), 710-716. doi:10.1111/j.1600-0463.2011.02780.x
13. 4 Fonya, Marcel, D., Fagundes, C., Rudge, M., Calderon, I., & Almeida-Pompei, A. (2011). Secretory IgA-Fc receptor interaction modulating phagocytosis and microbicidal activity by phagocytes in human omentum of diabetes. APMIS, 119(10), 710-716. doi:10.1111/j.1600-0463.2011.02780.x

Contact

Fernando Mendes:

fjmendes@estescoimbra.pt

Transfusion medicine

PJ-12

PJ-12

COIMBRA HEALTH SCHOOL

Monitoring Feto-Maternal Incompatibility in a Clinical Laboratory from Barcelona

Fernando Mendes[1,2,3,4*], Mari Raya Hinojosa[1,6*], John Quigley[6*], Lopez, M[5], Carrasco.P[5], Ana Valado[1], Nádia Osório[1], Armando Caseiro[1], António Gabriel[1]

*Both authors had the same contribution for the paper

1 - Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Department Biomedical Laboratory Sciences, Coimbra, Portugal;
2 - Biophysics and Biomathematics Institute, IBILI-Faculty of Medicine, University of Coimbra, Portugal;
3 - CIMAGO, FMUC-Faculty of Medicine, University of Coimbra, Portugal;
4 - CNC.IBILI, Universidade de Coimbra, Portugal;
5 - Departament of Transfusions Parc Salut Mar, Barcelona;
6 - Blood Transfusion Laboratory, Department of Pathology and Laboratory Medicine, The National Maternity Hospital, Dublin 2, Ireland.

Introduction

Haemolytic disease of the fetus and newborn (HDFN) continues to be a complication of early life in the newborn. Prophylaxis by administration of anti-D immunoglobulin IgG to Rh D-negative pregnant women at 28 weeks gestation and postpartum is standard practice and the study of irregular antibodies of all pregnant women has contributed to the detection of other antibodies capable of inducing HDFN .

Aims

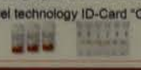
To identify all the non-sensitized Rh negative women should benefit with prophylactic administration of D IgG anti-D gamma globulin as well as the early detection of maternal erythrocyte alloimmunization to identify women at risk of inducing a HDFN. Since we haven't found any recent data about it, with this study we aim to analyze and clarify the prevalence of maternal alloimmunization and HDFN in Catalonia and Spain

Material and Methods

1 This was a retrospective study **7087** samples from pregnant women and **6808** neonatal samples during a two year period

2 We reviewed the positive results of **Indirect Antiglobulin Test** and **Direct Antiglobulin Test**.

3 Antibody screening was performed using plasma from pregnant women and testing against 3-cell screening cells - ID DiaCell I-II-III using gel technology ID-Card "Coombs Anti-IgG".



4 Antibody identification were performed using an 11 cell ID-panel in IAT and enzyme.

Results

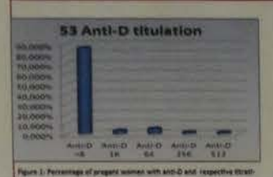


Figure 1: Percentage of pregant women with anti-D and respective titration.

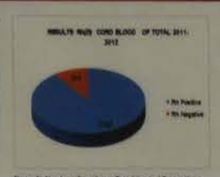


Figure 3: Number of newborns D positive and D negative.

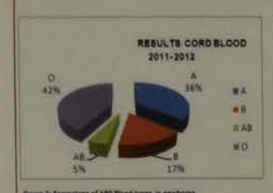


Figure 2: Percentage of ABO Blood types in newborns.

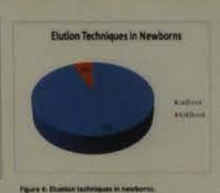


Figure 4: Elution techniques in newborns.

Table I: Distribution of specificities of alloimmunized mothers with multiples antibodies.

Antibody	Number of ca-	%
D	53	58
E	9	10
Kell	7	8
M	7	8
Lu^a	1	.
Kp^b	1	1
Le^a	1	1
D + E	4	4
D + Kell	1	1
D + C^w	1	1
D + c + G	1	1
E + C^w	1	1
C + e	1	1
Unspecific	3	3

Table II: Antibodies and respective impact on newborns.

	Titration Mothers		Impact on Newborns			
ANTIBODIES	1 - 128	>128	DAT -	DAT +	Acid Elution	HDFN
anti-D	51	2	33	3	3	1
anti-E	9	0	3	0	0	0
other Rh	9	0	3	1	1	0
anti- Kell	5	2	5	0	1	0
anti-M	7	0	3	0	0	0
anti-Kp^a	1	0	1	0	0	0
anti-Lu^a	1	0	1	0	0	0
anti-Le^a	0	0	0	0	0	na
nonspecific	0	0	1	0	0	na

Discussion

HDFN is a disease with a low prevalence (1 in 1500) in Spain, similar to what was previously reported in the Spanish literature of 1 per 1000 live births. One of HDFN cases detected by anti-D was from an emigrant. This case debuted with high titers (512) before the 28th week of gestation and corresponds to isoimmunization by the Rh D. In this study, the most frequently detected alloantibody laboratory was anti-D (58.24%). These antibodies have greatest clinical impact, with the peculiarity that 98% of cases were low titers of anti-D, associated with the history of administration of IgG anti-D prophylaxis to all pregnant D negative at 28 weeks of gestation. Between 2011-2012 studies we also detected positive studies regarding specificity other than D Ag. The most important are, apart from D, the antigens C, c, E, e, Kell, Duffy and Kidd Ag also have clinical significance. The second Ab in frequency was anti-E (9.9%) with low titers (<2) was pregnant D positive. The third most frequently detected Ab was anti-Kell (7.7%); pregnant women were D positive with initial titers 64 to 1024.

The acid elution test clarified the cause of hyperbilirubinemia, eluting with positive anti-D+C with anti-D (1/64) ant anti-C (1/1) titles, excluding anti-G. Regarding the Rh group of infants, the proportion of Rh positive fetuses born to D negative mothers negative is greater than what is described: in the Caucasian population it has been reported that the frequency of fetal D negative is 40 % of cases, while in the study we found only 25% of D negative fetus. This difference can be attributed to the variable gene frequency of D in our study population data show a high amount of mothers from Eastern, African and Central American origin. Regarding AB0 incompatibility, a small proportion of newborns was detected with positive DAT (3.19%) among 3.128 births.

Conclusion

⇒ We can conclude with our study that HDFN is detectable in a stable frequency, with 1 in 1500.

⇒ While we found a single case of alloimmune anti-D, other alloimmune Ab detected in maternal plasma corresponded to antigens belonging to the Rh system. The rest of anti-D Ab detected in our study are passive Ab, IgG remainders of the administration of anti-D prophylaxis.

⇒ Most infants with a positive DAT were AB0 incompatible.

⇒ Regarding the secondary objective, the proportion of mothers D negative who had children with D negative is slightly lower compared to that reported in the literature, which is about 40%.

⇒ We conclude that the initiative of screening for fetal RHD genotype in maternal plasma in all pregnant D negative women, to prevent the administration of IgG anti-D prophylaxis at 28 weeks if the fetus is also D negative, could be more cost-effective in populations with higher frequency of D negative fetal groups. Otherwise, routine scheduled antiglobulin injection for non-sensitized Rh negative mother with fetus which had Rh positive or unknown father is more reasonable.

Contact - Fernando Mendes: fmendes@estescoimbra.pt

PJ-13

Human platelet lysate: a source of animal serum-free growth factors for clinical grade BM-MSCs ex-vivo expansion

L.Piccini[1], P.Pavan[1], V.Becherucci[1], V.Gori[1], S.Bisin[1], V.Cunial[1], B.Bindi[1], R.Ceccantini[1], F.Gentile[1], C.Finocchi[2], F.Brugnolo[1], S.Ermini[1], F.Bambi[1]

[1]SOD Immunohaematology and Trasfusional medicine, Cell Factory Meyer, AOU Meyer, Florence, Italy
[2]Biochemical and Microbiological Laboratory, AOU Meyer, Florence, Italy

Introduction

Mesenchymal stromal cells isolated from human bone marrow (BM-MSCs) are emerging as hopeful candidates for cell-based therapy of numerous diseases. BM-MSCs as advanced therapy products must satisfy all the requirements for human use of medicinal products and so, the BM-MSCs manufacturing for clinical use should comply with the principle of Good Manufacturing Practices (GMP). BM-MSCs have been historically cultured in basal medium with the addition of fetal bovine serum (FBS) as a source of growth factors, raising concerns for health due to the possible diffusion of diseases. FBS has several disadvantages that make it inadequate for clinical use: high lot-to-lot variability, risk of contamination with infectious agents and potential for anaphylactic reactions. Thus, FBS is rated critically by the European Medicines Agency and should be avoided for clinical applications. Xeno-free supplements, particularly human platelet lysate (HPL), have been developed for clinical applications. HPL contains all factors platelets are composed of, and these can be easily derived by mechanical disruption of platelet concentrates via freezing and thawing. Subsequent centrifugation separates the platelet debris from the supernatant containing all bioactive platelet factors. The whole of growth-promoting factors are being established as a safe and efficient BM-MSCs culture supplement for robust BM-MSCs cultivation. The large-scale production of well-characterized media supplements is essential to maintain the cellular qualities required for the intended clinical application and minimizing risks of adverse events.

Aim of the Study

Generally BM-MSCSs expansion protocols use culture media supplemented with FBS. Due to the high risk of contaminations (virus positivity reported to be as high as 20–50%), FBS is critically rated by the European Medicines Agency. Because BM-MSCs internalize xenogenic proteins at high amounts, there is an additional risk of allergic reactions. FBS immunogenicity has already been demonstrated to compromise the therapeutic success. In view of these considerations xenogenic-free culture conditions appear desirable.

Platelets and their derivatives, in particular HPL, have already been considered as drugs and therefore produced following registered procedures, offer no doubt important advantages as potential substitutes for FBS. However, it has become necessary to standardize the process of production, and to investigate and understand the impact that all the components of the HPL would have on the BM-MSCs. This understanding would allow for the choosing and use of cells of known characteristics based on their final clinical purpose. Based on these considerations, we have designed a production process to obtaining HPL and we have

Materials and Methods

In our laboratory we developed two different methods to obtain HPL, both using buffy coats (BCs) as starting material. BCs were obtained by centrifugation of whole blood donations. In the first method a variable number of BCs (from 4 to 7) were pooled and resuspended in a volume of AB plasma to obtain a range of concentration of

1,5-2,4 x 10^6 plt/µl. BCs were used 24-48 hours after the withdrawal. Platelet were separated from BC by using an automated system. The product was frozen at -80°C then thawed at 37°C and centrifuged to remove platelet membranes. HPL was finally collected, aliquoted in bags (25ml) and cryopreserved at -80°C until use.

With experience, and basing our methods on literature, we tailored the procedure in order to standardized it, and to obtain a greater number of HPL's aliquots. In fact, in the second method BCs were processed within 24 hours from the withdrawal. HPL was produced using pools of 5 BCs; 8 pools (each one obtained from 5 BCs) were then pooled, washed and resuspended in AB plasma to obtain a range of concentration of 1,5-2,4 x 10^6 plt/µl. Suspension was frozen and thawed for three consecutive cycles and treated as described above.

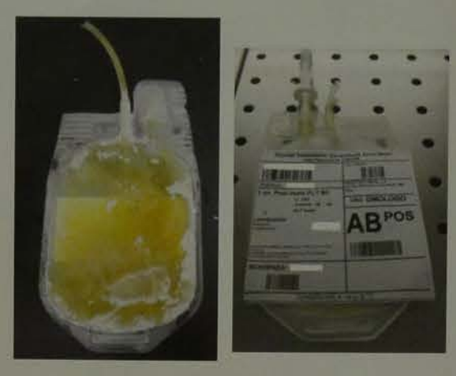
The effects of these HLPs produced following these guidelines were evaluated on 6 BM-MSCs cultures, at the Cell Factory Meyer. All batches were monitored for cell proliferation, morphology, immunophenotype, differentiative capacity and immunomodulatory activity.

Results

TAB. 1: batches obtained with first method

HPL BATCH N°	NUMBER OF BCs ASSEMBLED	PLATELET CONCENTRATION	NUMBER OF ALIQUOTS OBTAINED
1	5	1,54 x 10^6/ µl	5
2	5	1,58 x 10^6/ µl	4
3	7	1,98 x 10^6/ µl	4
4	7	1,80 x 10^6/ µl	4
5	6	1,60 x 10^6/ µl	6
6	5	1,90 x 10^6/ µl	4
7	5	1,70 x 10^6/ µl	5
8	6	1,60 x 10^6/ µl	2
9	4	1,70 x 10^6/ µl	2
10	5	2,08 x 10^6/ µl	3
11	7	1,88 x 10^6/ µl	4

With the first method we obtained 11 batches of HPL. The number of BCs used for each HPL is variable from 4 to 7, the platelets concentration was between 1.54 and 2.08x10^6/µl. From each batch of HPL we obtained from 2 to 6 aliquots of 25ml.



TAB. 2a: batches obtained with second method

HPL BATCH N°	POOL OF BCs	N° BCs ASSEMBLED	PLATELET CONCENTRATION
LP 1	1	5	1,92 x 10^6/ µl
	2	5	2,23 x 10^6/ µl
	3	5	2,27 x 10^6/ µl
	4	5	1,83 x 10^6/ µl
	5	5	1,89 x 10^6/ µl
	6	5	2,19 x 10^6/ µl
	7	5	1,82 x 10^6/ µl
	8	5	2,06 x 10^6/ µl
LP 2	1	5	1,92 x 10^6/ µl
	2	5	1,90 x 10^6/ µl
	3	5	1,68 x 10^6/ µl
	4	5	1,75 x 10^6/ µl
	5	5	1,78 x 10^6/ µl
	6	5	1,74 x 10^6/ µl
	7	5	2,10 x 10^6/ µl
	8	5	2,20 x 10^6/ µl

HPL BATCH N°	POOL OF BCs	N° BCs ASSEMBLED	PLATELET CONCENTRATION
LP 3	1	5	2,20 x 10^6/ µl
	2	5	2,50 x 10^6/ µl
	3	5	2,40 x 10^6/ µl
	4	5	2,30 x 10^6/ µl
	5	5	2,40 x 10^6/ µl
	6	5	2,10 x 10^6/ µl
	7	5	2,30 x 10^6/ µl
	8	5	2,00 x 10^6/ µl
LP 4	1	5	2,10 x 10^6/ µl
	2	5	2,00 x 10^6/ µl
	3	5	2,80 x 10^6/ µl
	4	5	2,60 x 10^6/ µl
	5	5	2,30 x 10^6/ µl
	6	5	2,10 x 10^6/ µl
	7	5	2,30 x 10^6/ µl
	8	5	2,30 x 10^6/ µl

With the second method we obtained 4 batches (LP1, LP2, LP3, LP4) of HPL.

TAB. 2b: batches obtained with second method

HPL BATCH N°	TOTAL N° OF BCs ASSEMBLED	PLATELET CONCENTRATION	N° ALIQUOTS OBTAINED
LP 1	40	1,89 x 10^6/ µl	25
LP 2	40	2,00 x 10^6/ µl	25
LP 3	40	2,27 x 10^6/ µl	21
LP 4	40	2,26 x 10^6/ µl	24

With the second method we obtained 4 batches of HPL. This method is more standardized than the first one: all the batchs were made using a total number of 40 BCs. The amount of aliquots we got was from 21 to 25 (each of 25ml).

FIG. 1: HPL induces morphologic changes on BM-MSC

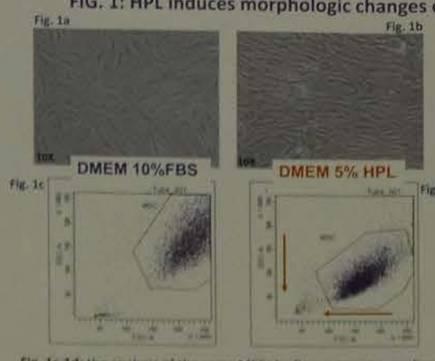
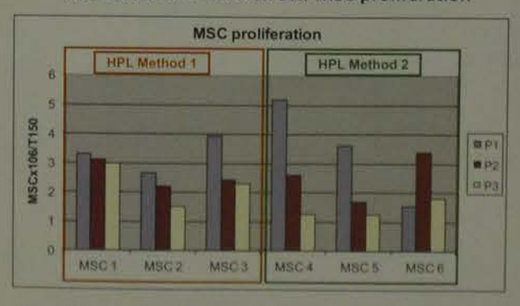


Fig. 1a-1b: morphology of MCSs cultured in FBS additionated medium and HPL additioned medium. When observed by inverted microscope (10X) MCSs cultivated in presence of HPL appear smaller, more refractive and less attached to plastic support than MCSs cultivated with FBS.

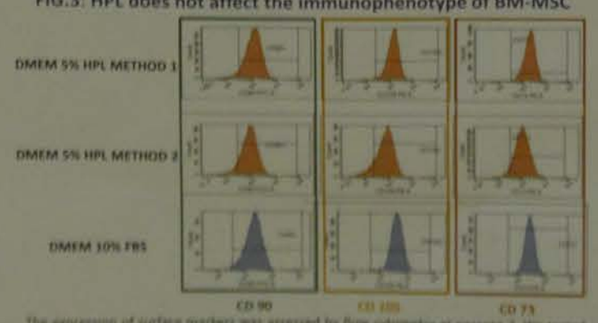
Fig. 1c-1d: the analysis of the same MSCs by flow cytometry confirms the features observed by microscope. SSC and FSC parameters reduction in fig. 1d indicate that MSCs with HPL are smaller and less complexes than MSCs cultivated with FBS.

FIG. 2: effects of HPL on BM-MSC proliferation



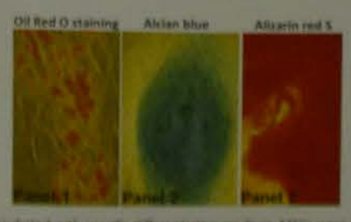
Valutation of HPL's effect on proliferation in 6 different batches of MSCs.
Cells were cultivated respectively:
- MSC 1 - MSC 3: DMEM additionated with 5% of HLP obtained with method 1
- MSC 4 - MSC 6: DMEM additionated with 5% of HLP obtained with method 2

FIG.3: HPL does not affect the immunophenotype of BM-MSC



The expression of surface markers was assessed by flow cytometry at passage 2. We tested the presence of the ISCT's characteristic markers, as CD90, CD105 and CD73. In this representative figure we compare BM-MSC cultivated in medium additionated with:
- 5% of HPL Method 1
- 5% of HPL Method 2
- 10% of FBS

FIG.4: HPL does not affect the differentiative potential of BM-MSCs



When incubated with specific differentiative medium, MSCs were able to differentiate in vitro towards fat, cartilage and bone lineages, as demonstrated by specific stainings (in panels 1, 2, 3 respectively).

Concluding Remarks

- The use of buffy coats as a source to obtaine HPL has many advantages compared with the standard method of platelet apheresis. The method to get HPL from BCs has a simple acquisition from the professional operator and moreover it resulted more cost affective.
- **Comparison between HPL obtained with Method 1 and Method 2:**
  - The second method is aimed at a process standardization. In fact the development of HPL batches from multiple sources (from 40 different donors instead of 4 or 7 donors), makes HPL a virtually standardized medium supplement in both a growth factor concentration and in inter-donor variability.
  - BCs processed within 24 hours from the withdrawal, instead 24-48 hours, reduce the presence of cytokines released from lymphocytes.
  - 3 cycles of freezing/thawing allow a greater release of growth factors from the platelets.
  - A greater number of aliquots permit to keep in culture a whole batch of BM-MSCs without changing batch of HPL.
- **HPL effects on BM-MSCs cultures:**
  - A substantial quantity of laboratory data on the preparation modalities and on the characterization of HPL have been produced, suggesting that BM-MSCs expanded in HPL grow faster when compared with BM-MSCs expanded in FBS-enriched media. The use of HPL obtained from both methods allows to collect a sufficient number of cells necessary for cell therapies.
  - All batches of BM-MSCs were monitored on cell proliferation, morphology, immunophenotype, differentiative capacity and immunomodulatory activity. In all cases, the HLPs stimulated cell proliferation, and no alterations or modification of phenotype, and functional qualities (data not shown) were observed.
- This study shows that HPL is a safe and efficient supplement for robust BM-MSCs culture. It offers certain advantages compared with FBS, especially in terms of cell growth. Moreover, it represents a good GMP-compliant alternative to animal serum for BM-MSCs clinical production.

Transfusion medicine

PJ-15

Effectiveness of Interventions to Reduce Blood Components Wastage: An experience from Taiwan

Li-Chuan Wang[1], Ya-Wen Tsai[1], Li-Ching Wu[1], Yu-Hsuan Yang[1], Hsin-Yin Chou[1], Jian-Cheng Lee[2]

[1]Department of Pathology, Chi Mei Medical Center, Tainan, Taiwan
[2]Department of Pathology, Chi Mei Medical Center, Chiali, Taiwan

Introduction

Blood transfusion is a life-saving medical procedure and blood products are a precious resource from voluntary donors. Health professionals should be responsible for ensuring sufficiency and quality of the blood transfusion. Therefore, this study investigated the causes of blood components wastage and developed strategic interventions for unnecessary wastage.

Materials and Methods

The study was conducted in a 1,300-bed medical center that issued 76,461units blood components, including red blood cells, platelets and clinical fresh frozen plasma. The causes of discarding blood components were analyzed in 2014 and several interventions were implemented. The post-intervention discarding rate was analyzed in 2015 to monitor the effectiveness of interventions.

Result

The blood component discarding rate in 2014 was 0.21% (167/81,597), which is higher than the threshold of 0.20%. The discarding rates showed that 0.04% for red blood cells (15/37,896), 0.10% for apheresis platelets(5/4,798), 0.30% for plasma (53/17583), and 0.54% for cryoprecipitated AHF(88/16184), respectively. The causes of discarding the blood components were categorized into the aspects of patients (58.1%) and health professionals (41.9%). Improvement of the patient's conditions and death of the patient were the reasons for discarding blood components. However, the reasons from the health professionals were expiry, improper transportation and storage. Several interventions were developed to minimize the unnecessary wastage from health care providers, including a) posting storage policies in nursing stations, b) affixing a note with expiry date and storage conditions on blood components, c) holding on-site education sessions for health professionals, d) designing transportation carts for transferring the blood components.

The post-intervention discarding rate in 2015 decreased to 0.15% (130/87,936), thus saving 36.7% of discarding expenditure, compared with that of 2014.

Conclusion

Enhancing awareness of blood components wastage was an important issue for the entire transfusion team. Through effective interventions, the wastage rate can be minimized.

PJ-16

Introduction

Materials and Methods

Result

Transfusion medicine

PJ-16

Frequency and specificity of Red Blood Cells Alloantibodies after Transfusions in Southern Taiwan

Li-Chuan Wang[1], Ya-Wen Tsai[1], Li-Ching Wu[1], Yu-Hsuan Yang[1], Hsin-Yin Chou[1], Jian-Cheng Lee[2]

[1]Department of Pathology, Chi Mei Medical Center, Tainan, Taiwan
[2]Department of Pathology, Chi Mei Medical Center, Chiali, Taiwan

Introduction

Alloimmunization after exposure to red cell (RBC) alloantigens depends on genetic and acquired patient-related factors, dose and route of administration. Previous studies indicated that the rate of alloimmunization in chronically transfused patients was as high as 60 percent and specificity of alloantibodies varied for different regions, ethnics, and diseases.

Materials and Methods

The study aimed to investigate the prevalence and type of unexpected red cell antibodies in a medical center with more than 1,200 beds. A total of 39,102 pre-transfusion records from 2012 to 2013 were retrospectively reviewed for antibody screening tests at the facility in southern Taiwan where Han Chinese dominantly inhabited. Descriptive statistics were applied to determine the figures, based on the time interval between transfusion therapy and antibody detections.

Result

The antibody screening test revealed positive in 337 of the 39,102 patients, indicating the overall alloimmunization rate of 0.86%. The most common alloantibody identified was anti-Mia (47.7%), followed by anti-E (20.8%), anti-M (6.2%), anti-Lea (5.0%), anti-E+c (4.7%). Additionally, 49.6 % (167/337) cases with alloantibodies were first identified after transfusion in this facility. Major antibodies detected after 7 days of transfusion included anti-Mia, anti-E, anti-P1, anti-Jka and anti-Jkb. Within one week, antibodies were found in 9 patients (5.4%), between 8-60 days in 48 patients (28.8%), between 2-24 months in 59 patients (35.3%), and more than 2 years in 51 patients (30.5%). Furthermore, 84 cases (50.3 %, 84/167) of alloantibodies were developed after 2 units packed RBC administered and 76 cases (45.5 %, 76/167) were detected alloantibodies after more than 3 units packed RBC administered. Seven cases (4.2%, 7/167) did them after 1 unit of Apheresis platelet was administered.

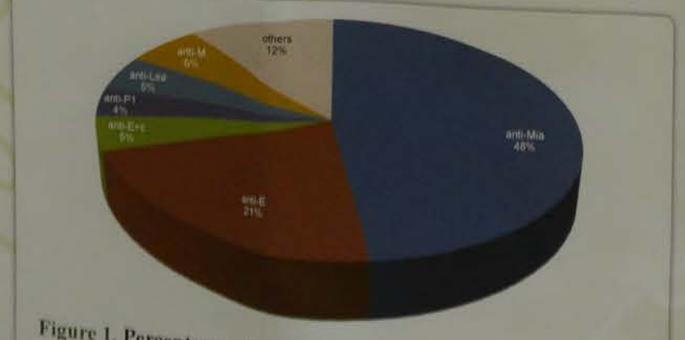


Figure 1. Percentages of Various Alloantibodies

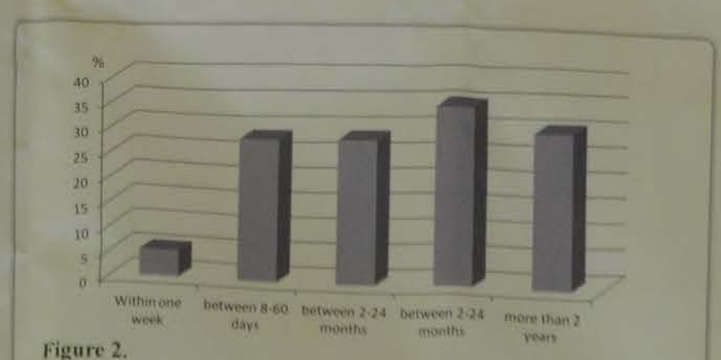


Figure 2.
Length of Time to Detect Alloantibodies after RBC Transfusion

Conclusion

Platelet products containing small volume of red blood cells can stimulate formation of alloantibodies. The alloantibodies develop on the fifth day at the earliest post-transfusions.

Transfusion medicine

PJ-17

PJ-17

The 32nd World Congress of Biomedical Laboratory Science | 2nd September 2016

ANTIGENS OF THE NEW HISTO-BLOOD GROUP FORSSMAN SYSTEM: EXPRESSION IN COLON CANCER TISSUES

R.Costa[1], P. Teixeira[1,2], C.Hesse[3], N.Osório[1] A.Valado[1], A.Caseiro[1], A. Gabriel[1], L Svensson[3], W. Abu Siba[4] Fernando Mendes[1,4,5,*]

[1]Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Department of Biomedical Laboratory Sciences, Coimbra, Portugal
[2]Pathology Service, Coimbra Hospital and Universitary Centre (CHUC)
[3]Biomedical Laboratory Sciences, Institute for biomedicine, Sahlgrenska Academy, University of Gothenburg
[4]Biophysics Unit-IBILI, Faculty of Medicine of University of Coimbra, Portugal
[5]CIMAGO, FMUC-Faculty of Medicine, University of Coimbra, Portugal
[6]Medical Laboratory Sciences Department – Al-Quds University, Abu Dis, Palestine
[*]CNC.IBILI, Faculty of Medicine of University of Coimbra, Coimbra, Portugal

Introduction

The FORSSMAN antigen, discovered in 1911 by Johann Friedrich Forssman, is expressed in erythrocytes, cells and organs. The antibody (Ab) anti-FORS may play a significant role binding complement and it could probably cause intravascular lysis of transfused FORS-positive red blood cells. Some authors propose that the antigen (Ag) might have a key role in carcinogenesis. According to several studies the FORS antigen is present in gastric, colon, and lung cancer and its expression in tissues could be related with the antibody titer in patient's plasma.[1-5] The anti- FORS titer is influenced by some features as age, sex and in cancer patients, also influenced by the histologic type of cancer. The Anti- FORS may function as anti-tumour Ab, this was recognized due to the decrease of FORS Ab titer caused by the establishment of anti- FORS – FORS immune complexes through the absorption of FORS Ag shed by the tumour. Therefore, serum level of FORS Ab could be used clinically to control the cancer reoccurrence in post-surgically cancer patients.[6-8]

The presence of FORS Ag was conclusively verified by *Ono et al* in the cytoplasm of colon goblet cells, especially those in the transitional mucosa adjacent to carcinoma.[9]

Nowadays, the numbers of new cases of cancer increases, turning it into a major public health problem. It's expected to be the main cause of death in the next few years. According to the latest cancer statistics, lung, colon and gastric cancer are amongst the most frequent new types of cancer.[10]

Aim

Our aim was to study 20 cases of colon cancer tissue in order to identify the FORS antigen expression and compare it in normal and neoplastic tissue.

Material and Methods

20 fresh surgical specimens of colon (7 female and 13 male) were collected at Centro Hospitalar e Universitário de Coimbra. Samples from neoplastic tissues were removed together with the transitional mucosa. All specimens were fixed in 4% buffered formaldehyde during 24 hours, processed through alcohols and xylenes and embedded in paraffin. All tissues were classified using TMN system, histotype and stage grading according to the Classification of Tumours criteria.[11]

Results

The results of tissue subjected only to antigen retrieval showed diffuse nuclear and cytoplasmic unspecific staining both in normal and tumor tissue without background staining as shown in figure 1.After testing with prior alkaline hydrolysis (potassium hydroxide) we were able to remove some unspecific binding without evident cytoplasmic staining. After performing immunohistochemistry with previous alkaline hydrolysis and neuraminidase digestion we were able to eliminate unspecific binding. However it seemed that the crypts of the colon tissue were immunostained but at high magnification we clearly see that there is no specific staining of the goblet cells.

Figure 1- Anti-Forssman labeling with rat monoclonal antibody Forssman (DAB, 100x)

Discussion/Conclusion

We believed that was required to execute antigenic recovery as it exposes antigens that may were blocked by the fixative agent and making possible and improve the formation of the antigen-antibody complex.[12] Previous studies demonstrated the presence of Forssman antigen in cytoplasm of colon goblet cells, in the transitional mucosa adjacent to carcinoma, and show that sialic acids might be linked to glycoproteins masking the Forssman antigenicity.[9] Due to that, using neuraminidase after alkaline hydrolysis endorsed to cleave sialic acids residues. The lack of observation of the immunostaining pattern in 16 samples may reflect the inability of the method used to identify the Forssman antigen or the inability of the supernatant of the cell culture to be used as antibody without any previous concentration. It is also considered that the cell culture supernatant, used as primary antibody, should be concentrated in order to ensure more specific binding.

According to Viktoria Dotz and Manfred Wuhrer, the various associations found between blood group glycans and different diseases, disease stages and health promoting factors are making histo-blood group glycans an intriguing topic in the field of personalized medicine.[13] Merging studies of genomic, proteomic, metabolomics and glycomic data containing histo-blood group antigen information may offer enhanced disease biomarkers as well as therapeutic targets in the future. Feng et al. hypothesize that the FORS Ag is tumor-specific and it has potential to be incorporate in carbohydrate conjugate vaccines used to mark different types of cancer in order to increase an immune response against the Fs pentasaccharide expressed on cancer cells surface.[14,15]

Therefore, we think that it's particularly significant to keep the study of this histo-blood group, bearing in mind all the potentialities and contributing to better reports of this histo-blood group.

References

[1]Storry JR, Castilho L, Daniels G, Flegel W a, Garratty G, de Haas M, et al. International Society of Blood Transfusion Working Party on red cell immunogenetics and blood group terminology: Cancun report (2012). Vox Sang [Internet]. 2014;107(1):90–6.
[2]Yamamoto F, Cid E, Yamamoto M, Blancher A. ABO Research in the Modern Era of Genomics. Transfus Med Rev [Internet]. Elsevier Inc.; 2012;26(2):103–18.
[3]Kawanami J. The appearance of forssman hapten in human tumor. J Biochem. 1972;72(3):783–5.
[4]Hirayama R, Hirokawa K, Takagi Y, Utsuyama M, Maejima S, Takemura K, et al. Changes in serum levels of Forssman-like antibody in patients with gastric cancer. Cancer [Internet]. 1989;63(8):1528–33.
[5]Kitamura H, Levine P, Cheng P, Egeli RA, Liu Y, Good RA, et al. Forssman-like Antibody Levels in Sera of Patients with Lung Cancer. Cancer Res. 1979;39(12):2909–14.
[6]Hirayama R, Hirokawa K, Takagi Y, Utsuyama M, Maejima S, Takemura K, et al. Changes in serum levels of Forssman-like antibody in patients with gastric cancer. Cancer [Internet]. 1989;63(8):1528–33.
[7]Kitamura H, Levine P, Cheng P, Egeli RA, Liu Y, Good RA, et al. Forssman-like Antibody Levels in Sera of Patients with Lung Cancer. Cancer Res. 1979;39(12):2909–14.
[8]Kitamura H, Levine P, Cheng P, Egeli RA, Liu Y, Good RA, et al. Forssman-like Antibody Levels in Sera of Patients with Lung Cancer. Cancer Res. 1979;123(1):22–6.
[9]Diagnostics O. Characterization of Anti - Forssman (ANTI-Fs) Antibodies in Human Sera : their specificity and possible changes in patients with cancer. 1979;123(1):22–6.
[10]Ono K, Hattori H, Uemura K, Nakayama J, Ota H, Kateoyama T. Expression of Forssman antigen in human large intestine. J Histochem Cytochem [Internet]. 1994;42(5):659–65.
[11]Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin [Internet]. 2015 Jan 5 [cited 2015 Jan 9];65(1):5–29.
[12]Leslie H. Sobin (Editor), Mary K. Gospodarowicz (Editor) CW (Editor). TNM Classification of Malignant Tumours, 7th Edition. 2009. 336 p.
[13]Werner M, Von Wasielewski R, Komminoth P. Antigen retrieval, signal amplification and intensification in immunohistochemistry. Histochem Cell Biol. 1996;105:253–60.
[14]Dotz V, Wuhrer M. Histo-blood group glycans in the context of personalized medicine. Biochim Biophys Acta - Gen Subj [Internet]. Elsevier B.V.; 2015;1860(8):1596–607.

raquelcosta1394@gmail.com

Transfusion medicine

PJ-18

Evaluation of a Novel Red Cell S
by Hemolysis and Antigenicity

Chun-Chang Lee[1]

Blood Bank, Department of Clinical Pathology, Cheng Hsin Ge

Background & Aim

storage of red cells causes a progressive increase in hemolysis and loss of antigenicity. In spite of the use of storage solutions for storage and filters for leucoreduction, certain extent of hemolysis and loss of antigenicity are still inevitable. In this study, we evaluated a novel red cell storage solution by these parameters.

Materials and Methods

Two sets of antibody screening cells of the same lot from Formosa Biomedical Technology Corp (Taiwan)were evaluated. One set was stored in the original solution and the other was placed into the novel storage solution. OD540 of the supernatant was used to evaluate hemolysis. Agglutination by antisera was used to evaluate antigenicity at subsequent storage. The antigenicity of the Rh, MNS, and Mia antigens were evaluated for 10 weeks. The other antigens (Kidd, Duffy, Lewis, and P1) were evaluated for 19 weeks. To avoid fluctuation in storage temperature, antisera were dispensed in advance as aliquots. To analysis the strength of the reactions, we used the scoring method as described in the Technical Manual of the AABB (4+ =12, 3+= 10, 2+=8, 1+=5, w+=2, negative=0). The scores of all the blood units tested were added together to obtain a score.

Results

Hemolysis (OD_{540}) progressively increased with the storage period in all the stored red cell units (Tab.1, original and novel solutions). On day 61 of storage, the hemolysis was slightly higher in origin as compared to novel (Fig.1). Antigenicity of Rh, MNS, and Mia was compared for 10 weeks (70 days) and no difference between two sets was found. Antigenicity of Duffy, Lewis, and P1 was not different between two sets for 19 weeks (132 days). However, antigenicity of Kidd in the original solution dropped over time. From week 10, Kidd antigens became weaker and from week 14 (96 days) they're almost undetectable in the original solution while antigenicity in the novel solution remained the same through time.

Table 1: Hemolysis in two RBC preservative solutions.

Day after collection	Hemolysis (OD_{540})	
	Original	Novel
0		
28	0.06	0.00
61	0.12	0.03
70	0.31	0.11
83	0.42	0.15
96	0.60	0.31
110	0.79	0.49
122	0.92	0.72
132	1.01	0.89
	1.05	0.93

Compassionate Heart, Dedicated Care

Transfusion medicine

PJ-18

Evaluation of a Novel Red Cell Storage Solution by Hemolysis and Antigenicity of Red Cells

Chun-Chang Lee[1]

CHENG HSIN GENERAL HOSPITAL

Blood Bank, Department of Clinical Pathology, Cheng Hsin General Hospital, Taipei, Taiwan[1]

Background & Aim

Storage of red cells causes a progressive increase in hemolysis and loss of antigenicity. In spite of the use of storage solutions for storage and filters for leucoreduction, certain extent of hemolysis and loss of antigenicity are still inevitable. In this study, we evaluated a novel red cell storage solution by these parameters.

Materials and Methods

Two sets of antibody screening cells of the same lot from Formosa Biomedical Technology Corp (Taiwan)were evaluated. One set was stored in the original solution and the other was placed into the novel storage solution. OD540 of the supernatant was used to evaluate hemolysis. Agglutination by antisera was used to evaluate antigenicity in subsequent storage. The antigenicity of the Rh, MNS, and Mia antigens were evaluated for 10 weeks. The other antigens (Kidd, Duffy, Lewis, and P1) were evaluated for 19 weeks. To avoid fluctuation in storage temperature, antisera were dispensed in advance as aliquots. To analysis the strength of the reactions, we used the scoring method as described in the Technical Manual of the AABB (4+ =12, 3+= 10, 2+=8, 1+=5, w+=2, negative=0). The scores of all the blood units tested were added together to obtain a score.

Results

Hemolysis (OD_{540}) progressively increased with the storage period in all the stored red cell units (Tab.1, original and novel solutions). On day 61 of storage, the hemolysis was slightly higher in origin as compared to novel (Fig.1). Antigenicity of Rh, MNS, and Mia was compared for 10 weeks (70 days) and no difference between two sets was found. Antigenicity of Duffy, Lewis, and P1 was not different between two sets for 19 weeks (132 days). However, antigenicity of Kidd in the original solution dropped over time. From week 10, Kidd antigens became weaker and from week 14 (96 days) they're almost undetectable in the original solution while antigenicity in the novel solution remained the same through time.

Table 1: Hemolysis in two RBC preservative solutions.

Day after collection	Hemolysis (OD_{540}) Original	Novel
0	0.06	0.00
28	0.12	0.03
61	0.31	0.11
70	0.42	0.15
83	0.60	0.31
96	0.79	0.49
110	0.92	0.72
122	1.01	0.89
132	1.05	0.93

Fig.1. Comparison between original and novel solution.

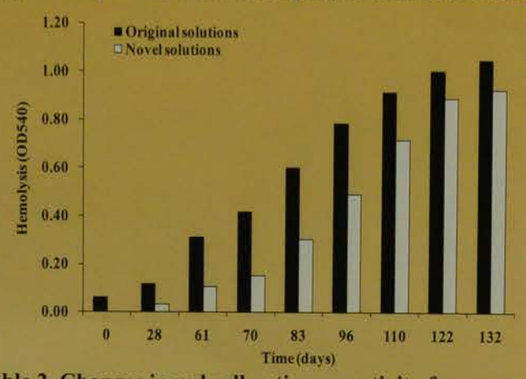


Table 2. Changes in red cell antigen reactivity for screen ells during storage with various preservative solutions

Storage Period		10 weeks					
Component		Screen 1		Screen 2		Screen 3	
	Preservative solution	Original	Novel	Original	Novel	Original	Novel
Rh	anti-D	5	8	2	2	2	2
	anti-C	0	0	5	5	5	5
	anti-E	5	5	0	0	0	0
	anti-c	8	8	0	0	0	0
	anti-e	0	0	2	2	2	2
MNS	anti-S	5	5	0	5	0	0
	anti-s	2	2	5	5	5	5
	anti-M	2	5	2	5	0	0
Other	anti-Mia	0	0	2	5	0	0

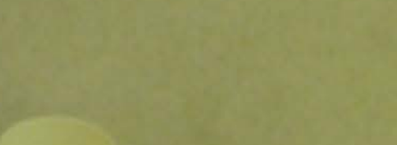
Storage Period		14 weeks		19 weeks		14 weeks		19 weeks	
Component		Screen 3				Screen 1			
	Preservative solution	Original	Novel	Original	Novel	Original	Novel	Original	Novel
Duffy	anti-Fya	5	5	2	2	/	/	/	/
	anti-Fyb	2	2	5	5	/	/	/	/
Kidd	anti-Jka	0	5	0	5	/	/	/	/
	anti-Jkb	0	5	2	5	/	/	/	/
Lewis	anti-Lea	5	5	5	5	/	/	/	/
	anti-Leb	/	/	/	/	5	5	5	5
P	P1	/	/	/	/	5	8	5	5

Note: scoring method, 4+ =12, 3+= 10, 2+=8, 1+=5, w+=2, negative=0, /= not tested

Conclusions

Hemolysis and loss of antigenicity of the red cells increase after long period of storage. The novel storage solution has better ability to keep antigenicity of Kidd. The use of this novel storage solution may extend the shelf life of commercial screening cells in Taiwan thus help to retain the availability of Mia-positive screening cells.

Compassionate Heart, Dedicated Care



Transfusion medicine

PJ-19

Frozen Platelet Rich Plasma Activity To Detect growth factor Concentration

PJ-19

[1]Chih-Man Yang, [2]Nai-Chi Chen, [3]Szu-Yun Huang, [2]Wei-Tso Chia
[1]Department of Laboratory, [2]Department of Orthopedics, [3] Nursing Department,
National Taiwan University Hospital, Shin-Chu Branch, Taiwan

台大醫院 NTUH Hsin-Chu Branch

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. it is also a concentration of the 7 funda-mental protein growth factors include the 3 isomeres of platelet-derived growth factor, 2 of the numerous transforming growth factors, vascular endothelial growth factor, and epithelial growth factor. Platelet-rich plasma (PRP) Commonly used to treat tendon injuries, including tendonitis, tendinopathy and tendinosis. Purpose: For improving the utilities of platelet rich plasma (PRP) and decrease the withdraw blood sample times from patient, we disclosed the PDGF and EGF concentrations observed activity of frozen PRP. Methods: We using MVon lymphocyte separation medium (MV-PRP02) for concentration the platelet. The platelet count used by CBC counter (Sysmex XT-2000i). Aliquot of stored plasma (at -80 ℃) and thaw at room temperature of our study group was used for assay of plasma platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) using RayBioR Human EGF ELISA Kit (RayBiotech, Inc.). Results:The MV-PRP02 kit can amplified the platelet count to 5.01+-0.86 Times(n = 6). After frozen and thaw, the active concentration time course of PDGF and The EGF concentration (plasma: under detection level) in 35 min. Conclusions: In this study, we demonstrate the properties of frozen and thaw PRP (FTPRP). The equipment can effectively concentrate the amount and increasing number of platelet. The FTPRP can and effectively preserve the activity of PDGF and EGF.

Introduction

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. it is also a concentration of the 7 funda-mental protein growth factors include the 3 isomeres of platelet-derived growth factor, 2 of the numerous transforming growth factors, vascular endothelial growth factor, and epithelial growth factor. Platelet-rich plasma (PRP) Commonly used to treat tendon injuries, including tendonitis, tendinopathy and tendinosis.

Purpose

For improving the utilities of platelet rich plasma (PRP) and decrease the withdraw blood sample times from patient, we disclosed the PDGF and EGF concentrations observed activity of frozen PRP.

Methods:

We using MVon lymphocyte separation medium (MV-PRP02) for concentration the platelet. The platelet count used by CBC counter (Sysmex XT-2000*i*). Aliquot of stored plasma (at -80 ˚C) and thaw at room temperature of our study group was used for assay of plasma platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) using RayBioR Human EGF ELISA Kit (RayBiotech, Inc.). CaCl2 (25mg/L) was aided as an activator then the samples were collected in time course. The procedures were done according to manufacturer's instructions.

Results

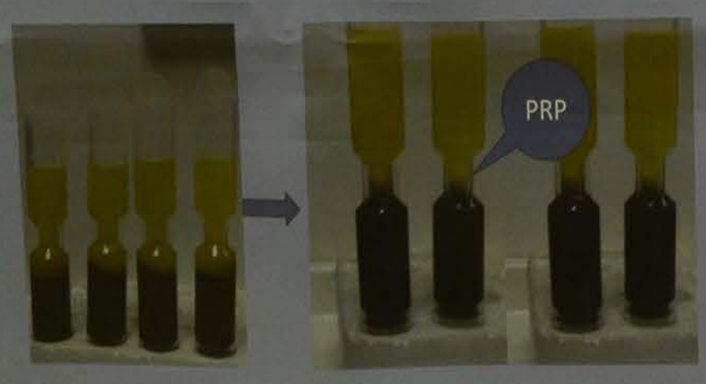


Fig1. Producted of PRP

Table1. autologous tissue regenrative healing system of pletelet count ,concentration multiple and volume

	control	c1	c2	c3	c4	c5	c6
Plt counting	242	1880	508	1003	2035	859	964
Multiple	1	7.77	2.10	4.14	8.41	3.55	3.98
Volume		200	500	500	100	1000	300

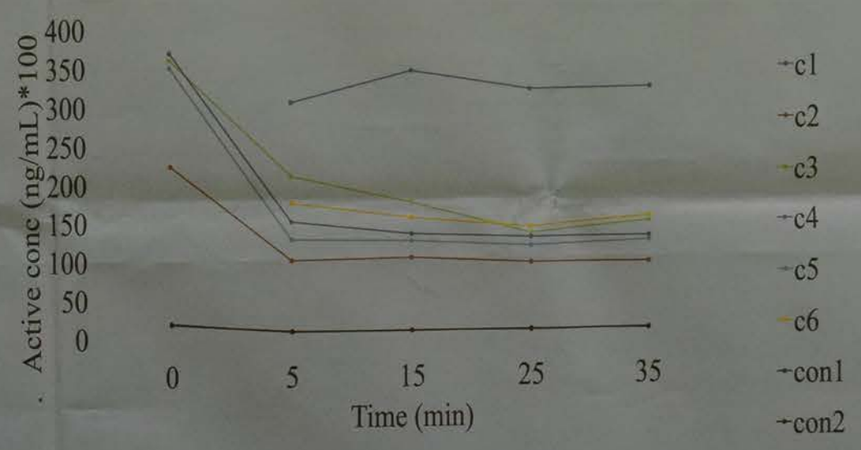


Fig2. The PDGF active concentration in time course

Table2. The EGF concentration in time course. .(ng/mL)*100

Sample \ Time (min)	0	5	15	25	35
1	11.8	7.88	7.52	7.24	7.04
2	2.9028	2.0784	1.8838	1.8838	2.0168
3	4.722	3.3576	3.6134	3.4144	3.3576
4	6.2	4.2104	3.8124	3.983	3.3007
5	4.9494	3.3292	3.3007	3.386	3.3292
6	7.2	4.3809	4.4378	4.1251	3.7271
Con1	<Min	<Min	<Min	<Min	<Min
Con2	<Min	<Min	<Min	<Min	<Min

PS: con: control Min : below of detected

Conclusions

In this study, we demonstrate the properties of frozen and thaw PRP (FTPRP). The equipment can effectively concentrate the amount and increasing number of platelet. The FTPRP can and effectively preserve the activity of PDGF and EGF.

2016_08_31_IFBLS

Transfusion medicine

PJ-20

Estimate the feasibility of gel card as the automated machine backup program

Chiung Ju Chen, Tong-Jong Chen
Department of Pathology and Laboratory Medicine, Shin Kong Wu Ho-Su Memorial Hospital

Aim

Blood typing and antibody screening are routine tests at blood bank. The most common methods have been used included tube method, automation instruments and solid phase, etc. which has its advantages and disadvantages. There are lots of related studies revealed the consistent. The aim of this study is to establish an alternative standardization method which can save the image of test results when automated blood bank analyzer is not available.

Materials & Methods

From february to April 2015,We compared the result correlation, operating time and costs among gel card, autovue and manual tube method. The specimens includes patient samples, CAP specimens and commercial antiserum. 39 samples for ABO/D grouping and 42 samples for irregular antibody screening were tested. All operate methods according to the product manual of manufacturer. The antibody screening and ABO subgroups were interpreted by two senior medical technologists. Subsequently, we compared test correlation, costs and time among three metohds.

table1 、Method and the manufacturer of reagent

	Methods	Tube method	AutoVue	Gel card
ABO and Rh typing	Anti-A/ Anti-B/ Anti-D	Immucor Gamma	Ortho Anti-A/Anti-B/Anti-D /Control/Reverse diluent card	Dia Clon ABO/D +Reverse Grouping ID-cards
	A_1 and B cells	Ortho 3~5% suspension	Formosa MeDiPro (A+B) cell(3~5%)	BioRed ID-ABO A_1 and B cells (0.8%)
Antibody screening	LISS	Immucor Gamma	BLISS	LISS/Coombs ID-cards
	Anti-human globulin	Immucor Gamma	Ortho Anti-IgG - C3d polyspecific Cards	
	Screening cells	Formosa 3~5% suspension	Formosa MeDiPro antibody screening cell(3~5%)	ID-Dia Cell I-II-III Asia+ID-Di^a cells (0.8%)

Figure 1: ABO/D typing

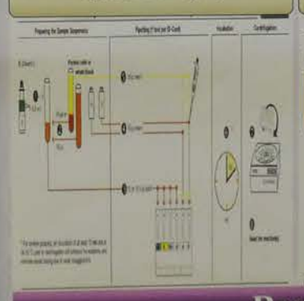
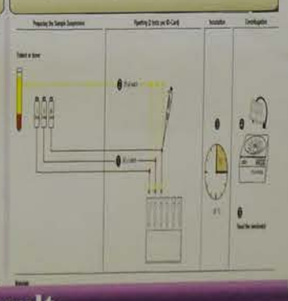


Figure 2: Irregular antibody screening



Result

There were 34 cases of ABO/Rh type were identified and the consistency was 100% (table2), all of them the titer to differ ≦1+. There are 5 cases Gel card, autovue and tube method are revealed the ABO discrepancies between red cell and serums tests(table3), One of the patient had been received BM transplant. One was received O+PRBC transfusion. Three patients are ABO subgroups (B_3^+(2) , A_{el}^+(1)) . 41 cases of irregular antibody were identified and the consistency of negative result was 100%(13/13) (table4). Because the Formosa MeDiPro antibody screening cell haven't K antigen, Anti-K is exclude. The Ab screening positive rate were 67.5%(27/40) for gel card and the LISS-AHG tube method and 65%(26/40) for autovue respectively.

Table 2 、The comparison of result of ABO/D typing

Blood groups	Gel card	Autovue	Tube method
A+	10	10	10
B+	6	6	6
O+	9	9	9
AB+	5	5	5
B-	4	4	4
ABO discrepancy	5	5	5

Table 3 、The comparison of result of ABO discrepancies

Sample No.	Gel card					Autovue					Tube method					Result
	Anti-A	Anti-B	Anti-D	A1 cell	B cell	Anti-A	Anti-B	Anti-D	A1 cell	B cell	Anti-A	Anti-B	Anti-D	A1 cell	B cell	
59008	0	0	4+	0	3+	0	0	4+	0	3+	0	0	4+	0	3+	BM transplant
59011	0	DP	4+	3+	0	0	MF	4+	3+	0	0	$3+^{mf}$	4+	3+	0	B_3+
00373	0	0	4+	2+	4+	0	0	4+	2+	4+	0	0	4+	2+	4+	A_{el}+
01034	0	DP	4+	4+	0	0	MF	4+	4+	0	0	$3+^{mf}$	4+	4+	0	B_3+
01010	DP	0	4+	0	2+	MF	0	4+	0	2+	$3+^{mf}$	0	4+	0	2+	O+ PRBC transfused

Table 4 、The comparison of result of irregular antibody screening：
*: MeDiPro antibody screening cell haven't K antigen

Antibody	LISS AHG tube method	AutoVue	Gel card
Anti-Mi^a	11	10	11
Anti-c	2	2	2
Anti-E	2	2	2
Anti-E+c	1	1	1
Anti-Mi^a+Le^a	1	1	1
Anti-D	1	1	1
Anti-M	2	2	2
Anti-S	1	1	1
Anti-Jk^a	1	1	1
Anti-Jk^b	1	1	1
Anti-K	0*	0*	1
Negative	13	14	13
DAT(+)	4	4	4

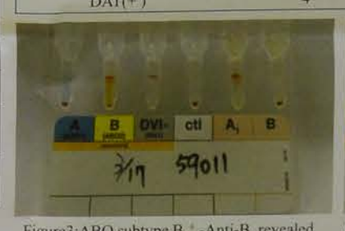


Figure3:ABO subtype B_3^+ -Anti-B revealed mixed field.

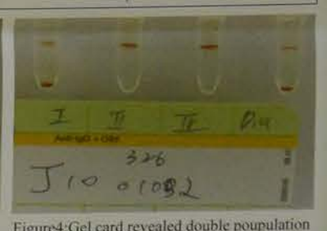


Figure4:Gel card revealed double poupulation -in screening I cell and Di^a cell

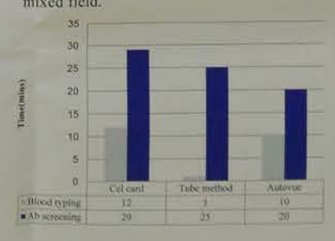


Figure5:The comparison of operating time.

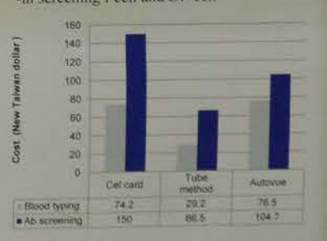


Figure6:The comparison of reagent cost.

Discussion

One case showed Anti-E double population in gel card because of fibrin interference, suggestion add serum first then add A_1/B cell into reaction chamber, *otherwise the cell will sediment in the column* bottom.11 anti-Mia Ab were identified and one revealed false negative result in autoVue. Blood type results of gel card is 100% consistency with tube method and autovue. The concordance rate of Ab screen is 96.3%between gel card and tube method and it's 92.6% between gel card and autovue.

Conclusion

The operation time: the gel card is longer than tube method(fig5).The cost of gel card and autovue is more expensive than tube method(fig6).Even though, the costs of gel card are higher, however, the results interpretation of gel card is easier for standardization than tube method. In conclusion, gel card is an optimal alternative method ofautomated blood bank analyzer.

專業・熱忱・服務　健康・希望・幸福

Transfusion medicine

PJ-21

Kaohsiung M

The amount of
with den

Chun-chi Hua

[1]Department of Lab
[2]Department of Public H
[3]Kaohsiung Me

Background

Dengue regions of the wo
tropical and subtropical co
are Aedes aegypti and Ae
distribution, including A
America, Middle East, So
and the Western Pacific. B
to 2013 in 10 years, 2007
and the local number of c
were more than one thou
2006, 2009 and 2013 the
cases, although not mor
thousand cases, also re
Example above, 2014
occurred over the years the
dengue epidemic, the numb
more than million cases o
were concentrated in Kao
Tainan. (Fig.1)

Purpose

Source platelet concentrate i
by volunteers, in order to r
risk of transfusion-transmissible
diseases, dengue positive patie
reduce the chances of platelet c
solution.

Method

Statistical analysis of 2014 to 2
number of dengue fever cases
amount of platelet components

Results

Monitor the statistics ammount of p
components from 2014 to 2015
finding that ammount of pla
components the period from Septe
to December that 2015 increas
15.28% than 2014, Check the pla
components in 2014 and 2015 in pat
with indications it found that 93 per

PJ-20

Transfusion medicine

PJ-21

Kaohsiung Municipal Hsiao-Kang Hospital

The amount of platelet components re lationships with dengue fever in southern Taiwan

Chun-chi Huang[1,2], Ching-mei Cheng[1], Yi-ping Chen[1]

[1]Department of Laboratory Medicine, Kaohsiung Municipal Hsiao-Kang Hospital
[2]Department of Public Health, College of Health Sciences, Kaohsiung Medical University
[3]Kaohsiung Medical University Hospital, Kaohsiung Medical University

Background

Dengue regions of the world, mainly in tropical and subtropical countries, there are Aedes aegypti and Aedes white line distribution, including Africa, South America, Middle East, Southeast Asia and the Western Pacific. Between 2004 to 2013 in 10 years, 2007, 2010, 2011 and the local number of cases in 2012 were more than one thousand cases in 2006, 2009 and 2013 the number of cases, although not more than one thousand cases, also reached 500 Example above. 2014 and 2015 occurred over the years the most severe dengue epidemic, the number of cases more than million cases of outbreaks were concentrated in Kaohsiung and Tainan. (Fig.1)

Purpose

Source platelet concentrate is provided by volunteers, in order to reduce the risk of transfusion-transmissible infections diseases, dengue positive patients should reduce the chances of platelet component solution.

Method

Statistical analysis of 2014 to 2015, the number of dengue fever cases and the amount of platelet components.

Results

Monitor the statistics ammount of platelet components from 2014 to 2015 , our finding that ammount of platelet components the period from September to December that 2015 increase of 15.28% than 2014. Check the platelet components in 2014 and 2015 in patients with indications it found that 93 percent of patients with the conditions of use are in compliance with recommendations of the AABB specification. All patients are less than the number of platelets 60000ul.

Analysis of the types of diseases in 2014 and 2015 platelet component solution, we find that to add a kind of disease - dengue in August to December 2015.

From January to December 2015 there are 1047 cases of dengue positive cases, but in August to December 2015, there are 1034 cases of dengue positive cases including 14 cases of severe cases.

Statistics August to December 2015 the amount of platelet components has 3.64% - 9.95% is dengue positive cases.

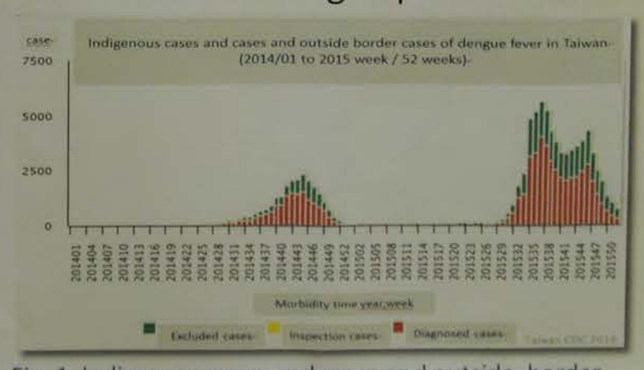


Fig. 1 Indigenous cases and cases and outside border cases of dengue fever in Taiwan

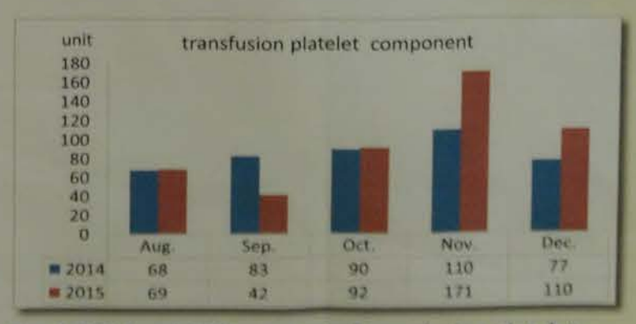


Fig. 2 2014 and 2015 amount of transfusion platelet component

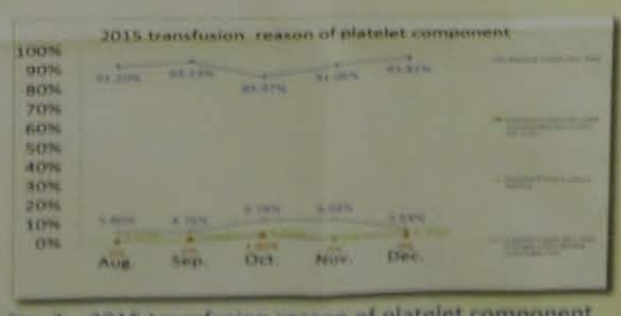


Fig. 3 2015 transfusion reason of platelet component

Transfusion medicine

PJ-22

Paper-based analytical devices for forward and reverse ABO blood group typing

Temsiri Songjaroen and Wanida Laiwattanapaisal

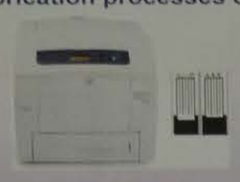
Department of Clinical Chemistry, Faculty of Allied Health Sciences
Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

Abstract

A paper-based analytical device (PAD) for simultaneous forward and reverse ABO blood group typing has been developed. The PAD was fabricated by wax printing technique. The six parallel channels were printed onto Whatman No. 4 filter paper. For reverse grouping, an LF1 blood separation membrane was used for the separation of plasma from whole blood. Haemagglutination was used to identify the blood groups. For forward grouping Anti-A, -B and Anti-A,B were immobilized on the test line of PAD, and inactivated Anti-A, -B and Anti-A,B were dropped on the control line. For reverse grouping, 30% standard A-cells, B- and O- were added to the test channel after plasma separation, and O-cells were used as a control. 0.9% normal saline solution (NSS) containing 1% Tween-20 was used for dilution of the blood sample and elution of the non-agglutinated red blood cells within the channels. The results can be visually analysed by compared the distance of agglutinated RBCs in each test line with the distance of non agglutinated RBCs in the parallel control line. The PAD has high reproducibility when 10 replications of the A, B, AB or O blood groups were performed The results of blood grouping by PAD were highly correlated with conventional methods (n = 76). The developed PAD has promise as point-of-care testing for blood typing

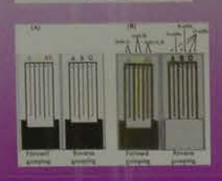
Methods

Fabrication processes of paper-based devices



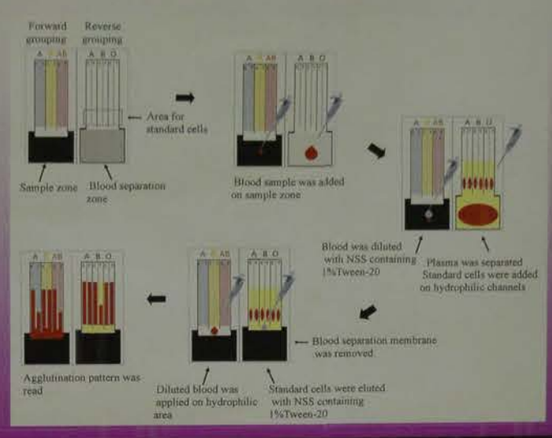
The pattern was printed on Whatman No.4 paper by a wax printer

The paper was heated on a hot plate at 150 °C for 2 min to melt and transfer wax into paper membrane



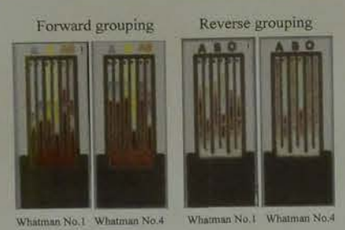
The antibodies were physically adsorbed on channels of the PADs

A diagram of blood group typing by forward and reverse grouping on paper-based devices



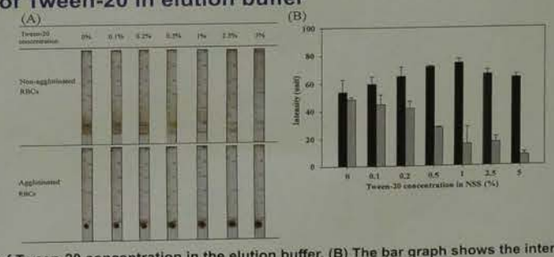
Results

Type of paper



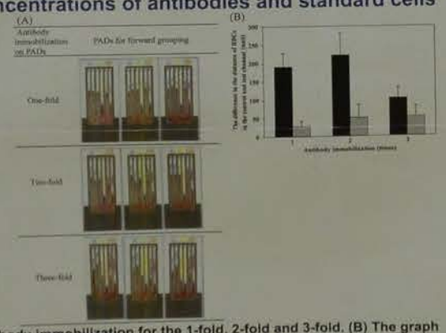
Type of paper for blood group typing on PAD; Left: Whatman No. 1 and Whatman No. 4 constructed for forward grouping; Right: PAD fabricated by Whatman No. 1 and Whatman No. 4 for reverse grouping

Effect of Tween-20 in elution buffer

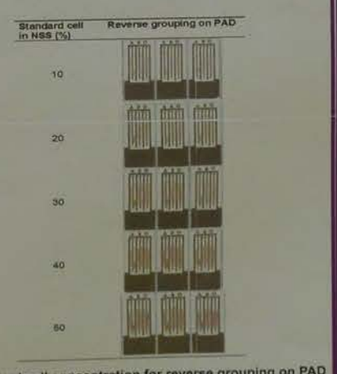


Effect of Tween-20 concentration in the elution buffer. (B) The bar graph shows the intensity of ■ aglutinated RBCs and ■ non-agglutinated RBCs on paper after the elution step

Concentrations of antibodies and standard cells

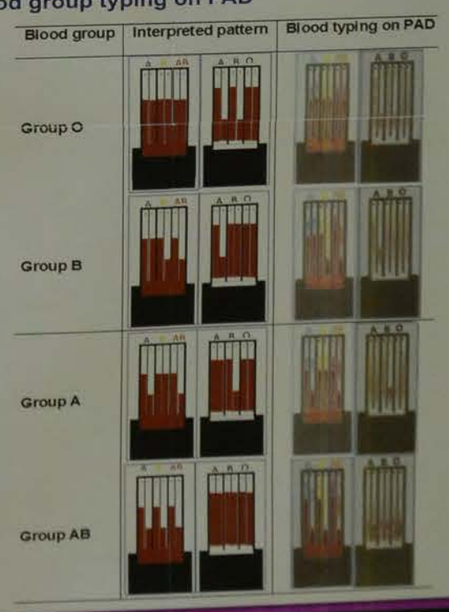


Antibody immobilization for the 1-fold, 2-fold and 3-fold. (B) The graph represents the difference in the distance of RBCs in the control and test channel versus immobilization times; ■ Distance of agglutinated RBCs compared with control; ■ distance of non-agglutinated RBCs compared with control



Standard cell concentration for reverse grouping on PAD

Blood group typing on PAD



Comparison of blood typing by PAD with conventional methods

Blood group	Number of samples	Interpretation				Accuracy (%)
		Forward grouping		Reverse grouping		
		Accuracy	Discrepancy	Accuracy	Discrepancy	
A	15	15	0	15	0	100
B	20	20	0	20	0	100
AB	14	14	0	14	0	100
O	27	27	0	27	0	100

Conclusions

- LF1 blood separation membrane was exploited for the separation of plasma from whole blood and used for reverse grouping
- A new platform of PADs for the simultaneous detection of forward and reverse ABO blood group testing has been reported
- The developed PADs could be used for detection of both RBC antigens and antibodies in plasma on the same device
- Tween-20 was an effective additive substance of the elution buffer for facilitating the elution of the non-agglutinated RBCs on the PAD
- The results of the blood group could be visually read
- The PAD is low-cost, simple, and portable for blood typing
- It can be used for quick detection of blood groups in remote areas where no laboratory facilities can be accessed, especially in developing countries

Acknowledgements

This research is financially supported from the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (WCU-58-002-HR). T.S. acknowledges the support from the Ratchadaphiseksomphot Fund for Postdoctoral Fellowship from Chulalongkorn University

Transfusion medicine

PJ-25

Ratio and reasons of blood products wastage in a Japanese Red cross hospital

Mikiko Endo[1)], Kazuyo Murakami[1)], Kazumi Naramoto[1)], Ako Futamura[1)], Kojiro Tunekawa[2)], Yukiyasu Ozawa[1)], Hideki Kato[2)], Norihiro Yuasa[2)]

1) Division of Blood Transfusion, 2) Division of Clinical Laboratory

Japanese Red Cross Nagoya Daiichi Hospital

The authors have no financial conflicts of interest to disclose concerning the presentation

Conclusion

Medical stuffs dealing with BP need to improve the skill, knowledge and management for reducing BP wastage across the job category.

Education on BP usage, storage and handling of BP should be enhanced.

Background

Blood products (BP) are made from blood donation by unpaid volunteers, so BP wastage should be reduced for effective use.

Reported wastage rate of Red blood cell (RBC) transfusion products:

England : 2.1~6.40%	G.A. Smith, et al. British Journal of Biomedical Science 2015.
U.S.A. : 0.1~6.7%	E.S. Heitmiller, et al Transfusion 2010
Japan : 0.18~10.1%	K. Tunekawa, et al. Japanese Journal of Transfusion and Cell Therapy 2011

Aim

To review our management and investigate the BP wastage rate and the causes during 18 years

Summary of results

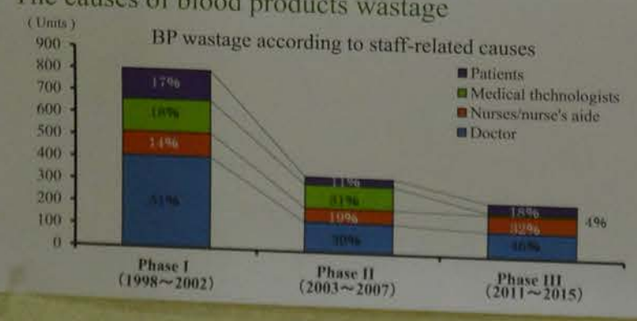
(1) Total BP wastage rate has been decreasing to 0.07% in phase III.
(2) The BP wastage caused by nurses/nurse's aide was increased in phase III.
(3) The BP wastage caused by miss-storage, miss-carriage and miss-handling were increased in phase III.

Result

Interventions for reduction of BP wastage and effects

Year	Interventions	Effects
1998		
1999	A cross-match/transfusion ratio was reported to chiefs surgical department	BP inventory for surgery was reduced.
2000	BP wastage causes were reported to the Blood Transfusion Therapy Committee	Measures for BP wastage cause were promoted.
	Introduction of blood transfusion management system	Information on blood transfusion and patients was easily obtained.
2001		
2002	Introduction of Blood transfusion ordering system	Doctor's BP ordering was facilitated.
	Introduction of fully automated blood transfusion testing equipment	Medical technologists workload was reduced.
2003	Introduction of Type & Screening	BP inventory for surgery was reduced.
	Learning sessions of BPs for hospital stuffs	Knowledge of the blood transfusion service was shared with hosipital stuffs.
2004	Transfusion urgency was settled in the hospital.	Urgent bloods transfusion was facilitated according to patient's condition.
2005	The latest patient hemoglobin concentration was reported to the doctor who ordered blood transfusion.	Proper transfusion was facilitated according to the outpatient condition.
	Recovering the BP left in operating rooms immediately after surgery.	Inappropriate BP storage was reduced.
2006	Providing BP segmented according to patient's condition or surgical bleeding	BP wastage due to improper storage was reduced.
	Increased number of medical technologist in the blood transfusion service.	Management ability of blood transfusion service was increased.
2007		
2008		
2009		
2010		
2011	Introducing full-time position to medical technologists of the blood transfusion service.	Blood transfusion safety was enhanced
	Introduction of the irradiated blood products.	
2012	Training general medical technologists for blood transfusion testing.	
2013		
2014		
2015		

The causes of blood products wastage



Material and Method

Blood products managed in blood transfusion service during 18 years (1998-2015)

Total	: 864,104 units
Red blood cells (RBC)product	: 191,488 units,
Fresh frozen plasma(FFP)	: 73,981 units
Platelets(PLT) product	: 598,685 units

Statistical analysis

Comparison between the multi-group was evaluated by Chi-square test or Multiple comparisons of Dunn's method
A significant difference was defined by $p < 0.05$.

Review period

phase I : 1998-2002
phase II : 2003-2007
phase III: 2011-2015

Causes classification

A: staff-related
- doctors
- nurses/nurse's aides
- medical technologists
- patients

B: BPs-related
- order
- storage
- carriage and handling
- management of BPs
- others

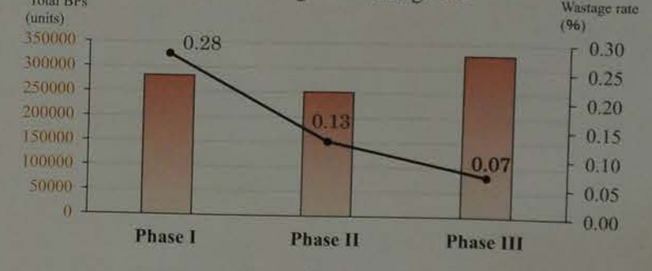
Result

BP wastage rate

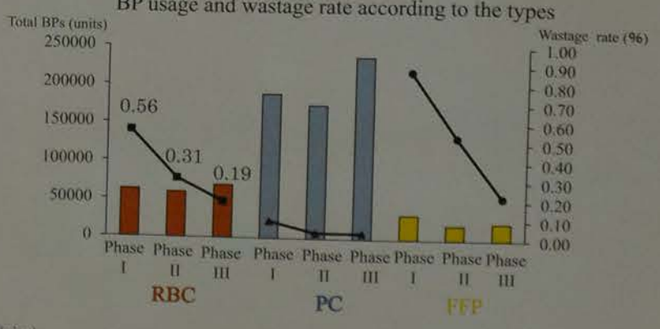
	Phase I (1998-2002)			Phase II (2003-2007)			Phase III (2011-2015)		
	BPs (units)	Waste	(rate)	BPs (units)	Waste	(rate)	BPs (units)	Waste	(rate)
RBC	62950	355	(0.56 %)	59370	185	(0.31 %)	69168	129	(0.19 %) *
PC	188160	164	(0.09 %)	173950	50	(0.03 %)	236575	62	(0.03 %)
FFP	32358	283	(0.87 %)	19132	101	(0.53 %)	22441	49	(0.22 %) *
Total	283468	802	(0.28 %)	252452	336	(0.13 %)	328184	240	(0.07 %)

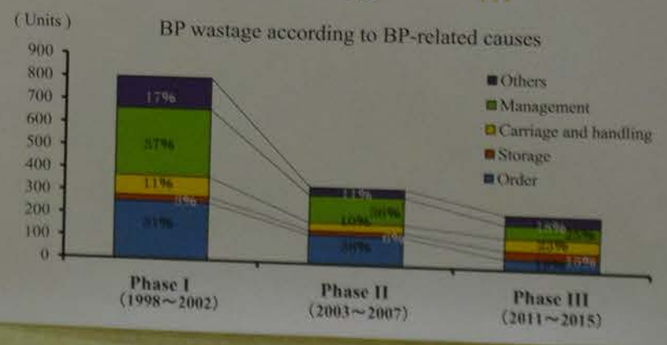
*: Phase II vs Phase III $p<0.001$: Phase I vs Phase III $p<0.001$

Total BP usage and wastage rate



BP usage and wastage rate according to the types





Transfusion medicine

PJ-26

FREQUENCY OF IRREGULAR ANTIBODIES FOR ERYTHROCYTE

Madoka Inoue, Manabu Yamaoka, Risa Kashimoto, Maki Osawa, Yukari Terashima, Misao Abe, Shuji Onishi

KANSAI MEDICAL UNIVERSITY HOSPITAL, OSAKA, JAPAN

INTRODUCTION

In general, there are antibodies (Abs), which don't react with a person's own antigens in the plasma, in the ABO blood group system. They are called regular Abs. On the other hand, Abs that react with the other blood group types are called irregular Abs. They are produced by immune responses to alloantigens of transfusion and pregnancy, and cause hemolytic transfusion reaction (HTR) for blood transfusion and hemolytic disease of the new born (HDN). Therefore, it is necessary to detect clinically significant irregular Abs quickly and correctly for patients who have irregular Abs.

Irregular Ab screening test is one of the most important tests to find irregular Abs. Most of patients are screened routinely and the number of the tests are increased year by year in our hospital. In this study, we retrospectively analyzed the frequency of irregular Abs of our screening tests.

METHOD

We analyzed the all patients with screening from January 2006 to December 2015 in Kansai Medical University Hospital.

RESULTS

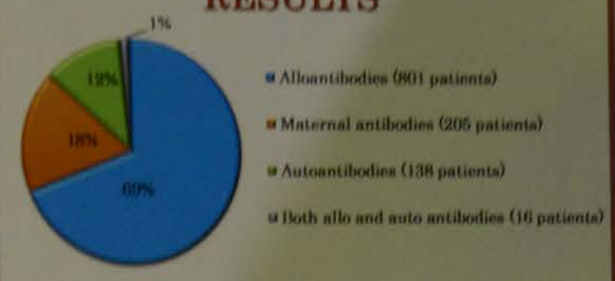


Fig.1 Irregular antibodies detected in screening (n=1,160)

Table.1 The number of cases and patients screened

	cases	patients
Screening	104,441	58,251
Positive	1,876	1,160
Positive-ratio	1.80%	1.99%

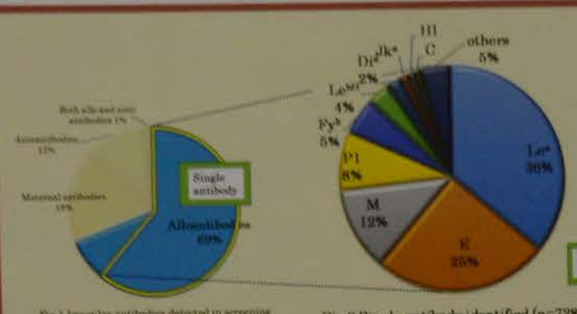


Fig.1 Irregular antibodies detected in screening (n=1,160)

Fig.2 Single antibody identified (n=728)

*Among the 801 patients who had alloAbs , a single type of Ab was identified in 728 (91.0%) patients.
*The single Ab identified were anti-Le^a (264 patients), -E (183 patients), -M (87 patients), -P1 (61 patients), -Fy^b (39 patients), -Le^bH (25 patients), -Di^a (15 patients), -Jk^a (9 patients), -HI (8 patients), -C (5 patients), others (32 patients).

Fig.1 Irregular antibodies detected in screening (n=1,160)

Fig.3 Multiple antibodies identified (n=73)

*Among the 801 patients who had alloAbs, multiple Abs were identified in 73 (9.0%) patients.
*The multiple Abs identified were anti-c+E (18 patients), anti-C+e (14 patients), anti-D+C (1 patient), Abs with anti-E or anti-C (22 patients), anti-Le^a+Le^bH (7 patients), anti-Le^a+Le^b (5 patients), other multiple Abs (6 patients).

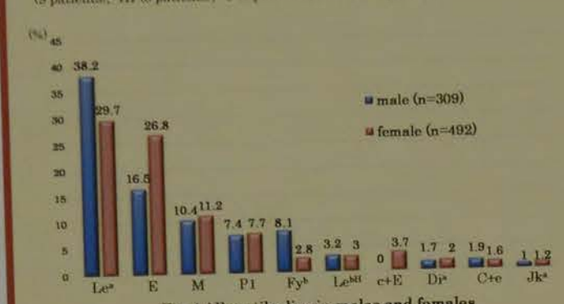


Fig.4 Alloantibodies in males and females

*The number of patients in male and female groups were 309 and 492 patients, respectively.
*Anti-E , and anti-c + E were more frequently observed in female than in male patients.

Fig.5 Alloantibodies in pregnant and non-pregnant females

*The number of patients in the pregnancy and non-pregnancy groups were 76 and 416 patients, respectively.
*Anti-M, anti-c + E and anti-C + e were more frequently observed in pregnant than in non-pregnant patients.
*Rh antibodies account for 48.7% of all alloAbs in pregnant patients.

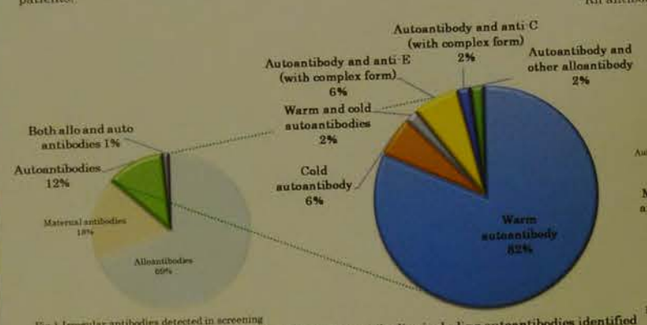


Fig.1 Irregular antibodies detected in screening (n=1,160)

Fig.6 Irregular antibodies including autoantibodies identified (n=154)

*The number of patients who had irregular Abs including autoAbs was 154.
* Among the 154 patients, those who had just warm autoAb were 126 patients ,those who had just cold autoAb were 9 patients, those who had warm and cold autoAbs were 3 patients, those who had autoAb and anti-E (with complex form) were 10 patients, those who had autoAb and anti-C (with complex form) were 3 patients and those who had autoAb and other alloAb were 3 patients.

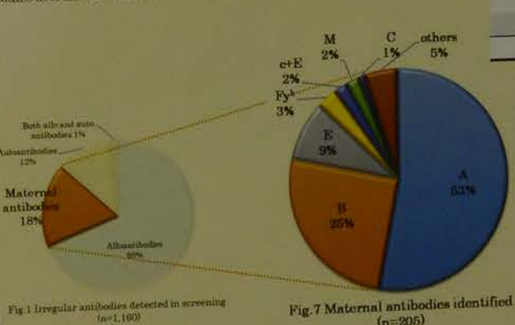


Fig.1 Irregular antibodies detected in screening (n=1,160)

Fig.7 Maternal antibodies identified (n=205)

*The maternal Abs identified were anti-A (109 patients), -B (52 patients), -E (18 patients), -Fy^b (6 patients), -c + E (4 patients), -M (4 patients), -C (2 patients), others (10 patients).

Table.2-1 The number of transfusion in patients who had alloAbs

Irregular antibodies	Patients	Irregular antibodies	Patients
anti-E	62	anti-Le^a (including multiple)	21
anti-C	3	anti-M	10
anti-D	1	anti-Di^a	7
anti- C + e	6	anti-Jk^a (including multiple)	7
anti- c + E	3	anti-Jk^b (including multiple)	2
Rh + autoantibody	10	anti-P1	6
Rh + Kidd	5	anti-Fy^b (including multiple)	6
Rh + Duffy	2	Anti-S	1
Rh + others	7	Total	159

Table.2-2 The number of transfusion in patients who had alloAbs

Irregular antibodies	Patients
anti-Le^a (including multiple)	36
anti-Le^bH	6
anti-Le^b	1
anti-P1	10
anti-M	5
anti-N	1
anti-HI	6
anti-I	2
anti-JMH	1
Total	68

*Among the 1,160 patients who had irrregular Abs, 308 (26.6%) were transfused.
*In those who had alloAbs, 227 patients were transfused.
* 159 patients of those 227 required antigen-negative red blood cell(RBC) and 68 patients of them used random RBC.(didn't require antigen-negative RBC)

Table.3 The number of transfusion in patients who had autoAbs

Irregular antibodies	RBC	Patients
warm autoantibody	E(-)c(-)	20
warm autoantibody	C(-)e(-)	4
warm autoantibody	E negative	2
warm autoantibody	c negative	1
warm autoantibody	E(-)c(-)S(-)	1
warm and cold autoantibodies	E(-)c(-)	1
warm autoantibody	random	23
warm and cold autoantibodies	random	1
cold autoantibody	random	7
Total		60

*In those who had autoAbs , 60 patients were transfused.
*We use RhCcDeE-matched RBC for patients who had autoAbs not to develop alloAbs. 29 patients required RhCDeE-matched RBC.

Table.4 The number of transfusion in patients who had maternal Abs

Irregular antibodies	RBC	Patients
anti-A	Exchange transfusion	4
anti-B	Exchange transfusion	5
anti-A	O positive	4
anti-B	O positive	3
anti-A	random	3
anti-B	random	2
Total		21

*In those who had maternal Abs, 21 patients were transfused.
*9 patients required an exchange transfusion and 7 patients used O positive RBC.

CONCLUSIONS

◎Dr. Toyama, in Tokyo University, reported that the frequency of Japanese irregular Abs is 1.90 % and that of alloAbs is 0.88 %. In this study, they were 1.99% and 1.38 %, respectively. Our results with the increase of irregular Ab screening tests might be attributed to an increase of more complex surgeries which require a lot of transfusions and of higher risk pregnancies who have irregular Abs.

◎Irregular Abs are classified with natural Ab and immune Ab, and contain alloAb and autoAb. AlloAbs are produced by immune responses to alloantigen of transfusion and pregnancy. AutoAbs, which attack a person's own red cells, cause hemolytic anemia. In this study, among the 154 patients who had autoAbs, 16 patients were found to have both alloAbs and autoAbs.Patients who have autoAbs easily produce alloAbs after transfusion, and Rh type-matched RBC is recommended for use in those patients. Therefore, we select the Rh type-matched RBC for patients who have autoAbs.

◎Dr. Takeshita, in Hamamatsu Medical University, reported that irregular Abs identified in Japan are anti-E(26.5%), anti-Le^a(25.7%), anti-P1(10.6%), anti-M(6.2%), anti-c + E(4.1%), anti-Le^b(3.0%), anti-D(1.6%), anti-Fy^b(3.7%), and anti-Di^a(3.3%). We also observed a similar result in our hospital.

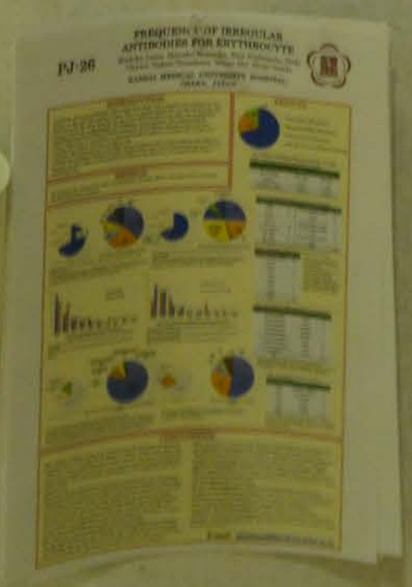
◎The frequency of irregular Abs depends on the frequency of antigens and the difference of immunogenicity to alloantigens. *Japanese Journal of Transfusion and Cell Therapy* clarifies the specificity of clinically significant irregular Abs and suggests that a person, who had developed a clinically significant irregular Ab, should be matched with antigen-negative RBCs.

◎The frequency of E(-)c(-) in Japan is 43 %. Indeed, among the 308 patients who had been given transfusions, 97 patients (31.5 %) required E(-)c(-), which was the highest type in our study.

We have experienced that a patient possessing irregular Ab was given antigen-positive RBCs in an emergency case and a titer of the irregular Ab of this patient was increased later. In such a case, it is necessary to monitor an onset of hemolytic diseases and the response of the transfusion.

In general, the selection of RBC and a cross-matched test take time. Therefore, irregular Ab screening in advance is prerequisite to prepare RBCs promptly even in emergency case. The results from this study would be useful for safe transfusion.

関西医科大学附属病院
KANSAI MEDICAL UNIVERSITY HOSPITAL



Transfusion medicine

PJ-27

Current status of perioperative blood transfusion in Cancer Institute Ariake Hospital

Rui Kojima, Kyoko Komatsu, Konosuke Nakayama, Takeo Juji, Masami Ogawa, Yumiko Okamoto, Megumi Suzuki

Blood Transfusion Department of The Cancer Institute Ariake Hospital

INTRODUCTION

Although the supply of blood transfusions from blood centers at this moment is satisfactory in Japan, there are growing concerns about shortage of transfusion blood for approximately one million people in 2027, due to decreasing blood donation and accelerating demographic aging.

In order to consider counter measures against this situation, we performed a usage survey of blood transfusions related to 25,037 surgeries during past three years in Cancer Institute Ariake Hospital. We also examined the longitudinal blood hemoglobin concentration of cases without blood transfusion between three days before and twenty-eight days after surgery.

METHODS

- We investigated the 25,037 operations of head and neck, orthopedics, urology, gynecology, gastroenterology, breast, respiratory cancer, and plastic surgery from January 2013 to December 2015 in Cancer Institute Ariake Hospital.
- We categorized patients into four groups according to the intraoperative blood loss as follows; group A 15% or more and less than 20%, group B 20% or more and less than 30%, group C 30% or more and less than 40%, and group D 40% or more and less than 50%. We investigated blood hemoglobin concentration of non-transfusion cases between 3 days before surgery and postoperative day(POD) 28.
- We did not investigate the cases of blood loss less than 15%, following the guideline by the Health, Labor and Welfare Ministry, which indicates that blood component transfusion should be performed for cases with more than 15% blood loss.

RESULT

(a) Blood loss and transfusion rate

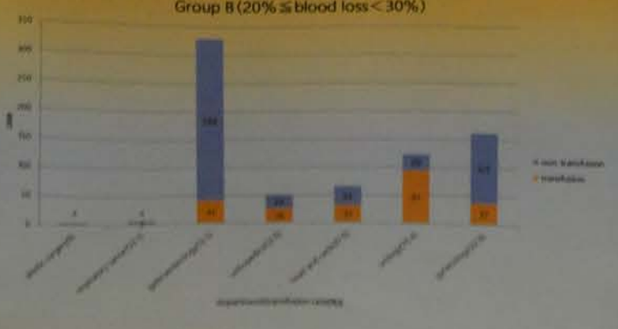
In this study, we focused on head and neck, orthopedic, and urological surgeries because of high transfusion rate. For group A, the lowest blood transfusion rate among these three surgeries were 29.4% of orthopedic (1), for group B, 47.6% of head and neck (2), for group C, 77.7% of head and neck (3), and for group D, 87.8% of urological (4), respectively. However, 81.7% of urological transfusion cases of all groups were transfused only autologous blood. (5)

RESULT- (1)

Group A (15%≦blood loss<20%)

RESULT- (2)

Group B (20%≦blood loss<30%)

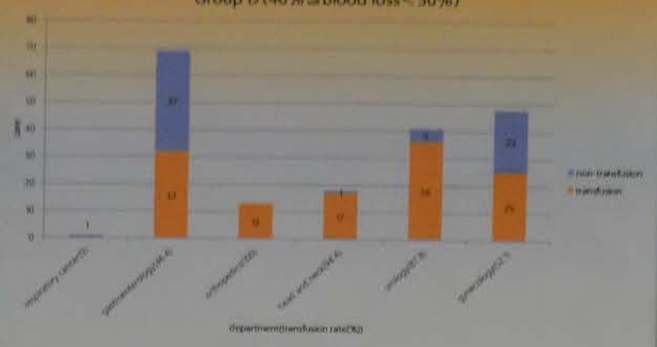


RESULT- (3)

Group C (30%≦blood loss<40%)

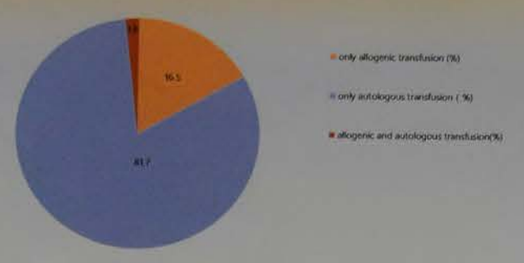
RESULT- (4)

Group D (40%≦blood loss<50%)



RESULT- (5)

Transfused group A~D(blood loss<50) in urological



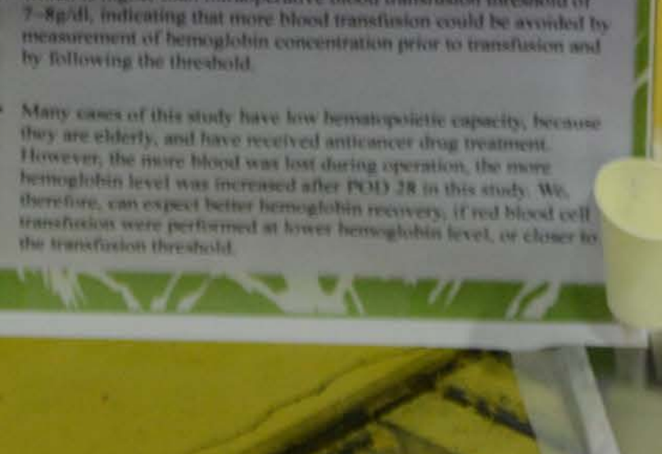
RESULT

(b) Longitudinal blood hemoglobin level of non-transfusion cases from 3 days before surgery until POD 28

The lowest mean hemoglobin level of non-transfusion cases of group A was 10.4g/dl on POD20, group B 10.0g/dl on POD18, group C 9.8g/dl on POD2, and group D 9.1g/dl on POD3, and hemoglobin level on POD28 was 10.8g/dl, 10.9g/dl, 10.8g/dl, and 10.7g/dl (6), respectively. The more blood was lost during operation, the more hemoglobin level was increased after surgery.

RESULT- (6)

Hemoglobin level of non-transfusion cases



DISCUSSION

- Covering more than 80 percent of urological transfusion cases, autologous blood transfusion can reduce red blood cell transfusion as expected.
- In order to promote more autologous blood transfusions, we have to overcome some barriers, such as time and cost of cancer cell elimination by leukocyte removal filter from patient blood, and autologous blood collection space and blood collecting technologists.
- In addition, blood removal by autologous blood collection stimulates hematopoietic capacity, which consequently leads to the reduction of red blood cell transfusion rate as well.

DISCUSSION

- The lowest hemoglobin level of non-transfusion cases was 9.1g/dl, which is higher than intraoperative blood transfusion threshold of 7–8g/dl, indicating that more blood transfusion could be avoided by measurement of hemoglobin concentration prior to transfusion and by following the threshold.
- Many cases of this study have low hematopoietic capacity, because they are elderly, and have received anticancer drug treatment. However, the more blood was lost during operation, the more hemoglobin level was increased after POD 28 in this study. We, therefore, can expect better hemoglobin recovery, if red blood cell transfusion were performed at lower hemoglobin level, or closer to the transfusion threshold.

Transfusion medicine

PJ-28

Time-dependent change of chemokine content and osmotic fragility of stored red blood cells

Shu Ogasawara[1)], Mihoko Kushibiki[1)], Ryoko Nakata[1)], Emi Ota[1)], Hiroyuki Kayaba[2)], Hideki Takami[3)]

1)Clinical Laboratory, Hirosaki University Hospital, Aomori, Japan
2)Department of Clinical Laboratory Medicine, Hirosaki University Graduate School of Medicine , Aomori, Japan
3)Department of Pathophysiological Laboratory Sciences, Hirosaki University Graduate School of Health Sciences , Aomori, Japan

HIROSAKI UNIVERSITY

Introduction

Non-hemolytic transfusion reaction is a type of transfusion reaction such as fever and allergy. It is known that long-term storage of red blood cells is associated with a significant increase of complications; however, the mechanism of the non-hemolytic transfusion reaction has not been well-understood[1)2)]. Red blood cells are known to scavenge and store Duffy antigen-binding chemokines.

We hypothesized that intracellularly stored chemokines may play roles in the development of non-hemolytic transfusion reactions.

Objective

We investigated the change of chemokine concentrations in stored red blood cells according to the storage duration. The change of red blood cell osmotic fragility was also investigated.

Methods

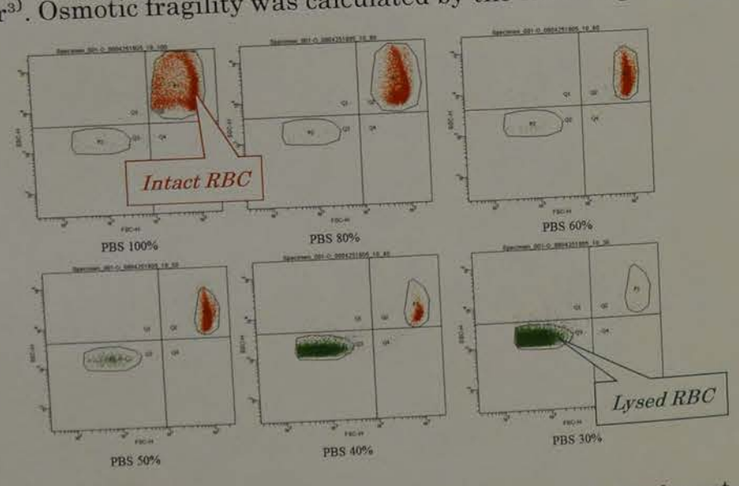
1. Chemokine concentrations in stored red blood cells

The extra and intracellular contents of RANTES, eotaxin-1 and MCP-1 were measured in stored red blood cells. The stored red blood cells (n=15) were divided into 3 groups according to the duration of storage.

Group	Blood type	Day of storage	average(day)±SD	median(day)
New (n=5)	B	9	9.4±0.9	10
	O	10		
	A	10		
	A	10		
	A	8		
Old (n=5)	O	20	19.4±1.5	20
	O	17		
	O	21		
	B	20		
	O	19		
Over (n=5)	B	46	42.4±2.6	42
	O	44		
	O	42		
	B	40		
	O	40		
Total (n=15)			23.7±14.4	20

2. The change of red blood cell osmotic fragility

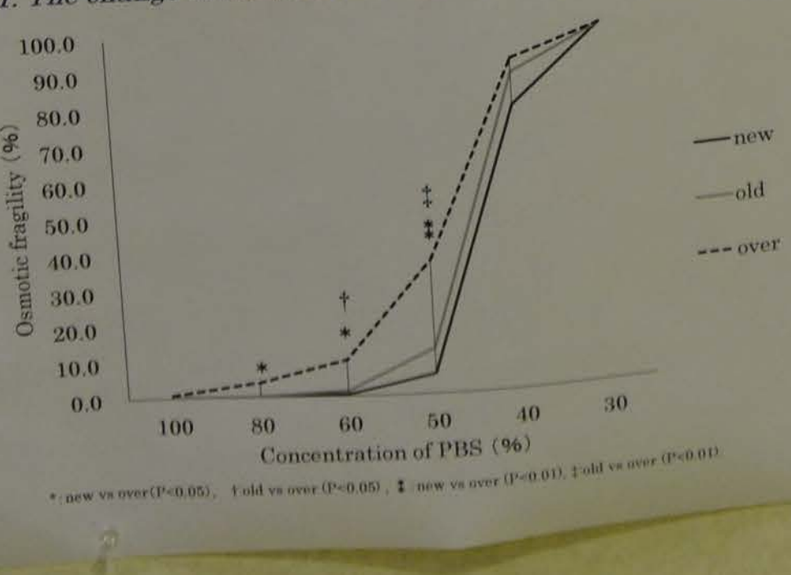
The change of red blood cell fragility was analyzed using flow cytometry. Red blood cells were suspended in phosphate-buffered saline (PBS) adjusted at various osmotic pressures with distilled water[3)]. Osmotic fragility was calculated by the following equation.



Osmotic fragility(%) = Count in lower left quadrat × 100 / Total count

Results

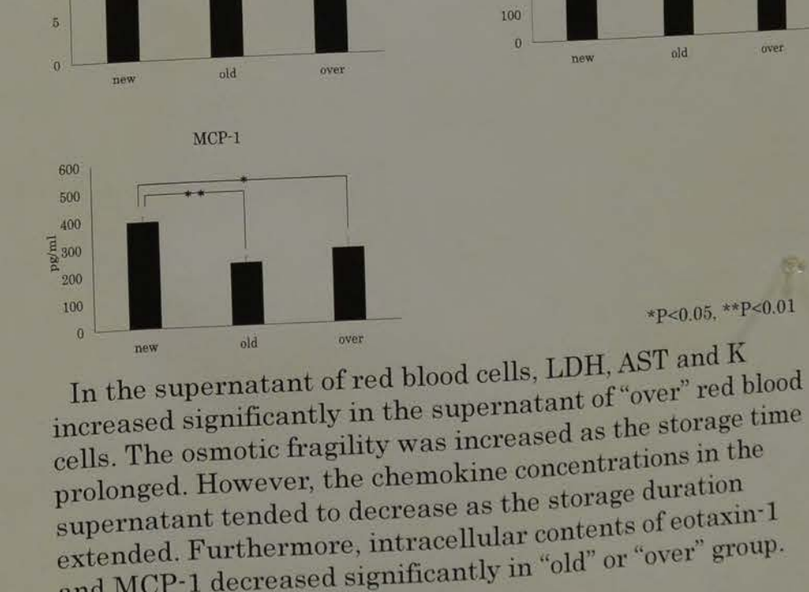
1. The change of red blood cell osmotic fragility



Results

2. Concentrations of chemokines and cytotoxic markers

Supernatant



Intracellular

*P<0.05, **P<0.01

In the supernatant of red blood cells, LDH, AST and K increased significantly in the supernatant of "over" red blood cells. The osmotic fragility was increased as the storage time prolonged. However, the chemokine concentrations in the supernatant tended to decrease as the storage duration extended. Furthermore, intracellular contents of eotaxin-1 and MCP-1 decreased significantly in "old" or "over" group.

Conclusion

Our results showed that chemokines in stored red blood cells decrease as the storage duration extended. The hypothesis that the intracellularly stored Duffy antigen-binding chemokines released into the supernatant of stored red blood cells may cause non-hemolytic transfusion reaction was, therefore, not supported in this study. However, the stored blood cells used in this study had not caused side effects at the preceded clinical use. Further investigation is needed to clarify the causes of non-hemolytic transfusion reactions.

Reference

1) Heddle N M, et al. The role of the plasma from platelet concentrates in transfusion reactions. N. Engl. J. Med., 331 : 625-628, 1994.
2) Koch C G, et al. Duration of Red - Cell Storage and Complications after Cardiac Surgery. N. Engl. J. Med., 358 : 1229-1239, 2008.
3) Yamamoto A, et al. Flow Cytometric Analysis of Red Blood Cell Osmotic Fragility. J. Lab. Automat., 19(5) : 483-487, 2014.

Transfusion medicine

PJ-29

Immunological responses after massive blood transfusions after cardiovascular surgeries

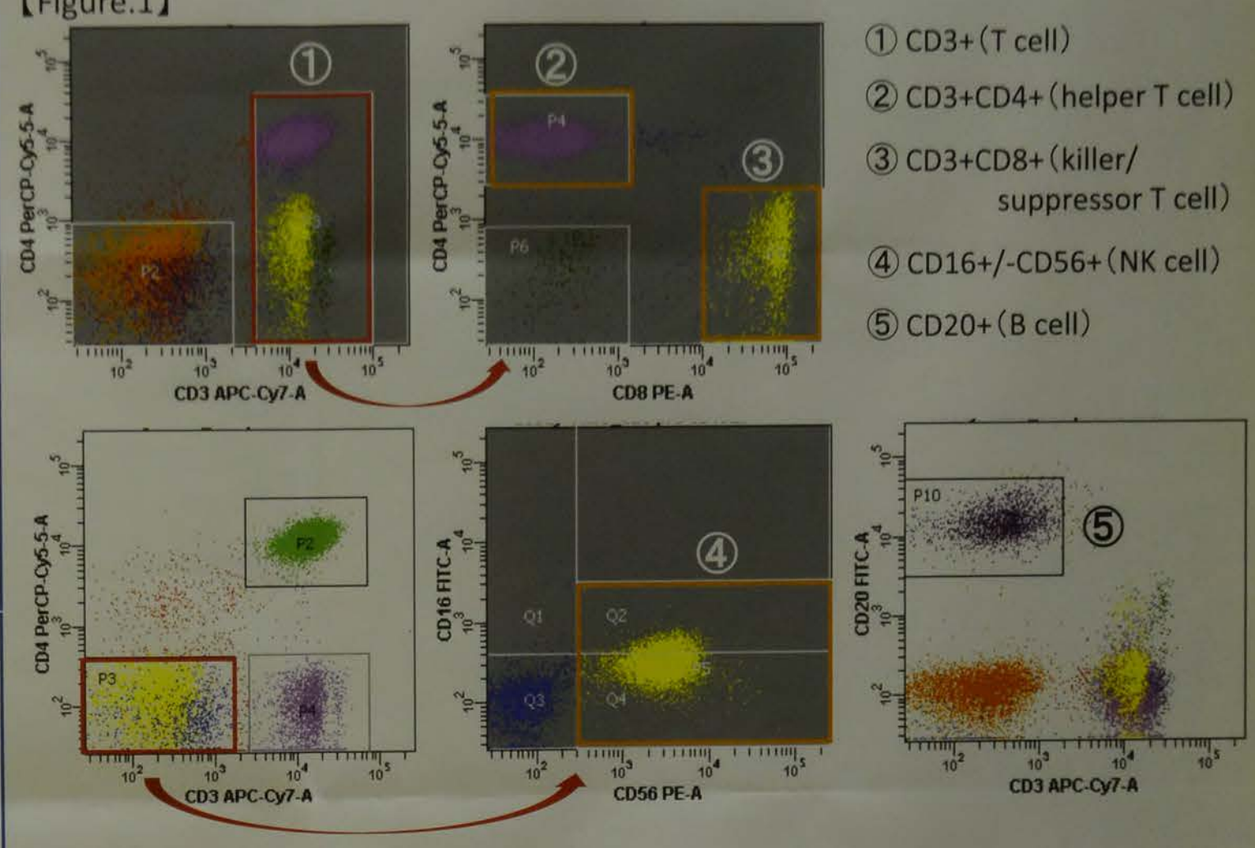
Marie Yamada[1)],Naotomo Yamada[1)],Nakao Mami[1)], Takanori Higashitani[1)], Yasushi Kubota[2)], Shinya Kimura[2)] and Eisaburo Sueoka[3)]

[1]Department of Laboratory Medicine, Saga University Hospital, [2]Department of Internal Medicine, Faculty of Medicine, Saga University, [3]Department of Clinical Laboratory Medicine, Faculty of Medicine, Saga University

Background :
It has been reported that allogeneic blood transfusions deteriorate the prognosis after the treatment. However, the reasons of this phenomenon have not been fully elucidated yet. We focused on the effects for immune system after allogeneic transfusions who received massive blood transfusions after cardiovascular surgeries. To analyze the activity of immune system before and after transfusions, we used flow cytometry for evaluation of immune status, and the results were compared with other clinical parameters and coincidences, such as infectious complications.

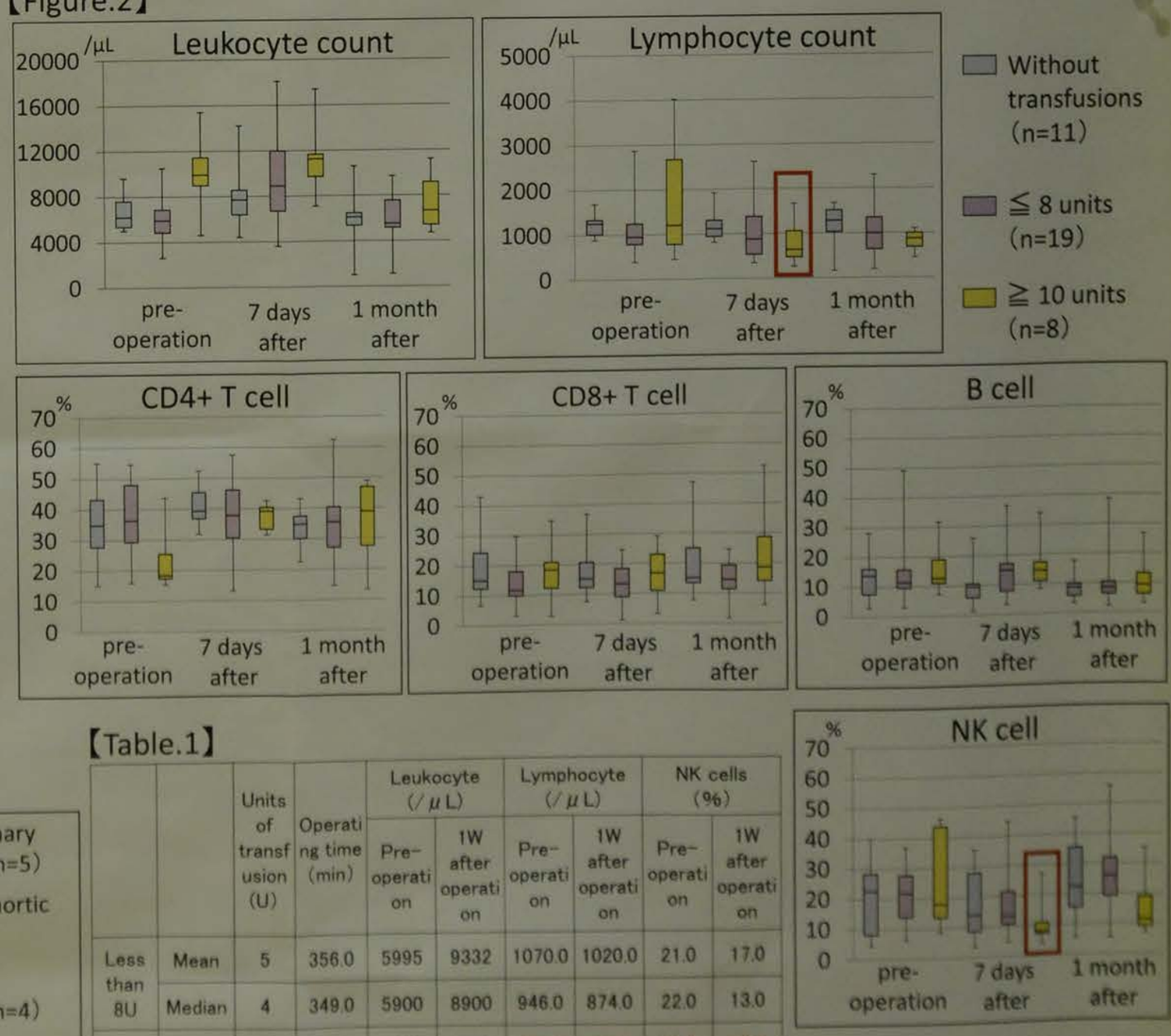
Methods :
Patients who received cardiovascular surgery were included in this study after obtained written informed consents. Flow cytometry analyses were conducted using peripheral blood collecting pre-operation, 7 days after and 1 month after operation. The surface antigens used for flow cytometry analysis of immunocytes are CD3, CD4 and CD8 for T cells, CD20 for B cells, CD56 for NK cells, respectively (Fig.1).

【Figure.1】

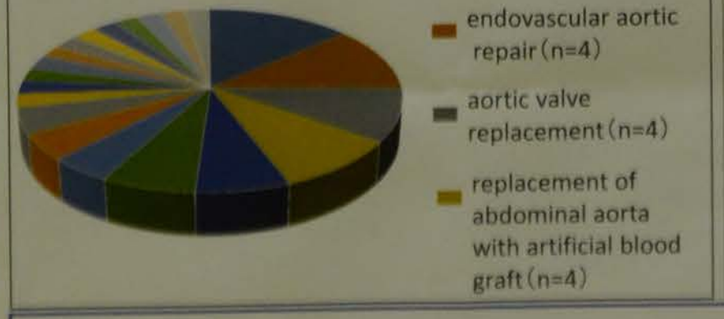


Results :
Among 51 patients, 38 patients who received one month's follow-up after the surgery were included in this analyses. 38 cases (25 male and 13 female) include 11 none-transfused, 27 transfused (19 cases; less than 8U red blood cell products, 8 cases; over 10U red blood cell products)(Fig.2). The most common operation method was autoperfusion apparatus for coronary artery bypass surgery under heartbeat (n=5), following endovascular aortic repair(n=4)(Fig.3). Post-operative infections were observed in 4 cases who received transfusions. Although the results were not statistically significant, the ratio of NK cells in the group with massive transfusion tended to be low compared with non transfused patients.

【Figure.2】



【Figure.3】



【Table.1】

		Units of transfusion (U)	Operating time (min)	Leukocyte (/μL) Pre-operation	Leukocyte (/μL) 1W after operation	Lymphocyte (/μL) Pre-operation	Lymphocyte (/μL) 1W after operation	NK cells (%) Pre-operation	NK cells (%) 1W after operation
Less than 8U	Mean	5	356.0	5995	9332	1070.0	1020.0	21.0	17.0
	Median	4	349.0	5900	8900	946.0	874.0	22.0	13.0
Over 10U	Mean	15	467.0	10125	11050	1752.0	822.0	25.0	10.0
	Median	14	378.0	9900	11250	1202.5	634.8	18.0	8.0
p			n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.

RBC: Red Blood Cells
n.s.: not significant

Conclusion :
Our preliminary results suggested that allogeneic transfusions induced decrease of immunocytes, such as T cells and NK cells after surgery.

【References】

(1) JP Cata, H Wang, V Gottumukkala, I Reuben, DI Sessler Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions. Br J Anaesth. 110:(2013) pp. 690-701
(2) Kanda M, Kobayashi D, Tanaka C, Iwata N, Yamada S, Fujii T, Nakayama G, Sugimoto, Koike M, Nomoto S, Murotani K, Fujiwara M, Kodera Y Adverse prognostic impact of perioperative allogeneic transfusion on patients with stage II/III gastric cancer. Gastric Cancer. 2016 Jan;19(1):235-43.
(3) Sun C, Wang Y, Yao HS, Hu ZQ. Allogeneic blood transfusion and the prognosis of gastric cancer patients: systematic review and meta-analysis. Int J Surg. 2015 Dec;13:102-10.
(4) Wang T, Lin J, Huang H, Yu J, Pan C, Cai X, Hu B, Yin K. Perioperative blood transfusion is associated with worse clinical outcomes in resected lung cancer. Ann Thorac Surg. 2013 May;97(5):1827-33.
(5) Acheson AG, Brookes MJ, Spahn DR.Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. Ann Surg. 2012 Aug;256(2):235-44.

Transfusion medicine

PJ-30

DETECTION OF ENZYME TECHNIQUES IN IRREGULAR ANTIBODY

Yumiko Maezawa, Tatsuya Sugimoto, Hitomi Yamaguchi, Akifumi Koyama, Fumiaki Yoshiba, Nobumasa Kobayashi.
Division of Medical Technology & Department of Blood Transfusion Service, Tokai University Hospital.

Introduction

- It is important to use indirect antiglobulin test(IAT) for detecting 37℃ reactive irregular antibody.
- On the other hand, ficin enzyme techniques(Enz) are highly sensitive assays for the detection of developing Rh antibodies.

Table1. Ratio of Antigen-negative blood in Japan

Blood group	Anti body	Ratio of Antigen-negative blood (%)	Selecting Antigen-negative Blood for Transfution	Blood group	Anti body	Ratio of Antigen-negative blood (%)	Selecting Antigen-negative Blood for Transfution
Rh	D	0.5	Yes	Lewis	Le^a	83	Yes(IAT +) No(IAT -)
	C	10.9	Yes		Le^b	27	No
	E	50.6	Yes	MNS	M	22.3	Yes(IAT +) No(IAT -)
	c	44	Yes		N	28.1	No
	e	8.6	Yes		S	88.7	Yes
Kell	K	≧99.9	Yes		s	0.5	Yes
	k	≦0.01	Yes	Diego	Di^a	90.8	Yes
Duffy	Fy^a	1.1	Yes		Di^b	0.2	Yes
	Fy^b	80.4	Yes	Jacobs	Jr^a	0.03	Yes
Kidd	Jk^a	27.2	Yes				
	Jk^b	22.4	Yes				

Reference) Japanese Association of Medical Technologists: technical textbook ISBN978-4-621-08973-6(輸血・移植検査技術教本), 2016

- In Addition, the most frequency of irregular antibody in Japanese is anti-E. (Table1) Reference) Akihiro Takeshita., et al, COLLABORATIVE STUDY ON IRREGULAR ERYTHROCYTE ALLOIMMUNITY IN JAPAN AND ASIAN COUNTRIES, Japanese Journal of Transfusion and Cell Therapy, Vol. 60. No. 3 6(0 3):435—441, 2014
- Many facilities in Japan adopt to use Enz for irregular antibody screening(Scr). (Table2)
- However, It is considered that antibodies detected by Enz alone were clinically non-significant. Reference) Wataru Ohashi., et al, CLINICAL SIGNIFICANCE OF ENZYME TECHNIQUES IN IRREGULAR ANTIBODY SCREENING, Japan Society of Transfusion Medicineand Cell Therapy Journal, Vol. 56.No.6 56(6):709—715, 2010
- The number of facilities do not use Enz has been increasing recently.

Table2. Technique of Scr in Japan

	Saline Techniques	Enzyme Techniques	IAT
Test	46.1%	71.9%	99.5%
Not test	53.8%	28.0%	0.4%
Unknown	0.1%	0.1%	0.1%

Reference) Japanese Association of Medical Technologists: control survey report, 2015

- Our facility adopt to use IAT and Enz for irregular antibody screening, so we carry out the investigation about irregular antibodies detected by Enz.

Objects/ Methods

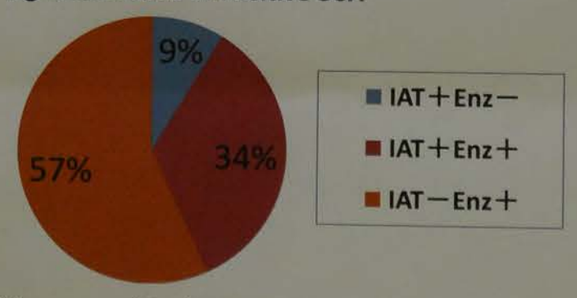
- Objects : A total of 36,357 tests were carried out for Scr by low ionic strength solution(Liss)-IAT and Enz between 2010 and 2015.
- Methods : We retrospectively analyzed the results of Scr.
  ① Positive ratio in Scr.
  ② This objects were analyzed about detected by Enz the kind of irregular antibodies.
- Instrument: Ortho clinical diagnostics brand AutoVue Innova (Gel Column Agglutination Technology).

Results: ① Positive ratio in Scr

Table3. Positive ratio in Scr.

Scr results		Enz +	Enz −
IAT	+	577 (1.6%)	145 (0.4%)
	−	954 (2.6%)	34,681 (95.4%)
Scr objects 36,357			

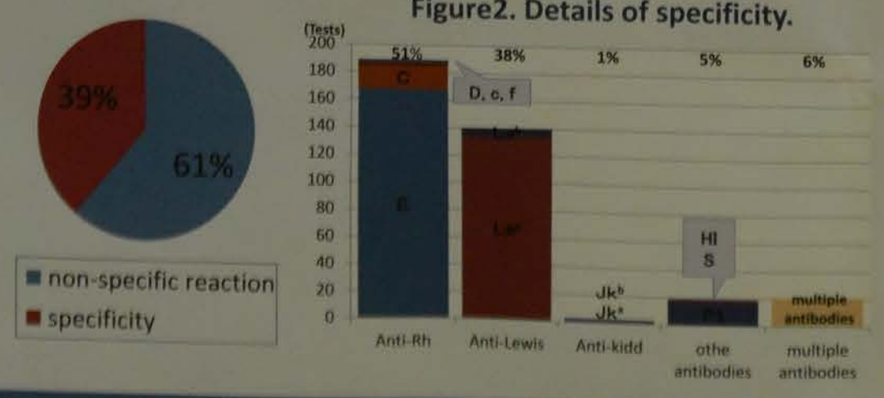
Figure1. Details of Positive Scr.



- 1,676 tests(4.6%) were positive by irregular antibody screening.
- In the all of positive reaction, 1531 tests (91%) were positive by Enz.

Results: ② Details of IAT− and Enz+

Figure2. Details of specificity.



- 61% of antibodies with positive by the Enz alone were non-specific reaction. Ratio of detected non-specific reaction in positive of Enz alone was significantly higher than positive of both IAT and Enz.
- 89% of antibodies with specific by Enz alone were Anti-Rh and Anti-Lewis.(Figure2)
- As for most one, Anti-Rh, the second were multiple antibodies in detected irregular antibodies by both IAT and Enz.(Figure3)
- 14% in detected irregular antibodies by both IAT and Enz were the combination of irregular antibodies with the enzyme sensitivity and other irregular antibodies.(Figure4)

Results: ② Details of IAT+ and Enz+

Figure3. Details of specificity.

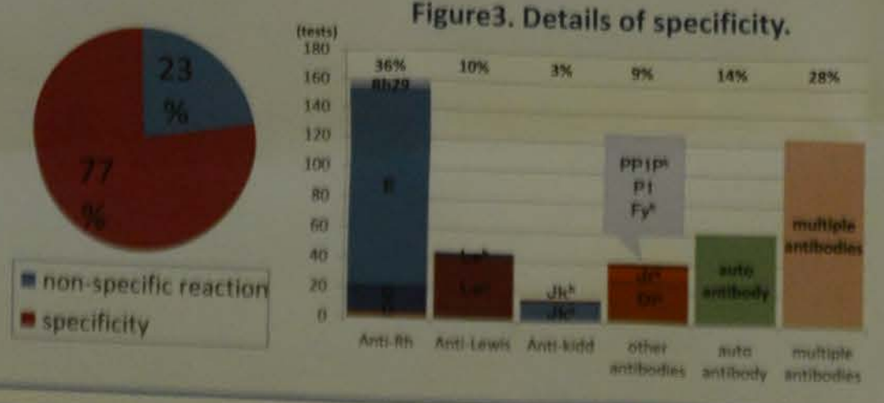
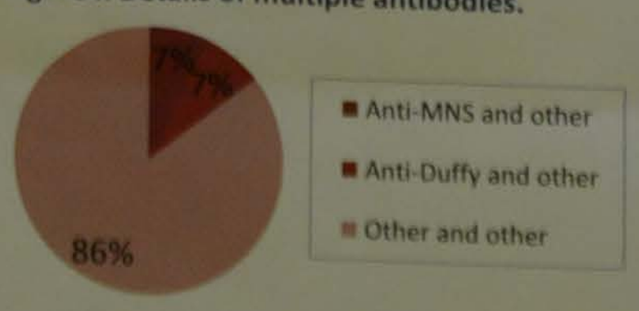


Figure4. Details of multiple antibodies.



Supplement: Necessary time of Enz in irregular antibody identification (Gel Column Agglutination Technology).

Conclusion

- 61% of antibodies with positive by the Enz alone were non-specific reaction. Ratio of detected non-specific reaction in positive of Enz alone was significantly higher($p \leqq 0.05$) than positive of both IAT and Enz.
- It is consider that irregular antibody detected by Enz alone was

Transfusion medicine

PJ-31

The strength of agglutination and plasma IgM and IgG levels in ABO reverse grouping

Kie Horioka M.T.(1), Akira Hayakawa M.D., Ph.D.(2). Nobuo Masauzi M.D., Ph.D.(3).

1) Technical Support Department, Faculty of Medicine, Hokkaido University, Sapporo.
2) Tokyo Medical Examiner's Office, Tokyo.
3) Dept. of Medical Laboratory Science, Faculty of Health Sciences, Hokkaido University, Sapporo, Japan.

Introduction

- ABO grouping is confirmed when the results of the forward grouping test agrees with that of the reverse grouping test.
- In ABO reverse grouping test, Anti-A and/or -B antibodies in plasma or serum samples agglutinate A and/or B red blood cells, respectively
- The agglutination strength considerably varies individually; the reason is unknown.
- In this study, we examined the agglutination strength and IgM and IgG levels in plasma samples to elucidate the correlation between them.

Materials & Methods

- Samples : 85 healthy adults. Age 20～30 years old.(41: blood type A, 21: blood type B, and 23: blood type O)

1. The strength of agglutination were measured with the routine method. (Fig.1.)
2. We measured total IgM levels of in plasma of 85 original samples by immunonephelometry. Reference ranges of IgM levels are 33~190mg/dl for male and 46~260mg/dl for female.
3. For 680 diluted samples, total IgM level in plasma of each sample was determined by calculating with each dilution rate.
4. Anti-A or -B antibody of IgM or IgG type was detected by ELISA. (Fig.2.)
5. All statistical analyses were performed with JMP pro 10 (SAS Institute, Inc. ,Cary, NC, USA). The difference among ABO blood groups were analyzed by one-way ANOVA and the Tukey-method.

Fig-1

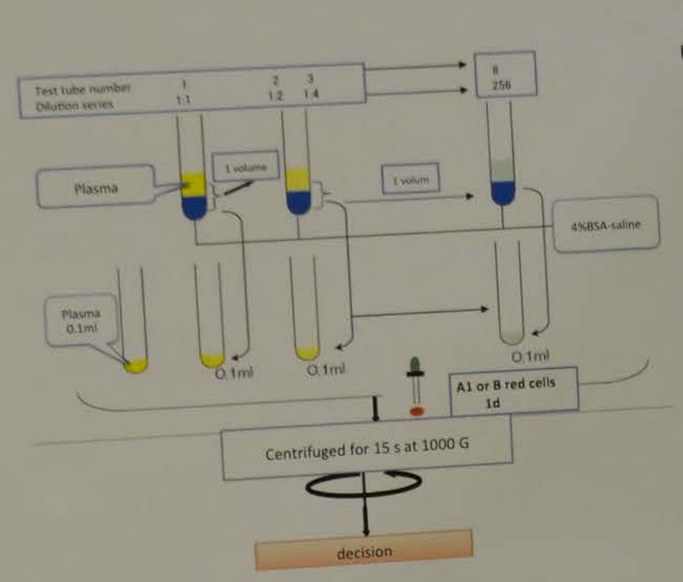
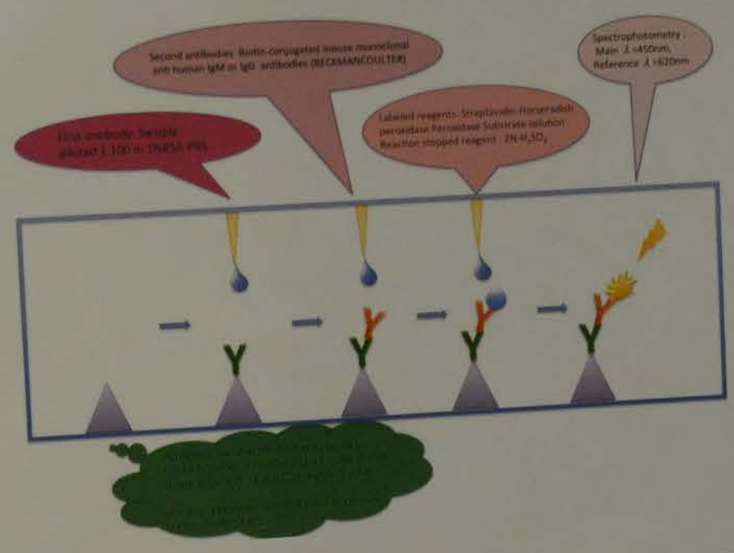


Fig-2



Result

Fig-3

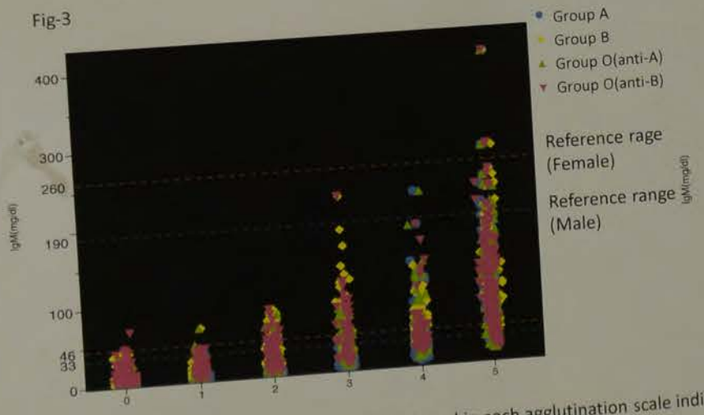


Fig-4

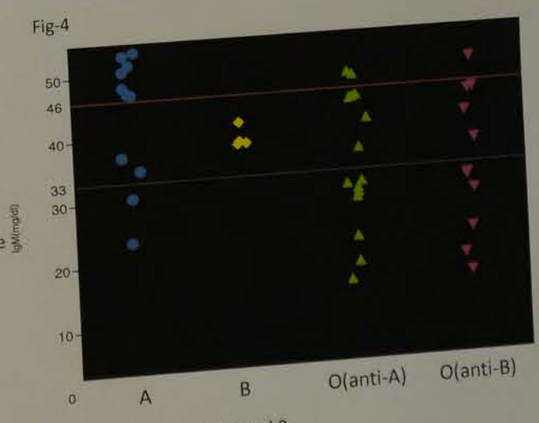


Figure-3: The total IgM levels were plotted in each agglutination scale indicated as 4+, 3+, 2+, 1+, w+ and 0.

Figure-4: The total IgM levels were plotted within agglutination scale 4 cases.
The number and the percentages in all 4+ cases (in parenthesis) in each scale, of which IgM levels were lower than reference range, ware indicated under each plots.

Fig-5

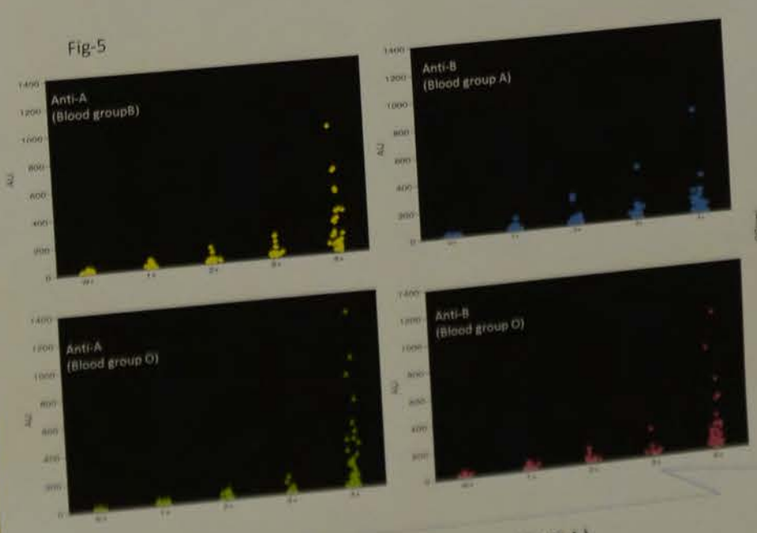
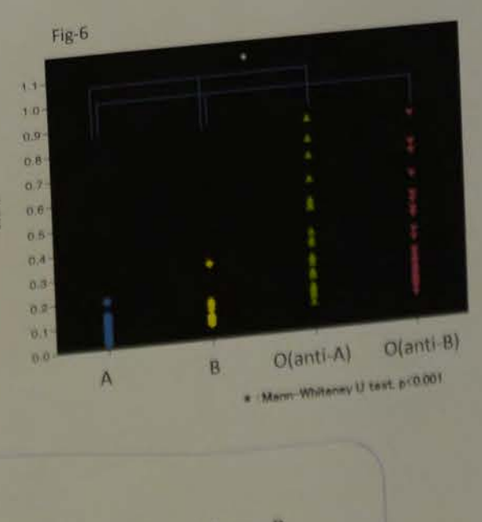


Fig-6



1.The strength of agglutination and mean IgM levels in plasma

Mean IgM levels (mg/dl) in each agglutination scale were indecated as follows, 4+; 99.1 in blood type A, 118.4 in blood type B, 87.9 in blood type O for A1 indicator cells, and 96.3 in blood type O for B indicator cells, 3+; 48.9, 43.9, 31.6 and 27.6, 2+; 28.3, 32.9, 21.6 and 17.6, 1+; 17.4, 15.7, 12.7 and 12.1, w+; 9.5, 6.9, 6.0 and 8.4, 0 ; 4.9, 5.1, 3.4 and 3.6, respectively.

The mean IgM levels in each scale were decreased to the lower as the agglutination strength became to the weaker. The IgM levels of 4+ cases widely varied. (Figure-3).

2. IgM in 4+ scale cases

The IgM levels of 4+ cases widely varied, and some cases indicated lower IgM level than reference range, which were more often recognized by cases in blood group O than the other blood groups. Such cases in each blood groups were as the following (the percentage of cases with lower IgM than lower reference range within all cases with 4+ scale of each blood group) ; 7%(4 cases), 13%(4 cases), 27% (12 cases) and 21% (9 cases), respectively, of the blood groups A (anti-B), B (anti-A), O (anti-A) and O (anti-B). (Figure-4).

3.Specific anti-A or –B antibody (ELISA)

No significant difference is observed in the absorbance of ELISA for anti-A or –B antibody of IgM type in plasma among blood group A, B, and O. (Figure-5).

Significant differences ($p<0.001$, Tukey-Kramer method) were observed in the absorbance of ELISA for anti-A or -B antibody of IgG type in plasma between blood group O and blood group A or B. (Figure-6).

Discussion

These results suggest that IgG might be involved in agglutination reaction in the clinical laboratory test more than we have been thought so far,especially in a case of O blood group.

We suggest that strong agglutination reaction occurs in blood group O, even if it is diluted, because blood group O contains more anti-A and -B antibodies of IgG type than other blood groups.

We are going to explore an further effective way for quantitative analysis of specific anti-A and -B antibodies.

Transfusion medicine

PJ-32

Auto Preparation of Autologous Fibrin Glue

Kaori Shimaya, Yuki Kobayashi, Takatoshi Osafune, Kumi Nakashizu, Kunihiko Miura, Chiharu Otokozawa

TEINE Keijinkai Hospital, Sapporo, Hokkaido, JAPAN

【Introduction】

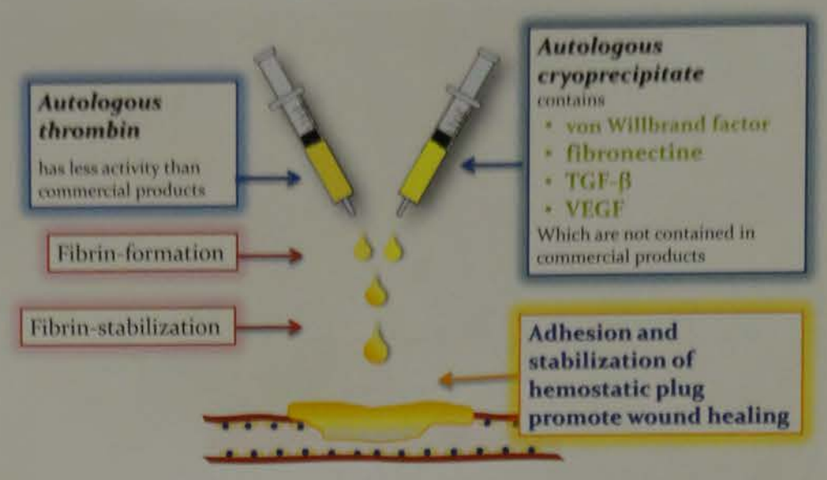
We started clinical use of autologous fibrin glue (AFG) and acquired an auto preparative machine.

The surgeon sprayed AFG on the affected area, evaluated effectiveness and assessed adverse events until day 7 after surgery.

【What's Fibrin Glue?】

Commercial products
Bolheal®
Tisseel®
Beriplast®....

【Coagulation Mechanism】



【Procedure】

•Obtain 200～400ml blood donation with 4 connected bags

•Centrifuge at 3500rpm for 10min. in a large centrifuge machine

•Separate plasma

• Register in the system

Autologous RBC Within 2~6℃

Autologous FFP Under −20℃

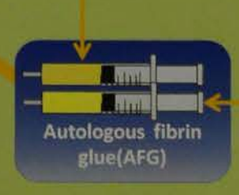
Thaw by the day before surgery

【 Use in Surgery 】

Thaw in the surgery room at 37℃

Register in the system and store

Auto preparation for 90 min.



Cryo poor plasma

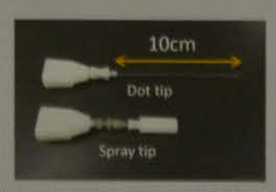
CryoSeal®
(Asahi KASEI Medical)

【Surgeries with AFG】 Age：18～81yrs Weight：50～81kg AFG volumes：4.4～10.0ml

Department	Digestive surgery (20casecs)	Gynecology (18cases)	Otological surgery (6cases)
Surgical method	• Pancreatoduodenectomy (11) • Distal pancreatectomy (2) • Esophageal cancer radical operation (2) • Total pancreatectomy (1) • Hepatectomy (4)	• Total laparoscopic hysterectomy (7) • Laparoscopic myomectomy (3) • Total abdominal hysterectomy (2) • Surgical removal of malignant tumor (2) • Extended hysterectomy (2) • Lymphnode dissection (2)	• Tympanoplasty (5) • Myringoplasty (1)
Surgeon's evaluation	✓AFG flows off the target because it gels slower than commercial products.	✓Need more volume ✓The tip in the kit is too short to operate laparoscopically.	✓The tip in the kit is unsuitable for application in limited space.

	Fib (mg/dℓ)	Thrombin activity (U/mℓ)	clotting time (min.)
AFG	2754	59.3	2.53
Bolheal®	8000	250	—
Beriplast®	8000	300	—

(Japanese Pharmacology and Therapeutics, 2012)



There are only 2 tips in the Cryoseal ® kit.

There are many tips in the Beriplast P combiset ® kit.

【Comparison between AFG and Commercial Products】

	AFG	Commercial products
Advantages	• No risk of infection • Reduces patients' anxiety, increases motivation and improves attitude .	• No need to prepare • It gels quickly • Variety of tips • Density uniformity
Dis-advantages	• Need to donate blood in advance • Need to procure equipment • Not covered by insurance in case AFG remains unused. (cost ¥72,300)	• Infection risk • Allergy reaction risk • Anaphylactic shock risk

【Adverse Events】

Surgical method	Date of occurrence	Contents
Esophageal cancer radical operation	POD7	Anastomotic leak delayed discharge, but improved by fasting.
Distal pancreatectomy	POD3	Pancreatic fistula improved after swapping drain tube.
Pancreato-duodenectomy	POD2	Intraperitoneal bleeding controlled after FFP transfusion. ※Patient had taken NOAC (Rivaroxaban®) before surgery.

【Conclusion】

We had started auto preparation of AFG, and it showed that this AFG is useful in surgery.

We need to assess the safety and effectiveness of auto-prepared AFG continuously.

Could you tell me about the current situation of autologous blood donation in your country?

Transfusion medicine

PJ-33

RECENT ADVANCES IN HLA TESTING: APPLICATION OF BEAD ARRAY TECHNOLOGY

Koki Fujiwara, Ph.D.
Department of Transfusion Medicine and Cell-processing,
Teikyo University School of Medicine

BACKGROUND: HLA testing is important in medicine when transplanting tissue or an organ.
In hematopoietic stem cell transplants, the HLA types that the donor and recipient have need to be the identical or match as closely as possible for a transplant to be successful and for the tissue to not be attacked or rejected by the recipient's immune system. HLA testing also includes screening transplant recipients for the presence of antibodies that might target the donated tissue or organ as part of an immune response. In addition, we previously reported that HLA antibodies, when the specificity corresponded to a mismatched antigen, had a negative effect on engraftment of unrelated cord blood transplants. Therefore, a reliable and high-throughput method is necessary for HLA testing. The recent introduction of high-throughput new technologies, such as Luminex (Luminex, TX, USA) (Fig. 1), has facilitated HLA typing (LABType; One Lambda, CA, USA) and detection of HLA antibodies (LABScreen; One Lambda). In contrast, a reliable and high-throughput method for HLA cross-matching has not yet been described.

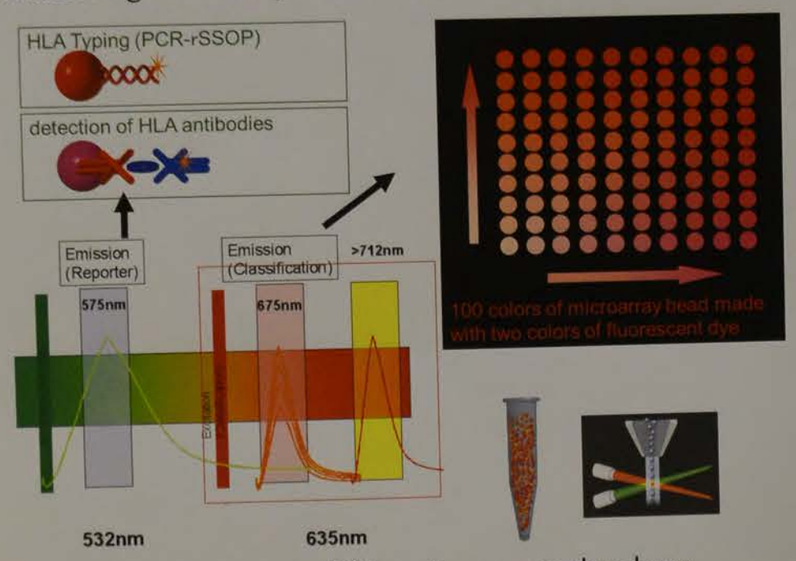


Fig. 1 Luminex[100] beads array technology

METHODS: Immunocomplex capture fluorescence analysis (ICFA) was established for high-throughput HLA cross-matching. This method was applied to flow cytometric analysis using Luminex microarray beads coupled with monoclonal antibodies specific for HLA class I and class II in order to detect HLA antibodies by capturing antigen-antibody complexes. The microarray beads were coupled to monoclonal antibodies specific for HLA class I and class II, respectively. Leukocytes that reacted with serum were lysed, and lysates were incubated with the above bead mixture in order to specifically capture antigen-antibody complexes against epitopes on each molecule (Fig. 2).

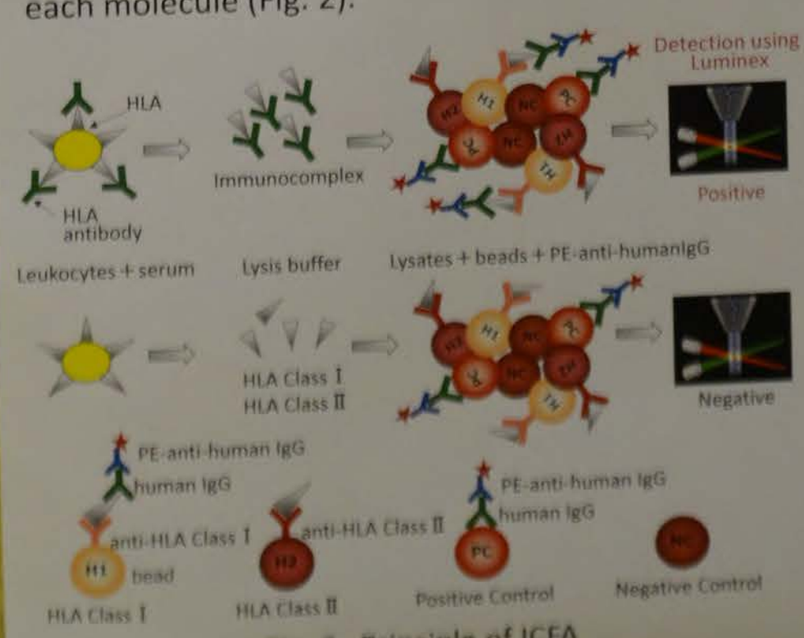


Fig. 2 Principle of ICFA

RESULTS: The method was validated with 65 HLA antibody samples. The method was able to detect all HLA antibodies with comparable sensitivity as purified HLA antigen-coated pooled-bead assay (LABScreen Single Antigen; One Lambda) (Table 1-4).

Table 1. Detection of HLA Class I antibodies in diluted antiserum samples by ICFA

No.	Sample	Panel	HLA Median	PC	BG	HLA Index	PC	BG	HLA Result	PC	BG	Specificity
1	NC	A26,- B39,61 Cw7,-	223	16556	71	1.00	74.25	1.00	-	+	-	NC
	QC04-S1 x4	A26,- B39,61 Cw7,-	579	16212	84	2.19	61.45	1.00	+	+	-	A26
	QC04-S1 x8	A26,- B39,61 Cw7,-	416	16993	64	2.07	84.54	1.00	+	+	-	
2	NC	A24, 2 B51,- Cw -,-	134	17331	28	1.00	129.82	1.00	-	+	-	NC
	QC04-S3 x4	A24, 2 B51,- Cw -,-	785	19202	59	2.79	68.26	1.00	+	+	-	B5
	QC04-S3 x8	A24, 2 B51,- Cw -,-	674	20737	56	2.52	77.66	1.00	+	+	-	
3	NC	A24,- B60,71 Cw7,10	376	18971	89	1.00	50.19	1.00	-	+	-	NC
	QC04-S5 x4	A24,- B60,71 Cw7,10	700	18303	72	2.29	59.85	1.00	+	+	-	A24
	QC04-S5 x8	A24,- B60,71 Cw7,10	650	18983	70	2.19	63.85	1.00	+	+	-	
4	NC	A 2,- B61,62 Cw7,-	374	19876	102	1.00	53.14	1.00	-	+	-	NC
	C1 x4	A 2,- B61,62 Cw7,-	411	21347	54	2.07	107.28	1.00	+	+	-	A2
	C1 x8	A 2,- B61,62 Cw7,-	361	20141	53	1.85	103.13	1.00	-	+	-	
5	NC	A24, 2 B51,- Cw -,-	357	21358	101	1.00	59.83	1.00	-	+	-	NC
	C2 x4	A24, 2 B51,- Cw -,-	459	22386	62	2.09	102.15	1.00	+	+	-	B51
	C2 x8	A24, 2 B51,- Cw -,-	351	21635	60	1.66	102.01	1.00	-	+	-	
6	NC	A24,26 B61,- Cw9,-	422	21245	128	1.00	50.34	1.00	-	+	-	NC
	FH x4	A24,26 B61,- Cw9,-	560	19904	82	2.07	73.62	1.00	+	+	-	B61
	FH x8	A24,26 B61,- Cw9,-	548	20899	86	1.93	73.71	1.00	-	+	-	
7	NC	A24,- B60,71 Cw7,10	301	18904	85	1.00	62.80	1.00	-	+	-	NC
	YH x4	A24,- B60,71 Cw7,10	472	19170	63	2.10	85.42	1.00	+	+	-	A24
	YH x8	A24,- B60,71 Cw7,10	405	18644	60	1.80	87.23	1.00	-	+	-	
8	NC	A11,24 B54,- Cw1,-	272	20074	78	1.00	73.94	1.00	-	+	-	NC
	13135 x4	A11,24 B54,- Cw1,-	615	17888	24	7.36	65.89	1.00	+	+	-	B7+22
	13135 x8	A11,24 B54,- Cw1,-	599	19601	68	2.53	25.48	1.00	+	+	-	

Serum samples containing HLA antibodies were diluted to a level not detectable by anti-Human immunoglobulin-complement dependent cytotoxicity (AHG-CDC) and were tested by ICFA. The sensitivity of ICFA was verified to be >eightfold higher than that of AHG-CDC, which is comparable to that of LABScreen Single Antigen.

CUT-OFF INDEX
(sample serum values / negative control serum values) / (sample serum background / negative control serum background).
A cut-off index of more than 2.0 was used for the designation of positivity.

Table 2. Reactivities of HLA Class I antibodies determined by LABScreen PRA, LABScreen SA, and ICFA

Sample	Specificity of HLA Class I antibodies detected by LABScreen PRA, LABScreen SA, and ICFA	detected by LABScreen SA, ICFA	detected by LABScreen SA
S01	B35 B62 B75 B56 B51 B52 B46	Cw10 B71	
S02	B7 B60 B61	B13 B48	
S03	A26		
S04	Cw8 A2		B37
S05	B60 B61 B13 B48		
S06	A26		
S07	B51 A24 B52 B44	B37 B13	
S08	B60 B7 B48 B61 B13		
S09	B62 B75	B13	B46
S10	B60 B61	B7 A11.1	
S11	B58 B51 A24 B52 B13 B44 B37 B59	B35	
S12	B56 B54 B75	B67 B55	
S13	A24		B51
S14	A24 B44	A2	A1
S15	B62 B75 B35 B56 B51 B46 B52 B54	Cw10 B71 B37	
S16	A1		
S17	A2 A24 B58 B35 B52	B51	
S18	A2 A24 B58	B51	
S19	B60 B61 B7 B13 B44 B48		
S20	B60 B48 B61 B13 B62 Cw8	B7	

LABScreen PRA (medium resolution ; One Lambda)
LABScreen Single Antigen (LABScreen SA : high resolution ; One Lambda)

Table 3. Detection of HLA Class II antibodies by ICFA

Sera	Panel	Median HLA-2	PC	NC	Index	Result
NC		222.5	21323.0	61.0		
DR4	DR4 / -	832.0	21343.0	29.0	7.87	+
DR9		285.0	21081.0	68.0	1.15	-
NC		180.0	20965.0	77.0		
DR9	DR9 / -	369.5	21421.0	45.0	3.51	+
DR15,16		201.0	21585.0	74.0	1.16	-
NC		113.0	21135.0	136.0		
DR15,16	DR15 / -	1422.0	21234.0	107.0	15.99	+
DR12		175.0	21451.0	174.0	1.21	-
NC		155.0	20548.0	43.0		
DR12	DR12 / -	539.0	19750.5	27.0	5.54	+
DR4		109.0	20223.0	29.0	1.04	-
NC		72.5	15376.0	35.0		
DR8	DR8 / -	329.0	15116.0	30.0	5.29	+
DR1		42.5	15200.5	22.0	0.93	-

Sera	Panel	Median HLA-2	PC	NC	Index	Result
NC		214.0	21499.0	45.0		
DR15,16	DR15 / -	1400.0	20839.0	64.0	4.60	+
DR4		45.0	20585.0	59.0	0.16	-
NC		172.0	19283.0	58.0		
DR4	DR4 / -	2006.0	20987.0	58.0	11.66	+
DR15,16		93.0	21363.0	59.0	0.53	-
NC		228.5	20492.0	90.0		
DR13,14	DR13 / -	988.0	19959.0	98.0	3.97	+
DR15,16		181.0	20646.5	59.5	1.20	-
NC		136.0	20816.0	36.5		
DR15,16	DR15 / -	489.0	20624.5	36.0	3.65	+
DR13,14		131.0	20020.0	30.0	1.17	-
NC		44.5	16545.0	59.5		
DR1	DR1 / -	1162.0	16879.0	42.0	36.99	+
DR8		47.0	16657.5	46.5	1.35	-

A cut-off index of more than 2.0 was used for the designation of positivity.

Table 4. Reactivities determined by AHG-CDC, LABScreen PRA, LABScreen SA, and ICFA in 65 HLA antibody samples

	AHG-CDC	LABScreen PRA	LABScreen SA	ICFA
Positive	35	60	65	65
Weakly positive	6	5	0	0
Negative	24	0	0	0

ICFA was validated using 65 serum samples containing HLA antibodies that were detectable by LABScreen PRA.

CONCLUSION: We developed a reliable and high-throughput method for Luminex-based HLA cross-matching, therefore all HLA testing technologies could adapt to the Luminex. In Japan, ICFA is widely used as an alternative method to the conventional complement dependent cytotoxicity test (CDC). Moreover, this method can be used to simultaneous detection of HLA and HPA antibodies by using different monoclonal antibody epitopes.

Transfusion medicine

PJ-34

STUDY OF THE PERFORMANCE AND USEFULNESS OF "ORTHO VISION Max" in TMDU

Department of Transfusion Medicine
Tokyo Medical and Dental University Medical Hospital

SHIHO Kobayashi, NAOKI Otomo, KAORU Okuyama, SHIHOKO Suwa, and MICHIKO Kajiwara

The automation of blood transfusion testing is useful for preventing transfusion errors due to human error. "ORTHO VISION Max (Ortho Clinical Diagnostics; OCD)" is new equipment that combines large-sample processing and emergency-sample processing to prevent human error and provide rapid test results. We examined the performance and usefulness of "ORTHO VISION Max."

METHODS

1) Stability of measurement according to the quality control sample
   (a) Simultaneous reproducibility (5 times of continuous measurements)
   (b) Reproducibility over the day (8 hours of measurement intervals)
   (c) Daily reproducibility (measurements for 7 days)
   (d) Interference in the result (bilirubin-F, bilirubin-C, hemolysis hemoglobin, rheumatoid factor, and milky fluid)

2) Sensitivity and specificity of the irregular antibody detection use the sample to fix that they are positive about.

3) Determination comparison among the instruments (Ortho AutoVue Innova and ORTHO VISION; OCD)
   (a) Blood typing and irregular antibody screening; agglutination grading of patient samples by the reading unit of each device
   (b) Comparison of turnaround time
   (c) Measuring time when implementing an emergency sample while measuring more than one sample
   (d) Agglutination grading of the adjusted-mixture cell population by the reading unit of each device

RESULTS

The following showed good measurement results: stability of measurement according to the quality control sample; simultaneous reproducibility; reproducibility over the day; and daily reproducibility.

Table 1: Comparison of hemolysis hemoglobin concentration, which has an influence on the reading of agglutination grading by the reading unit of the instrument

Method	Column	Hemoglobin concentration (mg/dL) 0	50	100	150	200	250	300	350	400	450	500
Indirect antiglobulin test	#1	4+	4+	4+	4+	4+	2+	4+	4+	4+	1+	4+
(BioVue IgG cassette)	#2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	#3	0	0	0	0	0	0	0	0	0	0	0
Enzyme (ficin) method	#1	4+	4+	4+	4+	4+	4+	4+	CI	CI	CI	CI
(BioVue Neutral cassette)	#2	4+	4+	4+	4+	4+	4+	4+	CI	CI	CI	CI
	#3	0	0	0	0	0	0	0	CI	CI	CI	CI

CI: contrast insufficient

Agglutination grading cannot be measured with hemolysis hemoglobin of 350 mg/dL due to insufficient contrast.

PHOTO: Screenshot of ORTHO VISION Max (edited)

Table 2: Sensitivity and specificity of irregular antibody detection

Antibody	Test result and antibody titer AV result	AV titer	OV result	OV titer	OVM result	OVM titer
Anti-E	Pos.	NT	Pos.	1:32	Pos.	1:32
Anti-Jk^a	Pos.	NT	Pos.	1:8	Pos.	1:8
Anti-Fy^b	Pos.	NT	Pos.	1:32	Pos.	1:32
Anti-S	Pos.	NT	Pos.	1:32	Pos.	1:32
Anti-Di^a *	Pos.	NT	Pos.	NT	Pos.	NT

* Diego A antigen is important for Mongoloids in Northeast Asia.

AV: AutoVue Innova; OV: ORTHO VISION; OVM: ORTHO VISION Max

Table 3: Measuring time when implementing an emergency sample (only blood typing) while measuring more than one sample

Loading length of time		Number of samples 2	4	6
		Measuring time of emergency sample		
1 minute later	AV	12	16	31 (min.)
	OV	21	28	30
	OVM	12	16	23
5 minutes later	AV	12	11	21
	OV	18	22	27
	OVM	8	12	17

AV: AutoVue Innova; OV: ORTHO VISION; OVM: ORTHO VISION Max

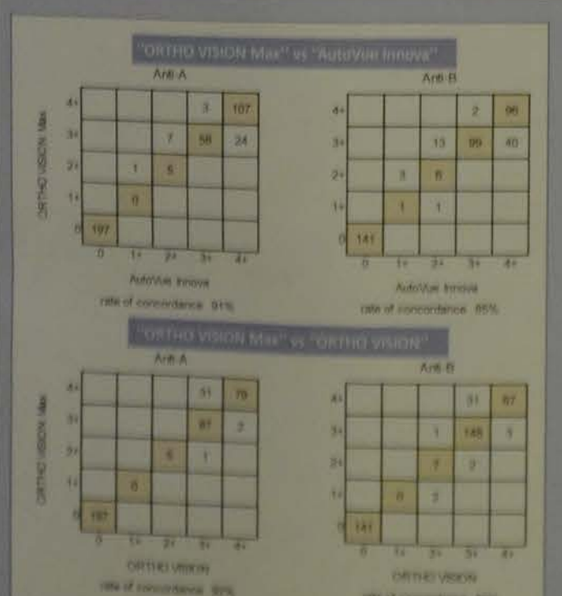


Figure 1: Comparison of the agglutination grading of blood typing: ORTHO VISION Max tends to read a higher agglutination grading than AutoVue Innova and ORTHO VISION.

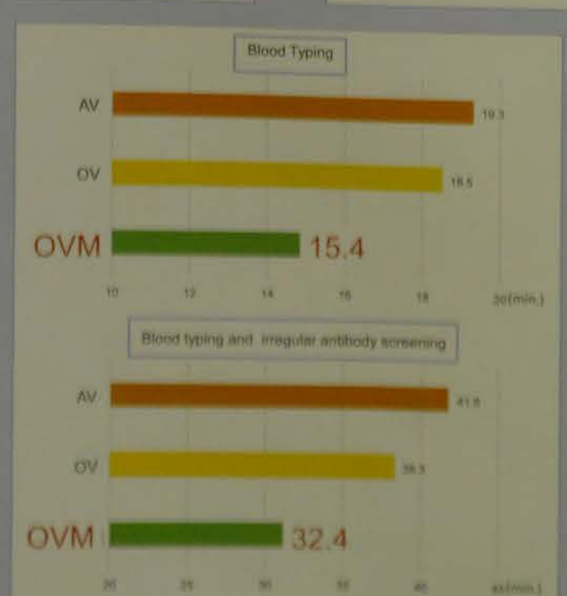


Figure 2: Comparison of turnaround time, which is necessary for the 10-sample determination

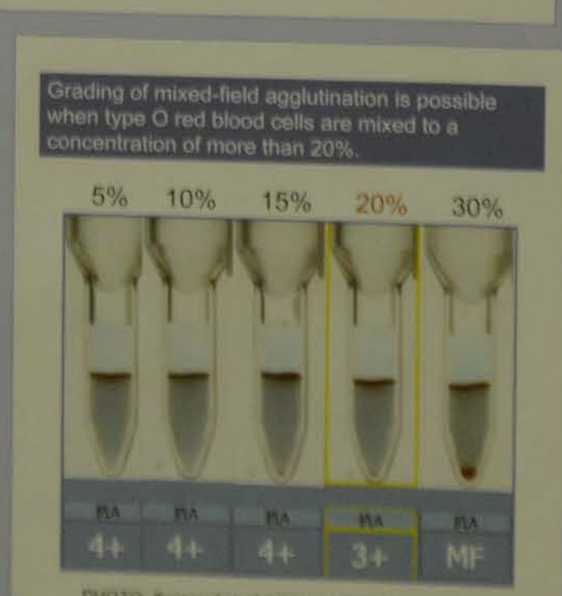


PHOTO: Screenshot of ORTHO VISION Max (edited)

Figure 3: Comparison among the instruments for agglutination grading using the samples, which adjusted the mixed-field agglutination by mixing type O red blood cells in various fractions

CONSIDERATION / CONCLUSION

Transfusion medicine

PJ-35

Serious autoimmune hemolytic anemia (AIHA) having warm autoantibodies with panreactive and relative specificity for RhD

Minakawa Keiji, Yasuda Hiroyasu, Kawabata Kinuyo, Saito Syunichi, Ikeda Kazuhiko, Ogawa Kazuei and Ohto Hitoshi

1) Fukushima Medical University Hospital Department of Blood Transfusion and Transplantation Immunology
2) Fukushima Medical University Hospital Department of Cardiology and Hematology

Background

- Autoimmune hemolytic anemia (AIHA) is a hemolytic disease caused by autoantibodies to red blood cells.
- Serious AIHA rarely occurs as a lethal manifestation of chronic GVHD after allogeneic stem cell transplantation.
- We report a case of AIHA due to anti-D specific autoantibodies triggered by the EB virus infection and chronic GVHD, leading to a struggle with choosing of red cell products and detection specificity.

Patient profile

30's male, blood type: A RhD-positive

【Health history】

- Philadelphia chromosome-positive ALL.
- Allogenic bone marrow transplantation.
  〈Unrelated donor: type A DccEE, HLA 1-locus mismatch (DRB1)〉
- Engraftment confirmed on Day 26, discharged after 3 months.
- There was limited-stage chronic GVHD affecting skin and liver.

	result
AST(IU/L)	32
ALT(IU/L)	24
LDH(IU/L)	580
TB(umol/L)	47
DB(umol/L)	7
WBC(x10^9/L)	4.2
RBC(x10^{12} /L)	3.23
Hb(g/L)	85
PLT(x10^9/L)	82
CRP(mg/L)	2.77

【History of present illness】

- 5 months post-transplant, hospitalized with hemolytic anemia.
- Hemolysis, mild anemia, thrombocytopenia.
- Direct anti-globulin test: positive anti IgG: 3+, anti-complement: 2+
- EB virus-LQ: Positive (200copies)

Panel Results

- Autoantibodies with relative specificity for anti-D

Antigen	D	C	E	c	e	Saline	PEG-IAT		
						x1 Serum	x1 Serum	x10 Serum	x10 Elution
RBC1(R1R1)	+	+	0	0	+	2+	4+	2+	2+
RBC2(R2R2)	+	0	+	+	0	2+	4+	3+	2+^{s}
RBC3(rr)	0	0	0	+	+	2+	4+	1+	1+
Di(a+)RBC	+	0	+	+	+	2+	4+	2+	2+

RBC Choice

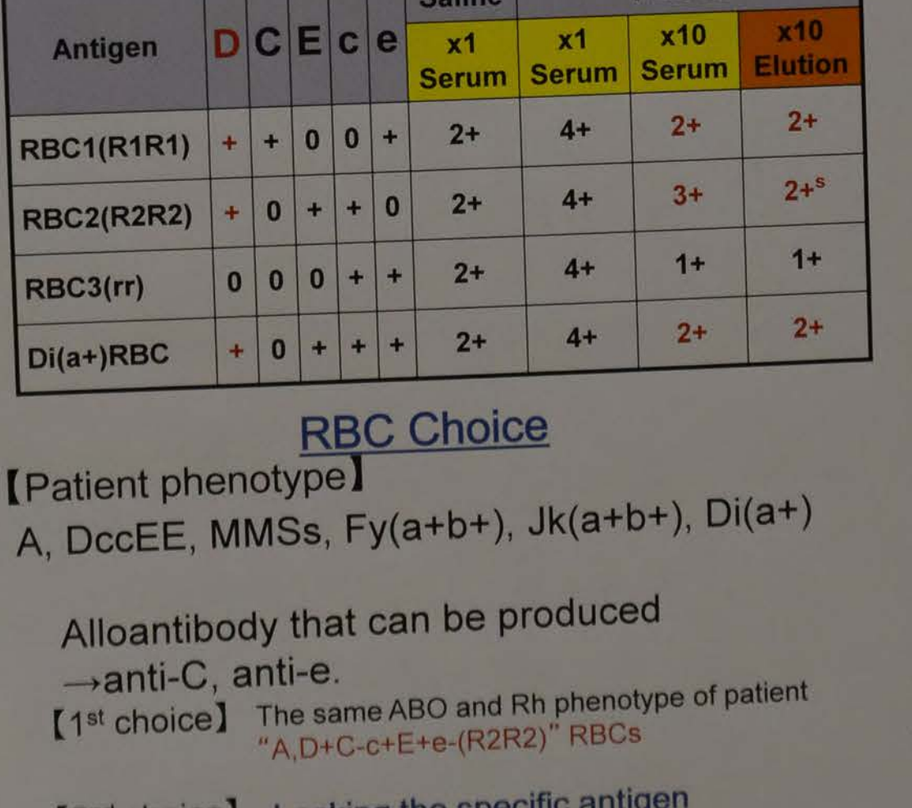
【Patient phenotype】
A, DccEE, MMSs, Fy(a+b+), Jk(a+b+), Di(a+)

Alloantibody that can be produced
→anti-C, anti-e.

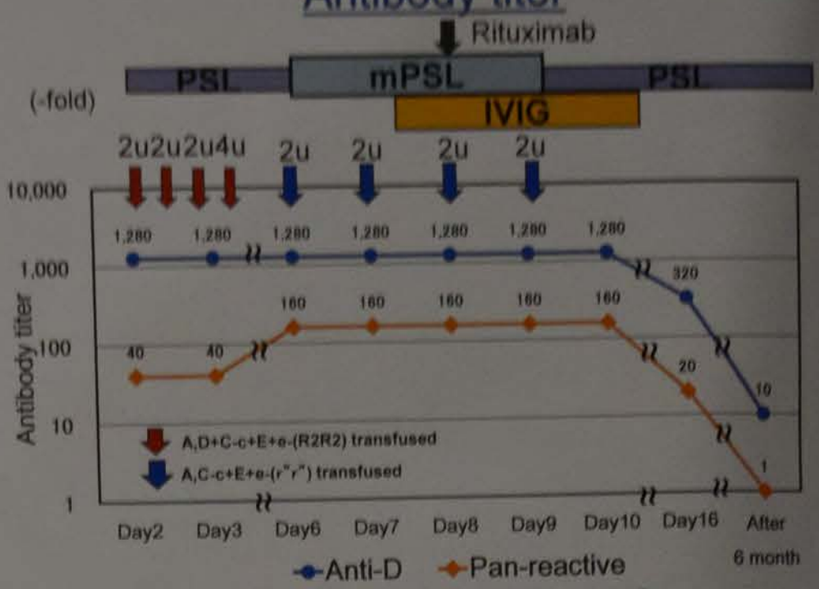
【1st choice】 The same ABO and Rh phenotype of patient "A,D+C-c+E+e-(R2R2)" RBCs

【2nd choice】 Lacking the specific antigen "A,D-C-c+E+e-(r''r'')" RBCs

Clinical course



Antibody titer



What about anti-LW?

Anti-LW reacts like anti-D.

DTT-treated D-positive RBCs are a useful way to differentiate anti-D from anti-LW; anti-D will be react while anti-LW will not.

Even after the treatment of panel RBCs with 0.2M DTT, apparent reactivity for anti-D was detected by indirect anti-globulin test (data not shown).

Thus, anti-LW was ruled out.

Monocyte monolayer assay (MMA)

	Red cell	Reactivity(%)
Day2 Serum	R2R2	6.1
	r''r''	2.9
Day7 Serum	R2R2	28.7
	r''r''	7.7
Day10 Serum	R2R2	7.8
	r''r''	5.4

D+C-c+E+e- (R2R2)
D-C-c+E+e-(r''r'')

*Reactivity: Percentage of the monocyte attached or phagocytosed RBCs. Over 1% is positive.

- D-positive RBCs showed higher reactivity than D-negative RBCs.
- Reaction was consistent with their specificity.

IgG Subclass

	Red cell	IgG$_1$	IgG$_2$	IgG$_3$	IgG$_4$
Day2 Serum	R2R2	1.13	1.15	1.12	1.00
	r''r''	1.44	1.03	0.99	1.00
Day7 Serum	R2R2	3.26	1.31	1.04	1.00
	r''r''	1.86	1.35	1.01	1.00
Day10 Serum	R2R2	3.26	1.34	1.11	1.00
	r''r''	1.46	1.08	1.01	1.00
Day10 Elution	R2R2	7.03	1.83	1.00	1.00
	r''r''	3.32	1.45	1.10	1.00
Positive control(IgG$_2$+IgG$_3$)	R2R2	1.12	2.18	73.80	1.00
Positive control(IgG$_1$)	R2R2	2.41	1.20	1.10	1.00

S/N ratio(Sample/Negative Control)
* S/N ratio > 2: Positive

D+C-c+E+e-(R2R2)
D-C-c+E+e-(r''r'')

Anti-D specific IgG$_1$ was detected.

Conclusions

- Chemotherapy like a steroids, rituximab and IVIG were often insufficient during acute phase AIHA due to anti-D relative specificity and panreactive autoantibodies after stem cell transplant.
- D-negative RBC transfusion was lifesaving. In addition, effect of steroids and rituximab was delayed, but helpful to suppress the production of autoantibody.
- If an autoantibody with anti-D relative specificity has a hemolytic activity, it is appropriate to consider D-negative RBCs transfusion.

Transfusion medicine

PJ-36

A FAMILY CASE OF DEFY SEROLOGICAL CLASSIFICATION THE RARE SUBGROUP A

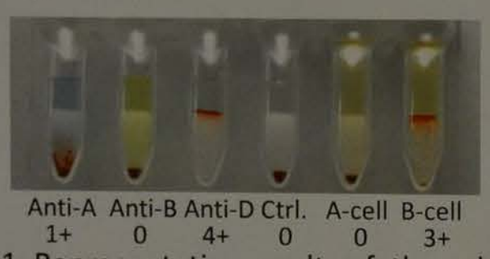
R. Maejima,, K. Osone, H. Kasai, H. Namba, K. Fujiwara

Department of Transfusion Medicine and Cell-processing, Teikyo University School of Medicine

BACKGROUND: The propositus was a 45-year-old healthy female who was admitted to our hospital for a detailed examination of ABO blood types because it was difficult to classify at another hospital. Family studies were also performed from her 70-year-old mother as well as her 19-year-old son and 17-year-old daughter.

STUDY DESIGN AND METHODS: Serological analyses including adsorption and elution assay, plasma transferases activity assay, and flow cytometric analysis were performed to determine the unique phenotypes of these samples. DNA sequencing and polymerase chain reaction-reverse sequence-specific oligonucleotide (GENOSEARCH™ ABO, MBL, Nagoya, Japan) were performed to further investigate the relationships between the genetic characteristics and phenotypic features of these samples.

RESULTS: Blood type test using automated instrument AutoVue showed weak reactivity (1+) to anti-A (Fig. 1).



Anti-A 1+ | Anti-B 0 | Anti-D 4+ | Ctrl. 0 | A-cell 0 | B-cell 3+

Fig. 1 Representative results of the column agglutination method.

Further examinations by tube tests using anti-A, anti-B, anti-A_1 lectin, and anti-H lectin showed 1+, negative, negative, and 4+, respectively. Plasma A-glycosyltransferase activity was not detected (Table 1).

Table 1. ABO blood typing data, including anti-A adsorption-elution test and plasma A-glycosyltransferase activity

	Proposita	Son	Daughter	Mother
Column agglutination technology				
Anti-A	1+	1+	1+	0
Anti-B	0	0	0	0
A_1 cell	0	0	0	4+
B cell	3+	3+	3+	4+
Tube test				
Anti-A	1+	1+	1+	0
Anti-B	0	0	0	0
Anti-A_1	0	0	0	N.T.
Anti-H	4+	4+	4+	N.T.
Anti-A, B	0	0	0	N.T.
A_1 cell	0	0	0	4+
B cell	3+	3+	3+	4+
Anti-A adsorption-elution test				
A_1 cell	3+	3+	3+	N.T.
B cell	0	0	0	N.T.
O cell	0	0	0	N.T.
A-glycosyltransferase activity				
(reference titer = 1:128)	0	0	0	N.T.

Flow cytometric analysis of the expression level of A-antigen demonstrated similar histogram pattern to Ax (Fig. 2).

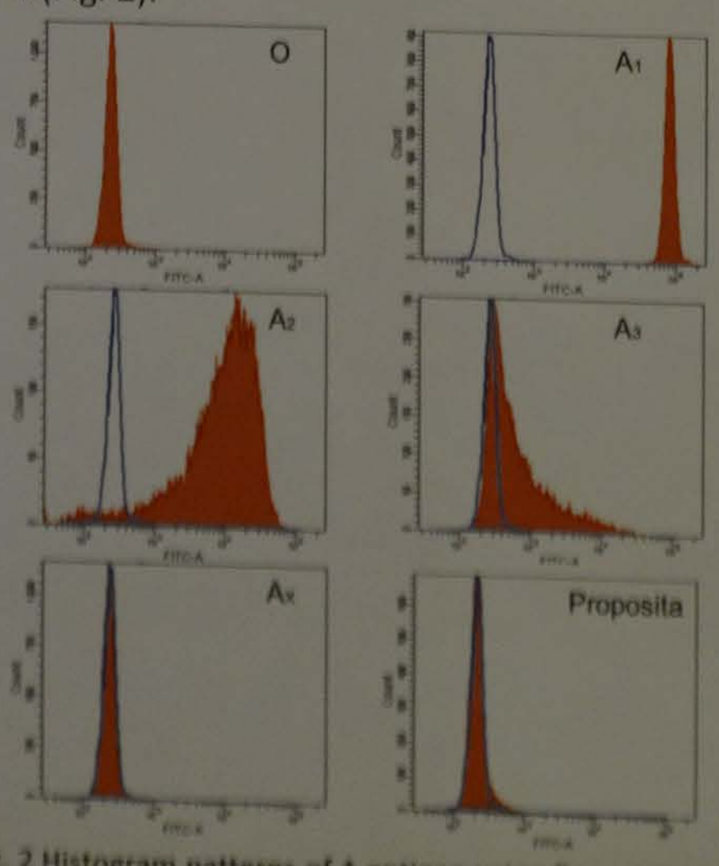


Fig. 2 Histogram patterns of A antigen using flow cytometry

Table 2 shows the results of the genotyping using GENOSEARCH™ ABO. The genotyping results of two children were identical to the proposita. Although the combination of *ABO* alleles estimated from allele frequency in the Japanese population was *A102/O01*, 189 ambiguous allele combinations existed(A). The genotyping result of mother was *O01/-*. Therefore 13 ambiguous allele combinations existed when one of proposita *ABO* allele was *O01*(B)

Table 2. Results of Genoseach ABO

A : Frequent ABO genotypes and their ambiguity.

Sample	Type	Ambiguity
Proposita	*A102, O01*	189
Son	*A102, O01*	189
Daughter	*A102, O01*	189
Mother	*O01, -*	54

B : Ambiguity allele coinherited with *O01*.

Allele		intron 6		exon 7											
A101		163	179	425	467	539	543	556	564	745	761	767	820	860	893
(Reference)		T	C	T	C	G	G	A	C	C	C	T	G	C	C
O01	*A102*				T										
O01	*A103*				T				T						
O01	*A105*	C	T		T										
O01	*A208*				T	C									
O01	*A306*				T								A		
O01	*A307*				T					T					
O01	*Ael05*				T							C			
O01	*Ael06*			C	T										
O01	*Am01*				T						T				
O01	*Aw12*				T		G								
O01	*Ax09*				T	T									
O01	*Ax11*				T									T	
O01	*O14*				T										T

Nucleotide sequences of exons 6 and 7 of the *ABO* gene revealed the genotypes of *A307/O01* (Fig. 3). The genotyping results of two children were identical to the proposita.

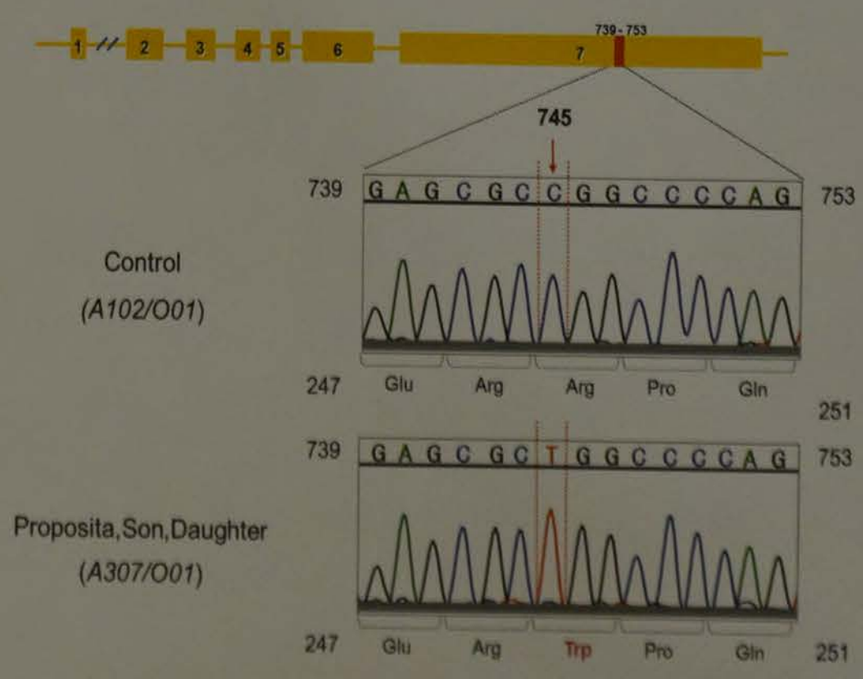


Fig. 3 Nucleotide sequence of exon 7 of *ABO* gene.

CONCLUSION: According to the allelic enhancement phenomenon, the different expression of the rare *A307* allele could be due to coinherited alleles. The original case reported a Taiwan family classified A_3B serologically, in which the *A307* was coinherited with *B101*, and the expression level of A-antigen was enhanced (Li L *et al*., Transfusion, 2007).

In contrast, here the *A307* was coinherited with *O01*, and the weakly expressing A-antigen was detected in our reported family, which was weaker than A_3.

Transfusion medicine

PJ-37

Fulminant aplastic anemia that was successfully treated by frequent transfusion of HLA -matched platelet concentrate

Takahiro Ishii[1], Megumi Numanoi[1], Yuriko Okazaki[1], Naomi Kojima[1], Kumiko Sekiguchi[1], Yasushi Takagi[1], Hiroaki Onishi[2], Takashi Watanabe[2]

1) Department of Clinical Labolatory, Kyorin University Hospital
2) Department of Laboratory Medicine, Kyorin University

Introduction

Fulminant aplastic anemia is defined as the most severe form of aplastic anemia that does not show an increase in the neutrophil count after the administration of granulocyte colony stimulating factor (G-CSF). We report a case of fulminant aplastic anemia in which severe bleeding was prevented by frequent transfusion of human leukocyte antigen (HLA)-matched platelet concentrate (PC-HLA).

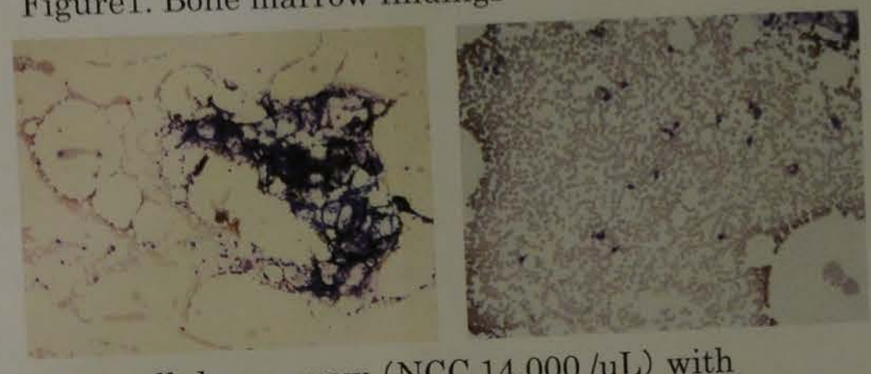
Case report

A woman in her 50s was referred to our hospital due to petechiae in her lower limbs and thrombocytopenia. Serious pancytopenia was observed, and she was diagnosed with stage V aplastic anemia based on bone marrow biopsy findings.

Table1. Result of laboratory test

Hb	6.6	g/dL	Reti	1.1	×10⁴/μL	TP	6.6	g/dL
Ht	22.5	%	PT	13.9	sec	Alb	3.7	g/dL
RBC	250	×10⁴/μL	PT-INR	1.10		BUN	15.9	mg/dL
MCH	31.5	pg	APTT	32.9	sec	Cre	0.63	mg/dL
MCV	90.1	fl	Fbg	409	mg/dL	LDH	159	IU/L
MCHC	35.0	%	FDP	4.3	μg/mL	AST	7	IU/L
PLT	0.3	×10⁴/μL	DD	1.31	μg/mL	ALT	8	IU/L
WBC	400	/μL	AT-Ⅲ	94.0	%	Fe	232	μg/dL
Neut	24	%	FMC	9.7	μg/mL	TIBC	263	μg/dL
Mono	4	%	V.B12	350	pg/mL	UIBC	31	μg/dL
Lymph	72	%	folate	9.4	ng/mL	ferritin	180	ng/mL

Figure1. Bone marrow findings



Hypocellular marrow (NCC 14,000 /μL) with remarkable decrease in the number of immature granulocytes, erythroblasts, and megakaryocytes.

Clinical course

Despite immunosuppressive therapy combined with administration of G-CSF, hematopoietic effect was not obtained, and she was diagnosed with fulminant aplastic anemia.

Since transfusion of platelet concentrate (PC) was found to be ineffective, 63 out of 72 times. she was tested for serum anti-HLA antibody and was found to have positive results. Therefore, transfusion of PC-HLA was attempted, and it was effective 45 out of 53 times.

The corrected count increment (CCI) of 24 hours after transfusion of PC-HLA was 9,200±6,100/μl (mean ± standard deviation). On the other hand, CCI of 24 hours after transfusion of random PC was 180±4,600/μl. As a result, she did not suffer serious bleeding for 10 months while she waited for a bone marrow transplantation.

Table2. Severity classification of aplastic anemia

International	Japan		Neutrophil	Reticulocyte	Platelet	Condition
			Other than the following			
non-severe	stage Ⅰ	mild				
	stage Ⅱ	moderate	<1,000 /μL	<60,000 /μL	<50,000 /μL	Requried more than two criteria
	stage Ⅲ					
severe	stage Ⅳ	severe	<500 /μL	<20,000 /μL	<20,000 /μL	
	stage Ⅴ	most severe	<200 /μL			

stage Ⅲ : Monthly red blood cells transfusion is a necessary condition.
stage Ⅴ : Neutrophil count of <200 /μL is a necessary condition.

Figure3. Clinical course

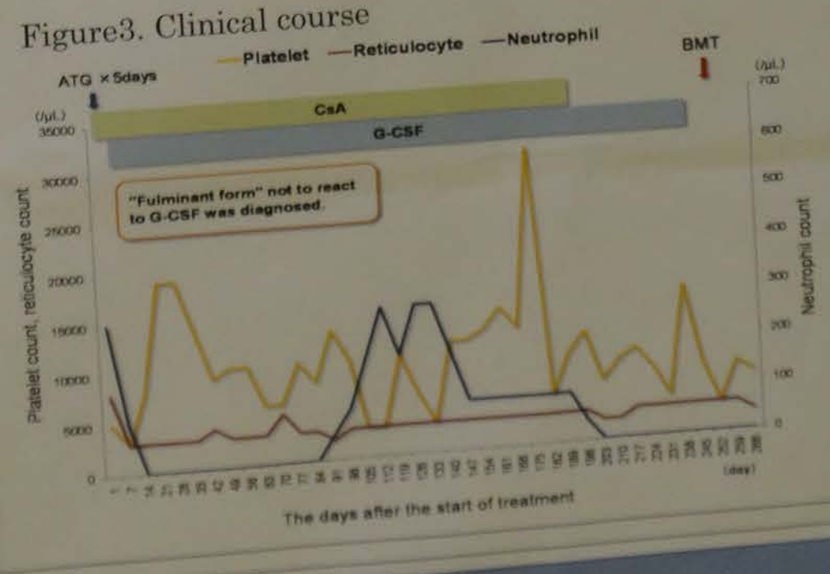
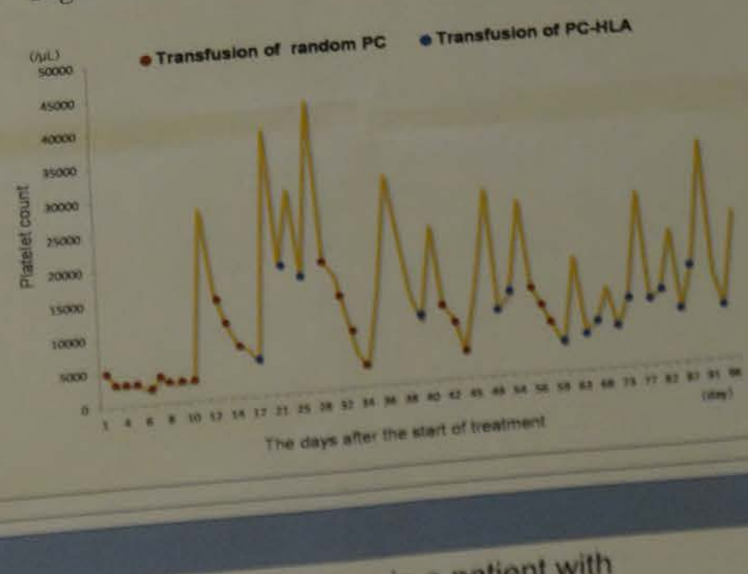


Figure4. Effect of PC-HLA transfusion



Conclusions

Multiple transfusions of PC-HLA were effective for the management of bleeding in a patient with fulminant aplastic anemia with anti-HLA antibodies that was refractory to platelet transfusion. Based on our experience, we recommend tests for anti-HLA antibodies and transfusion of PC-HLA for even in patients with a most severe form of aplastic anemia.

Transfusion medicine

PJ-38

Cryopreservation of autologous blood from an Rh_{mod} patient in cooperation with Hyogo Blood Center

Yuki Harada[1], Hiroki Sugiyama[1], Shinya Otsuka[1], Hitomi Onomoto[1], Noriko Okuda[1], Satoshi Yoshihara[2], Masaya Okada[2], Yoshihiro Fujimori[2]

[1]Center for Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine
[2]Department of Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine

BACKGROUND

Rh_{mod} is one of the rare Rh variants with extremely suppressed Rh antigen expressions in red blood cells (RBC) by suppressor gene ($X^{Ø}$). Rh antigen-expression level varies, ranging from which can be agglutinated by commercial antiserum to which can be detected only by Adsorption-elution test.

Transfusions from regular donors potentially result in the production of irregular alloantibodies to overall Rh system antigens. Additionally, Rh_{mod} individuals are often ineligible for blood donation due to chronic anemia. Therefore, storage of autologous blood is highly recommended in preparation for the need for transfusions.

We cryopreserved Rh_{mod} autologous blood as a form of frozen red blood cells (FRC) in cooperation with Japanese Red Cross Hyogo Blood Center.

CASE

The patient was first diagnosed with hemolytic anemia when she was 20 years old. Detailed analysis revealed that chronic hemolysis resulted from RBC membrane fragility due to shortage of Rh antigenicity associated with Rh_{mod} blood type. She delivered two children uneventfully. Her autologous frozen red cells (FRC) was first processed when she was 35 years old.

Six years later, she presented hemolytic crisis following parvovirus infection, which resulted in severe anemia (Hb 3.8 g/ dL). She received autologous frozen thawed red cells (FRTC) and O Rh_{mod} RBC from two donors at another hospital.

Since this episode, we started storage of her autologous blood upon her request.

Table 1. Laboratory data before autologous blood collection.

T-bil	1.9 mg/dL	WBC	34×10^2 /μL
D-bil	0.5 mg/dL	Stab	3.0 %
AST	19 U/L	Seg	58.0 %
ALT	19 U/L	Lym	26.0 %
LDH	292 U/L	Mono	8.0 %
γ-GT	28 U/L	Eos	5.0 %
UN	6 mg/dL	Bas	0.0 %
UA	8.9 mg/dL	RBC	257×10^4 /μL
CRE	0.59 mg/dL	Hb	8.4 g/dL
Na	142 mmol/L	Ht	25.8 %
K	3.7 mmol/L	MCV	100.4 fL
Cl	108 mmol/L	MCH	32.7 pg
CRP	0.3 mg/dL	MCHC	32.6 %
		Ret%	11.4 %
		PLT	13.9×10^4 /μL

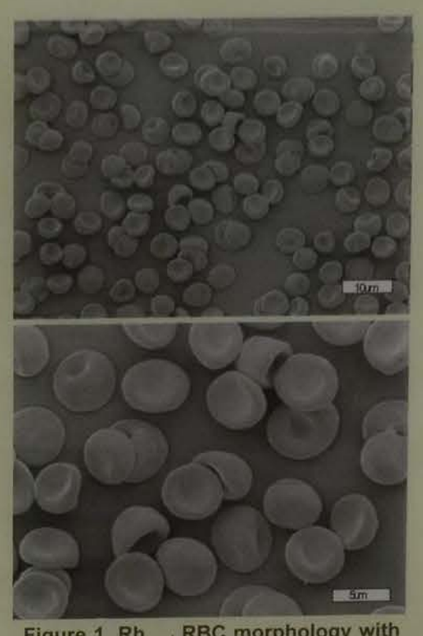


Figure 1. Rh_{mod} RBC morphology with a scanning electron microscope.

METHODS & RESULTS

We collected her blood for five times between 2003 and 2016. ABO typing showed B with Rh negative in both of the tube and column agglutination tests. Indirect antiglobulin test using patient's RBC and an IgG anti-D showed negative for D antigen. No alloantibodies to RBC were detected by column agglutination test. Rh typing was all negative by monoclonal antiserum.

Whole blood was transferred to the blood center, where it was processed and cryopreserved as a form of FRC. In the process, hemolysis was observed after centrifugation, which suggested the fragility of Rh_{mod} RBC membrane structures.

FRC were transferred to our hospital and can be stored for less than ten years. When necessary, FRC, we have five bags at present, will be transferred to the Blood Center, where FRC will be thawed and washed, and then transferred back to our hospital for transfusions.

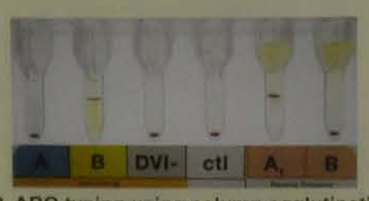


Figure 2. ABO typing using column agglutination test.

Table 2. Rh typing using commercial antiserum.

	Anti-D	Anti-C	Anti-c	Anti-E	Anti-e
Monoclonal	0	0	0	0	0
Polyclonal	0	NT	NT	NT	NT

NT: not tested.

Figure 3. Difference in color of a supernatant plasma layer after blood bag centrifugation between normal blood (left) and Rh_{mod} (right).

CONCLUSION

There have been limited information regarding Rh_{mod}. Furthermore, there are only a few reports regarding blood transfusions to Rh_{mod} patients. Our current system would serve as a suitable model for the management of the patients with rare blood types.

Transfusion medicine

PJ-39

Late-onset pure red cell aplasia after cord blood transplantation accompanied with de novo irregular antibodies

Noriko Okuda[1], Yuki Harada[1], Reiko Irie[1], Rie Murata[1], Junko Ikemoto[1], Satoshi Yoshihara[2], Masaya Okada[2], Yoshihiro Fujimori[2]

[1]Center of Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine , Hyogo, Japan
[2]Department of Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine , Hyogo, Japan

Introduction

Pure red cell aplasia (PRCA) is one of the rare complications after hematopoietic stem cell transplantation (HSCT). While the majority of early-onset PRCA is caused by isohemagglutinin derived from recipient antibody-producing cells under a major ABO blood type mismatch, the pathogenesis of late-onset PRCA remains unclear. We report the case of PRCA developed in twenty-two months after cord blood transplantation (CBT).

Case

A 59-year-old male underwent CBT for follicular lymphoma in third complete remission.

Clinical course of CBT (Figure 1)

HLA 2-loci mismatched cord blood cells were transplanted with reduced intensity conditioning consisted of fludarabine, cyclophosphamide and 3 Gy total body irradiation. There was a minor ABO mismatch between the donor (O Rh+) and the recipient (A Rh+). Recipient irregular antibody screening was negative before CBT. Neutrophil engraftment (neu >500/μL) was achieved on day 19 and RBC engraftment (reticulocyte >1%) was achieved on day 28. The patient developed skin GVHD (stage 2, grade I). Early clinical course was uneventful and he was discharged 3 months after CBT. Patient blood type changed to donor type (O Rh+) 4 months after CBT.

Development of late-onset PRCA (Figure 2)

Twenty-two months after CBT, he developed flu-like symptoms followed by severe anemia. Bone marrow examination showed severe erythroid hypoplasia, which was compatible with the diagnosis of PRCA. Parvovirus B19 DNA was not detected by polymerase chain reaction (PCR). We performed irregular antibody screening and detected anti-E and anti-Di^a. Rh blood typing was DCCee and Di^a antigen was negative. The patient initially received prednisolone at 20 mg/day with limited response. He then received rituximab followed by cyclosporine A. Direct antiglobulin test (DAT) turned negative and hemoglobin recovered to normal range. He received 48 units of RBC transfusion during the treatment.

Effect of pre- and post-treatment serum on BFU-E colony formation (Figure 3)

Erythroid burst colony formation (BFU-E) was suppressed by the addition of patient's pre-treatment serum, but not by post-treatment serum in a methylcellulose hematopoietic stem cell culture of the patient's recovered bone marrow cells (Figure 3). This finding suggests that some humoral factors in the patient's serum are responsible for the development of PRCA.

Discussion

Most reported cases of early-onset PRCA occur after major ABO-mismatched HSCT, showing that anti-A or anti-B causes PRCA . Yamaguchi et al. reported that late-onset PRCA in blood type-matched HSCT and we here reported late-onset PRCA in a minor ABO mismatched HSCT, suggesting that isohemagglutinin other than anti-A or anti-B may also cause the PRCA. De novo irregular antibodies including anti-E and anti-Di^a were detected in the course of PRCA, but these would not cause PRCA because of lack of corresponding antigen on engrafted hematopoietic cells. Other humoral factors might cause PRCA in this case (Figure 3).

Conclusion

We presented a rare case of late onset of PRCA, accompanied with the appearance of de novo irregular antibodies, and was successfully treated with the suppression of humoral and cellular immunity. This case highlighted the importance of close examinations including the irregular antibody screening in patients who underwent allogeneic HSCT.

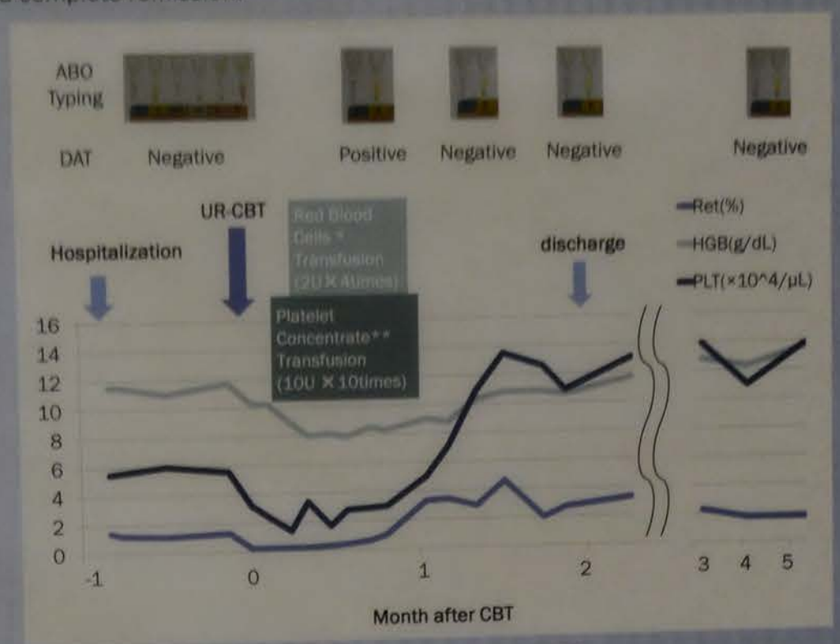


Figure 1 : Clinical course of CBT

*Red Blood Cell 2U : approximately 280mL derived from 400mL of whole blood
**Platelet Concentrate 10U : approximately 2.0×10^{11} platelets collected by apheresis

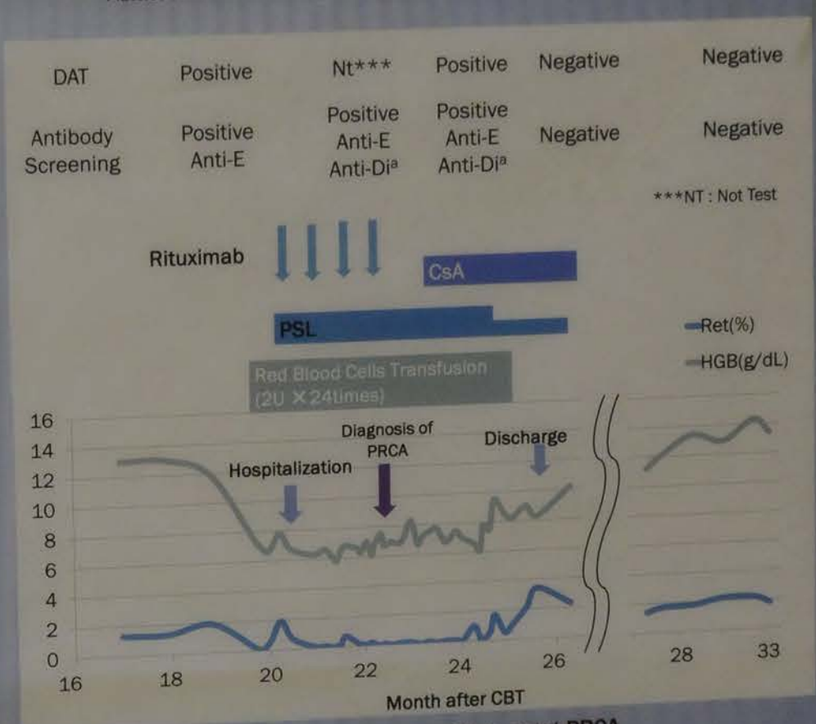


Figure 2 : Development of late-onset PRCA

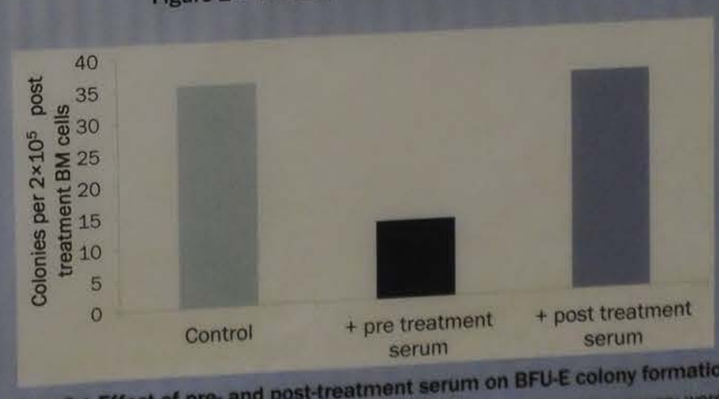


Figure 3 : Effect of pre- and post-treatment serum on BFU-E colony formation.

Patient's bone marrow cells at 2×10^5 cells/dish after hematopoietic recovery were cultured in the presence of pre- or post- treatment serum in the semisolid MethoCult H4034 medium, and BFU-E colonies were counted at day14.

Transfusion medicine

PJ-40

The first report of the reference ranges of rotational thromboelastometry (ROTEM®) in Japanese

Daisuke Hanada, Yoshie Kawahara, Kouhei Ohtsuka, Hiroyuki Takahashi, Yutaka Tomoda, Satoshi Fujii

Asahikawa Medical University Hospital Medical Laboratory and Blood Center

Background and Aim

ROTEM® , monitoring device with the principle of rotational thromboelastometry, quickly reflects clotting ability of the whole blood. ROTEM® can evaluate the clotting ability to help appropriately judge the need of blood transfusion in patients with massive bleeding. Because there are no available data on the reference ranges of ROTEM® parameters in Japanese and clotting ability can be diverse among various populations, we aimed to determine the reference ranges of INTEM (intrinsic coagulation systems), EXTEM (extrinsic coagulation systems) and FIBTEM (fibrinogen contribution to coagulation).

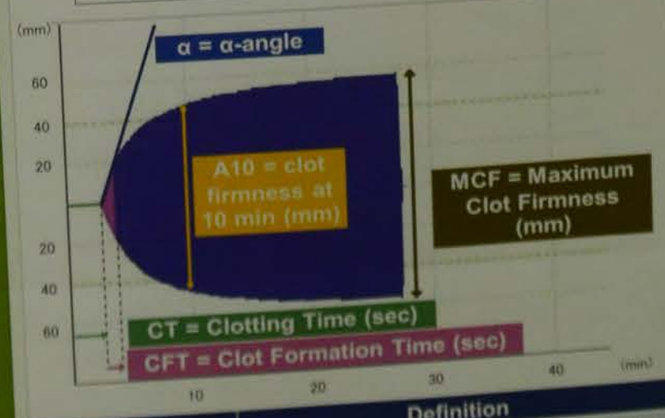
Methods

The study was approved by the ethics committee and informed consent was taken. Blood sample obtained from healthy donors (30 males and 21 females) were subjected to ROTEM® . Clotting time (CT), clot formation time (CFT), clot firmness at 10 min (A10), maximum clot firmness (MCF), and α-angle (α) were measured to evaluate INTEM, EXTEM, and FIBTEM. Moreover, we simultaneously carried out conventional coagulation tests: activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen levels. The reference ranges (95% reference limits) were determined with parametric method, and the gender differences were investigated with Mann-Whitney U test. The relation between ROTEM® parameters and conventional coagulation tests were determined with regression analysis.

Overview of ROTEM®

Whole blood (300 µl, anticoagulated with citrate) is placed into the disposable cuvette using an electronic pipette. A disposable pin is attached to a shaft which is connected with a thin spring and slowly oscillates back and forth. The signal of the pin suspended in the blood sample is transmitted via an optical detector system. The test is started by adding appropriate reagents. The instrument measures and graphically displays the changes in elasticity at all stages of the developing and resolving clot.

Graph and Parameters of ROTEM®



Parameter	Definition
CT (Clotting Time)	The CT is the latency time from adding the start reagent to blood until the clot starts to form.
CFT (Clot Formation Time)	The CFT is the time from CT until a clot firmness of 20 mm point has been reached.
A10 (Clot firmness at 10 min)	The A10 is the vertical amplitude of the trace at 10 min.
MCF (Maximum Clot Firmness)	The MCF is the greatest vertical amplitude of the trace.
α (α-angle)	The α is intercept represented the firm velocity.

Results

	Study group
Age (yrs)	38.8 (26.2-51.4)
Gender (female/male)	30/21

Table 1. Basic characteristics.
Data are presented as mean (range) or number

	INTEM	EXTEM	FIBTEM
CT (sec)	145-220	41-82	36-77
CFT (sec)	49-102	47-119	
A10 (mm)	47-62	45-65	7-17
MCF (mm)	51-66	52-70	7-17
α (°)	70-80	67-80	

Table 2. The reference ranges obtained by measurement.
The results by ROTEM®. The date are calculated as 95% reference limits.

		p value			*p* value			*p* value
	CT	0.061		CT	0.527		CT	0.970
	CFT	0.199		CFT	0.076		CFT	
INTEM	A10	0.453	EXTEM	A10	0.477	FIBTEM	A10	0.557
	MCF	0.850		MCF	0.465		MCF	0.877
	α	0.120		α	0.141		α	

Table 3. The results of gender differences.
The gender differences were investigated with Mann- Whitney U test ($p<0.05$) .

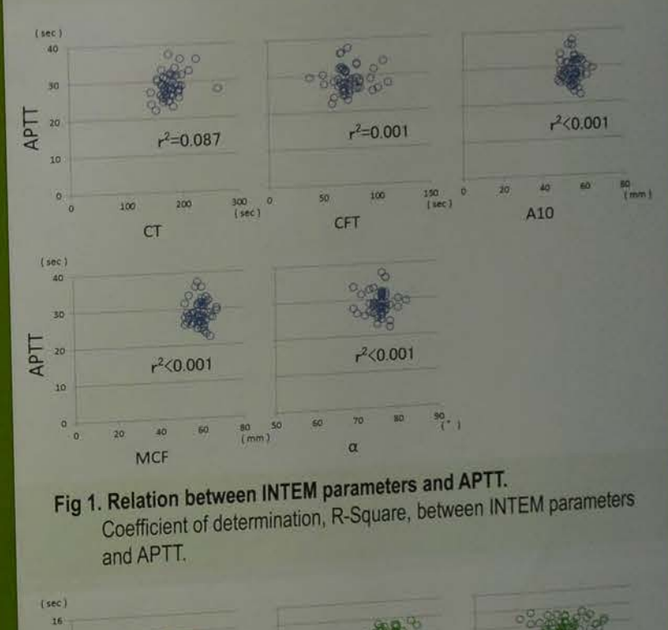


Fig 1. Relation between INTEM parameters and APTT.
Coefficient of determination, R-Square, between INTEM parameters and APTT.

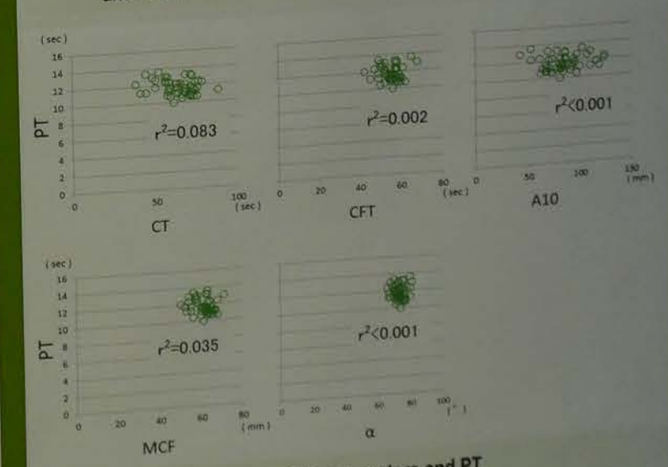


Fig 2. Relation between EXTEM parameters and PT.
Coefficient of determination, R-Square, between EXTEM parameters and PT.

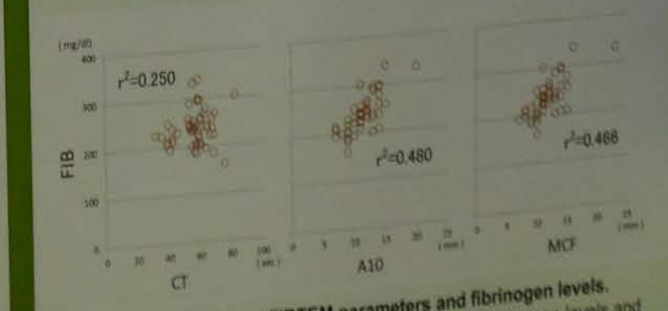


Fig 3. Relation between FIBTEM parameters and fibrinogen levels.
Coefficient of determination, R-Square, between fibrinogen levels and FIBTEM parameters.

Discussion

We determined the reference ranges of ROTEM® in Japanese.
The results of this study was comparable to the results reported by Lang T[1] and Ganter MT[2]. The material of this study was adult. The results of study in paediatric age groups[3] were similar.

Conclusion

Reference ranges of ROTEM® were determined for the first time in Japanese. These values will be utilized to assess the necessity of blood transfusion in patients with massive bleeding, and to compare the bleeding and clotting tendency among different populations.

References

1) Lang T, et al. Blood Coagul Fibrinolysis. 2005;16:301-310.
2) Ganter MT, et al. Anesth Analg. 2008;106:1366-1375.
3) Oswald E, et al. Br J Anaesthesia. 2010;105:827-835.

Transfusion medicine

PJ-41

Revision of the guideline for pre-transfusion testing in Japan

Akira Kokubunji[1], Makoto Okuda[2], Ken Ishimaru[3], Nobumasa Kobayashi[4], Tomoya Takahashi[5]

1 Hiroshima International Univ., Faculty of Health Sciences, Japan
2 Toho- Univ. Medical Center Omori Hosp., Div. of Blood Transfusion, Japan
3 Japanese Red Cross Blood Service Headquarters, Japan
4 Tokai Univ. Hosp., Div. of Medical Technology & Dept. of Blood Transfusion Service, Japan
5 Yoshidagakuen Medical and Dental College, Dept. of Medical Technologist, Japan

Background

Transfusion in Japan is being conducted even in a small hospital. It is necessary to enforce the blood transfusion system at each hospital in accordance with the policies and guidelines of the Ministry of Health, Labour and Welfare (MHLW) of Japan. Therefore, the "Red blood cell type inspection (erythroid inspection) Guidelines" has been created by the guidelines committee of the Japan Society of Transfusion Medicine and Cell Therapy(JSTMCT) in 2003. This guideline seems to be made well-known widely as a guide of a domestic pre-transfusion testing, and to contribute to improvement of safety in blood transfusion.

Aims

Because the addition of much inspection items and the revision of contents had been carried out in the guidelines on other countries, we have also revised in this guideline.

Methods

We took the guideline in Transfusion Medicine on British Blood Transfusion Society into account. In addition, we followed the guideline of transfusion practice of MHLW in Japan.

Results

3 items of ABO GROUPING, RhD TYPING and Crossmatch were added newly, and it was the construction by 8 items in total. The amount of contents was increased to approximately two times.

The Japan Society of Transfusion Medicine and Cell Therapy

The guideline for pre-transfusion testing -revised edition-

CONTENTS

Section 1 CLINICAL SIGNIFICANCE OF RED BLOOD CELL ANTIBODIES
Clinically significant antibodies destroy red blood cells with antigens which correspond in vivo, and are red blood cell antibodies which causes the side effects.

Section 2 SAMPLES
Transfusion or pregnancy may stimulate the production of unexpected antibodies against red cell antigens through either a primary or secondary immune response.
Serological studies should be performed using blood collected no more than 3 days in advance of the actual transfusion when the patient has been transfused or patients, with the exception of neonates and baby within 4 pregnant within the preceding 3months.

Section 3 ABO GROUPIN (NEW Section)
A full ABO group comprises a forward group and a reverse group. A full group must be performed on all samples from months, where the reverse group is unlikely to be helpful, as any ABO antibodies are likely to be maternal in origin.

Section 4 RhD TYPING (NEW Section)
It's checked using Rh control reagent simultaneously with anti-D reagent.
Positive and negative controls should be used on a regular basis. Controls for manual and automated procedures are shown in Table 1.

Section 5 ANTIBODY SCREENING
The following antigens should additionally be present in the screening cell set: C, c, D, E, e, Di^a, Di^b, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P1, Le^a and Le^b.
The screening cell set should include at least one cell with homozygous expression of the C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens.

Section 6 ANTIBODY IDENTIFICATION
Table 2 shows the likely clinical significance of red cell alloantibodies, and recommendations for the selection of blood for patients with their presence.

Section 7 AUTOANTIBODY
If possible, rhesus phenotype compatible blood should be selected for patients with autoimmune hemolytic anemia (AIHA) in order to prevent alloantibody formation.

Table 1 Use of positive and negative controls for ABO/D grouping

Reagent	Positive control cells	Negative control cells
Anti-A	A	B
Anti-B	B	A
Anti-D	D positive	D negative

Table 2 Likely clinical significance of red blood cell alloantibodies, and recommendations for the selection of blood for patients with their presence

Specificity	Likely clinical significance in transfusion	Recommendation for selection of red blood cells for transfusion
Rh	Yes	Antigen negative
Duffy	Yes	Antigen negative
Kidd	Yes	Antigen negative
Diego	Yes	Antigen negative
S, s	Yes	Antigen negative
Kell	Yes	Antigen negative
M (active 37◦C)[1]	Yes	Antigen negative
M (not active 37◦C)[2]	No	N/A[3]
Le^a (active 37◦C)[1]	Yes	Antigen negative
Le^a (not active 37◦C)[2]	No	N/A
P1, N, Le^b	No	N/A
Xg^a	No	N/A
Antibodies to high frequency antigens JMH, Knops, Cost, Chido/Rodgers	No	N/A
Jr^a	Yes	Antigen negative is desirable
Antibodies to others high and low frequency antigens	Different depending on Specificities and cases	Consult Physician and Medical Technologist certified by JSTMCT, or reference lab

[1] A case of a positive result by Salt-IAT at 37℃ for 1 hour.
[2] A case of a negative result by Salt-IAT at 37℃ for 1 hour.
[3] N/A: not applicable

Section 8 CROSSMATCH (NEW Section)
In the major cross match, it should be performed by an appropriate method including the indirect antiglobulin test (IAT) which can detect clinically significant antibodies.
Polyethylene glycol (PEG) or low ionic strength solution (LISS) is desirable for enhancement reagents to use in IAT.

Conclusions

A public comment was put into effect in case of this revision. We expect to become the guideline more useful for transfusion medicine based on many opinions.