SOUTH CENTRAL
Association of Blood Banks

2021 | ABSTRACT Journal

Title:
Correlation of antihuman globulin test tube strength and clinical significance of antibodies by MMA

Sol Haberman AWARD WINNER

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ABSTRACT

Blood transfusions are a widely used relatively safe medical treatment. Risks arise when compatible blood is not readily available for patients with multiple clinically significant alloantibodies and/or with an antibody to a high prevalence antigen. RBC antibodies are generally considered clinically significant if they cause shortened survival of the cells possessing their targeted antigen, there is laboratory evidence of hemolysis and/or clinical signs and symptoms of a hemolytic transfusion reaction or hemolytic disease of the fetus and newborn. The monocyte monolayer assay (MMA) is an in vitro procedure that mimics extravascular hemolysis and can predict the clinical significance of an antibody to guide transfusion recommendations. This study was designed to assess the correlation, if any, between antibody reaction strength at the antihuman globulin (AHG) phase of testing and predicted clinical significance using the MMA. Multiple examples of anti-K with varying strengths were used to sensitize K+k+ donor cells then incubated with monocytes harvested from a healthy donor. A minimum of 400 monocytes were counted for each example and the monocyte index (MI) was calculated and compared. The MI is a percentage of RBCs adhered, ingested or both by the monocytes. Results show no correlation between the antibody strength and its significance according to MI results.[4]

INTRODUCTION

Today blood transfusions are a relatively safe low risk medical treatment in large part because of years of research and innovation in compatibility testing, blood collection and storage, testing for blood borne diseases, mitigation of bacterial contamination and safeguards put in place to accurately identify patients and their donors. Risks still arise when compatible blood or blood components are rare or not readily available for patients with multiple clinically significant red cell alloantibodies and/or with an antibody to a high prevalence or unidentified antigen. When an antibody is detected it must be identified to evaluate its likely significance. Antibodies that bind complement, IgM and some subclasses of IgG (IgG_1 and particularly IgG_3), can cause intravascular hemolysis and are always considered more significant than those that do not. Any antibody is generally considered clinically significant if it causes shortened survival of RBCs possessing its corresponding antigen, there is laboratory evidence of hemolysis and/or clinical signs and symptoms of hemolytic transfusion reaction (HTR) or hemolytic disease of the fetus and newborn (HDFN).[1] These antibodies are usually IgG, react at 37°C and/or the antiglobulin or antihuman globulin (AHG) phase of the antibody screen/panel and are the result of previous antigen exposure via transfusion and/or pregnancy. Antibodies generally deemed insignificant are 5 often IgM antibodies that react at low temperatures (RT or 4c), historically do not cause HTR or HDFN and do not bind complement or cause hemolysis.[2] Not all antibodies fit into either category and may need to be evaluated case by case. Antibodies capable of causing extravascular hemolysis or shortened cell survival can vary in significance. Years of published reports of transfusion reactions and/or HDFN caused by RBC alloantibodies provide immunohematologists with general characterizations on their clinical significance.

When antigen negative units are readily available for those with multiple antibodies or antibodies to high prevalence antigens, there is no need to question the clinical significance of each antibody. Antigen negative units are simply AHG crossmatched to provide compatible units. However, with each new antibody detected the availability and likelihood of finding suitable units becomes increasingly more difficult. Some may be tempted to rely on antibody strength to determine significance, but in vitro antibody strength is highly subjective and is not a reliable indicator of clinical significance.[3] Antibody strength is dependent on many variables including but not limited to the test methodology (tube, gel or solid phase testing), enhancement media, antibody titer, the number of antigen sites present on the RBCs (dosage) as well as, incubation pH, time and temperature.[2]

The monocyte monolayer assay (MMA) is an in vitro procedure that has been use for more than 40 years to predict in vivo survivability of incompatible RBCs. The MMA mimics extravascular hemolysis of red blood cells and may be used to guide transfusion recommendations or to assess the likelihood of HDFN in a pregnancy.[4] Macrophages (monocytes that migrated from the blood stream to the spleen, liver and lymph nodes) have Fc receptors that can bind to IgG_1, IgG_2, IgG_3 or C_3 coated RBCs and may or may not remove the cells from the circulation.[4] Macrophages/monocytes do not have the proper Fc receptor sites to bind cells coated with IgG4.[2] Macrophages may either phagocytize or adhere to an antibody and/or complement coated RBC or ingest small portions of the cell forming spherocytes that have shortened survival. In cases where the availability of antigen negative units becomes difficult, the MMA may be used to help predict the clinical significance of a particular antibody.[5] It may be necessary and is often advantageous to "challenge" some or all of a patient's antibodies in vitro with the MMA to assess whether one or more antibodies are clinically insignificant and can be mismatched before proceeding with transfusion.[3] This research was designed to assess the correlation between the antibody strength by graded tube testing (1+ to 4+) and titer with the predicted significance of the antibody using the MMA. The results of parallel MMA testing of sixteen 7 aliquots from donor plasmas known to contain anti-K, varying in reaction strength from 1+ to 4+, revealed poor correlation of graded strength and the predicted significance by MMA.

MATERIALS AND METHODS

The first steps of the experiment involved AHG tube testing and performing titers on plasma aliquots known to contain anti-K of varying strength. Reactivity of each sample was confirmed by testing cells from a heterozygous (K+k+) donor at AHG using 60-minute saline incubation. The titer of each antibody sample was determined by serial dilution and four samples of comparable antibody strength and titer were selected for MMA testing. Four samples determined to react 4+, 3+, 2+ and 1+ were chosen that had comparable titer results. (See titer results Table I).

Three (8mL) BD Vacutainer® CPT TubesTM and two serum tubes were freshly drawn from a healthy volunteer. The two serum tubes were placed in a 37°C dry cabinet to clot. The CPT tubes were gently mixed and spun for 30 minutes at 1800 RCF (relative centrifugal force). After centrifugation, about half of the plasma layer was aspirated and discarded from each tube. The remainder of the plasma and the underlying whitish layer containing mononuclear cells and platelets was collected with a Pasteur pipette and transferred to three separate 15 mL conical centrifuge tubes. Fifteen mL of phosphate buffered saline (PBS) was added to each conical tube. The tubes were capped, inverted five times then centrifuged for 15 minutes at 300 RCF as a first wash. After centrifugation, as much supernatant as possible was removed without disturbing the cell pellet. The pellet was gently vortexed, ten ml of PBS was added and the tubes were centrifuged for ten minutes at 300 RCF.[6] The supernatant was aspirated and discarded and the cell pellets from all three tube was combined and resuspended in 7.5 mL of RPMI-1640 medium with 5% Sigma-Aldrich® Fetal Bovine Serum (FBS). This mononuclear cell suspension was equally divided into seventeen of the wells of three individual Lab-Tek II Chamber Slides w/ Cover. The three chamber slides were covered and placed in a 37°C incubator with an atmosphere of 4-5% CO2 for 60 minutes to create the monocyte monolayer. Simultaneously, EDTA cells from a K+k+ donor were manually washed three times with PBS and resuspended to a 3% cell suspension. A separate positive control was set up using an equal volume of pooled R1R1 and $R2R_2$ screening cells, washed and resuspended to a 3% cell suspension. During the first wash of the monocytes, the 'two-stage sensitization' of the washed red cells begins. Stage one - four drops of each test cell was added to a labeled tube; one positive control cell tube and sixteen K+ cell tubes. (Figure 1 Chamber Slide Maps) Eight drops of the anti-D positive control serum was added to four drops of the 3% suspension of Rh+ screening cells. Eight drops of each individual anti-K sample was added to its labeled tube containing four drops of the 3% suspension of K+ cells. All tubes were incubated at 37° C for 60 minutes without enhancement. Stage two - The two serum tubes were centrifuged and ABO compatible serum was harvested to serve as a fresh complement source. At the end of the 60 minute incubation of the red cells, eight drops of fresh complement serum was added to each of the K+ sensitized cell suspensions. All tubes were incubated for an additional 15 minutes, then washed three times and resuspended to 3% in PBS. This completes the 'two-stage sensitization' method.[7] One drop of each sensitized cell suspension was tested using anti-IgG and anti-C3b/C3d separately. All tubes were centrifuged for 60 seconds, decanted, mixed gently and resuspended to 3% in RPMI-1640 medium with 5% Sigma-Aldrich® Fetal Bovine Serum (FBS). After the first 60 minute incubation, the chamber slides were removed from the CO2 incubator and the excess fluid was aspirated from each chamber and discarded.

The prepared sensitized red cells were gently added to the appropriate chamber well and the chamber slides were returned to the CO2 incubator for a final 60 minute incubation. The chamber slides were removed from the incubator, gently washed with 0.9% saline and dried at 37°C for 30 minutes in the dry incubator cabinet. The chamber slides were then stained with SIGMA-ALDRICH Wright-Giemsa stain, and dried for 30 minutes at 37°C.7 Each Lab-Tek II Chamber Slide has eight wells. Three Chamber slides were used. Well one of chamber slide #1 was used for the positive control. The remaining wells, two through 17, contained the sixteen selected anti-K samples used to sensitize the same K+k+ RBC donor. A minimum of 400 monocytes were counted microscopically in each well under oil immersion. The monocyte index (MI) was calculated for each chamber well. The MI is the percentage of RBCs adhered, ingested or both (for the total) verses free monocytes. (See Figure 3 Microscopic Image of MMA). An MI of 'Zero' or O indicates there were no adhered or phagocytized red cells. Experience with this procedure has been similar to others; in that MI values of ≤5 have indicated that incompatible blood can be given without the risk of an overt hemolytic transfusion reaction but it does not guarantee normal long-term survival of those RBCs. MI values ranging from 5–20 have a reduced risk of clinical significance, but signs and symptoms of transfusion reaction may occur. Similarly, an MI of >20 indicates the antibody has clinical significance, which may range from abnormal RBC survival to clinically obvious adverse reactions.

Figure 1 Chamber Slide Maps

Chamber Slide # 1

1. Positive Control	2. 11115071 (4+)	3. 5758282 (4+)	4. 9619786 (4+)
5. 19756058 (4+)	6. 4463382 (3+)	7. 17511864 (3+)	8. 17495344 (3+)

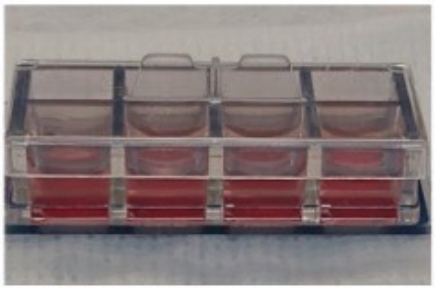
Chamber Slide # 2

9. 20930172 (3+)	10. 20981128 (2+)	11. 20976210 (2+)	12. 20970437 (2+)
13. 20947714 (2+)	14. 20979117 (1+)	15. 20969743 (1+)	16. 20936274 (1+)

Chamber Slide # 3

17. 20934846 (1+)	N/A	N/A	N/A
N/A	N/A	N/A	N/A

Figure 1 Chamber Slide Maps illustrates the assigned location anti-D (positive control) and the strength of reactivity (1+ to 4+) of the anti-K samples (2-17) at AHG phase of tube testing.



Lab-Tek II Chamber Slide

RESULTS

The antibody graded strength and titer results of the sixteen samples of anti-K selected for this study are noted in Table I. The goal of the study was to find four anti-K samples of each graded strength (1+ to 4+) within a comparable titer range to test using the MMA to assess whether antibody strength correlates with clinical significance.

Results of the calculated MI values of each tested sample are shown in Table II. The MI values of the 4+ strength anti-K samples ranged from 13.75-41.50, indicating that all would be considered clinically significant. MI values of the 3+ strength ranged from 17.00-37.75, indicating all would be considered clinically significant. MI values of the 2+ strength ranged from 14.50-23.5, indicating that all would be considered clinically significant. Even the results of the 1+ anti-K samples ranged from 9.5-39.75, indicating they all would be considered clinically significant.

These results show no correlation between tubes testing AHG antibody strength and the clinical significance of an antibody (anti-K) as assessed by the MMA.

Table I- Antibody strength and titer results of each anti-K sample

	Unit ID	1:1	1:2	1:4	1:8	1:16
1	Positive Control	4+	N/A	N/A	N/A	N/A
2	11115071	4+	2+	2+	1+	1+
3	5758282	4+	2+	2+	1+	W+
4	9619786	4+	3+	2+	2+	1+
5	19756058	4+	2+	1+	1+	W+
6	4463382	3+	2+	1+	1+	W+
7	17511864	3+	2+	1+	W+	0
8	17495344	3+	1+	1+	0	0
9	20930172	3+	3+	2+	1+	0
10	20981128	2+	2+	2+	1+	W+
11	20976210	2+	W+	0	0	0
12	20970437	2+	W+	0	0	0
13	20947714	2+	1+	W+	0	0
14	20979117	1+	0	0	0	0
15	20969743	1+	1+	1+	W+	0
16	20936274	1+	0	0	0	0
17	20934846	1+	0	0	0	0

W+ = weak macroscopic agglutination

Table II-MMA results for each anti-K sample

Antibody Source	Specificity	RBC	Slide Position	AHG		Macrophages counted	Adhered RBCs	Ingested RBCs	MI adhered RBC	MI ingested RBC	MI total
				IgG	C3b/C3d						
Positive Control	anti-D	Rh D+	1	4+	N/A	400	70	95	17.50	23.75	41.25
11115071	anti-K	K+	2	4+	0	400	38	54	9.50	13.50	23.00
5758282	anti-K	K+	3	4+	0	400	3	58	0.75	14.50	15.25
9619786	anti-K	K+	4	4+	0	400	10	45	2.50	11.25	13.75
19756058	anti-K	K+	5	4+	3+	400	105	61	26.25	15.25	41.50
4463382	anti-K	K+	6	3+	0	400	21	99	5.25	24.75	30.00
17511864	anti-K	K+	7	3+	2+	400	24	90	6.00	22.50	28.50
17495344	anti-K	K+	8	3+	2+	400	4	64	1.00	16.00	17.00
20930172	anti-K	K+	9	3+	1+	400	72	79	18.00	19.75	37.75
20981128	anti-K	K+	10	2+	2+	400	25	52	6.25	13.00	19.25
20976210	anti-K	K+	11	2+	0	400	2	60	0.50	15.00	15.50
20970437	anti-K	K+	12	2+	1+	400	20	74	5.00	18.50	23.50
20947714	anti-K	K+	13	2+	1+	400	31	27	7.75	6.75	14.50
20979117	anti-K	K+	14	1+	2+	400	16	22	4.00	5.50	9.50
20969743	anti-K	K+	15	1+	3+	400	37*	122	9.25	30.50	39.75
20936274	anti-K	K+	16	1+	0	400	51	19	12.75	4.75	17.50
20934846	Anti-K	K+	17	1+	0	400	71	52	17.75	13.00	30.75

Formula used to calculate the Monocyte Index (MI):

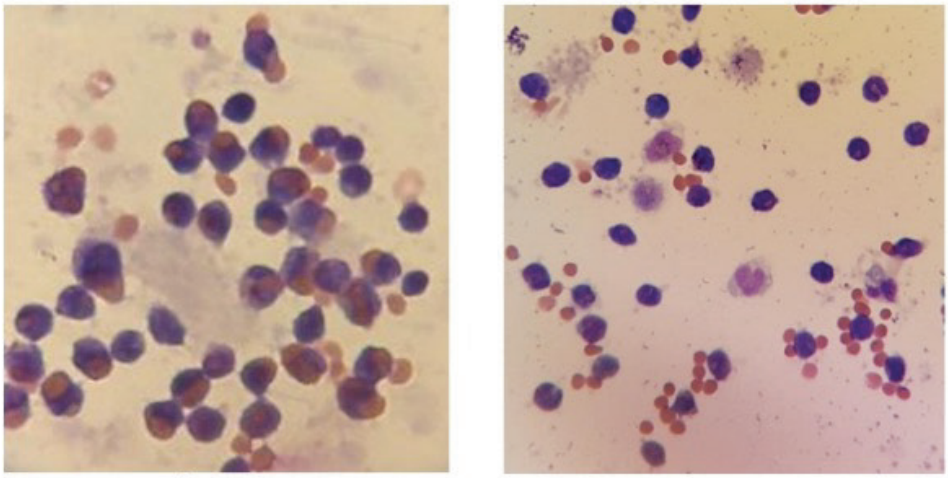
$$MI = \frac{\text{total \# of monocytes with adhered RBC and/or phagocytized RBC}}{\text{Total number of monocytes counted}} \times 100$$

Figure 2

Ranges of clinical significance	Monocyte Index (MI)
Non Clinical significant	< 5
Moderate Clinical significant	5 - 20
Clinical significant	21 - above

Correlation of MI range and predicted clinical significance of an antibody

Figure 3 Microscopic Image of MMA



Ingested/Phagocytized RBC **Adhered RBC and Free Monocytes**

DISCUSSION

Blood transfusions are a relatively safe low risk medical treatment. Problems arise when compatible blood is rare or not readily available for patients with multiple clinically significant alloantibodies, antibodies to a high prevalence antigen or when the antibody specificity is in question. During pre-transfusion or prenatal testing if a red cell antibody is detected it must be identified to determine its likely significance. Years of documented published reports of antibodies causing adverse transfusion outcomes or severe HDFN aid in categorizing specific antibodies and their significance. This historical data aids in transfusion recommendations and deciding which antibodies can be challenged if antigen negative units are not available. Although it may be tempting to rely on this data and or antibody strength as an indicator of clinical significance this study demonstrates in vitro antibody strength is highly subjective, variable and is not a reliable indicator of clinical significance. While anti-K is always considered clinically significant, as demonstrated in this study, it is also one of the easiest to find compatible units for and is rarely challenged. The initial purpose of this study was to ascertain if antibody strength correlated with predicted in vivo clinical significance. The findings demonstrate the strength of antibody-antigen reactivity in vitro has little or no correlation with predicted RBC survival in vivo with the MMA. The obvious exception is when hemolysis is noted during antibody screening and identification or compatibly testing. Hemolysis is always an indicator of a probable acute hemolytic transfusion reaction. Unlike antibodies known to cause intravascular hemolysis, those capable of causing extravascular hemolysis and shortened cell survival can vary greatly in significance and may be advantageous and worth challenging especially if finding suitable blood is difficult. This is especially true of antibodies to high prevalence antigens, especially where historical data is lacking. The MMA is mediated by monocytes to predict extravascular hemolysis occurring in the liver, spleen and lymph nodes. Variables such as immunoglobulin class and subclass, and complement activation may affect the clinical significance of an antibody despite MMA predictions. It also must be noted that antibody clinical significance can and does change with repeated transfusions and exposure to the antigen being challenged, so the MMA, similar to a RBC compatibility testing must be repeated regularly. Perhaps additional testing should be performed with antibodies whose reaction strength and clinical significance if known to be variable.

SUMMARY

Although blood transfusions are a relatively safe medical treatment, risks arise when compatible blood is rare or not readily available for patients with multiple clinically significant alloantibodies and/or with antibody to a high prevalence or unidentified antigen. Antibodies considered clinically significant cause shortened survival of RBCs possessing the targeted antigen, laboratory evidence of hemolysis, and/or clinical signs of hemolytic transfusion reaction and or Hemolytic disease of the fetus and newborn. The MMA is an in vitro procedure that mimics extravascular hemolysis that may be used to predict the clinical significance of an antibody to guide transfusion or to assess the likelihood of HDFN in a pregnancy. Although it may be tempting to rely on antibody strength as an indicator of clinical significance when antigen negative units are not readily available, the results of this study demonstrated no correlation between the graded strength an antibody (in this study anti-K was addressed) and its significance according to MMA test results.

REFERENCES

1. Cohn CS, Delaney M, Johnson ST, Katz LM. AABB technical manual 20th ed. Bethesda, MD: AABB 2020.

2. Harmening DM. Modern blood banking and transfusion practices. 7th ed. Philadelphia, PA: F.A. Davis Company 2019.

3. Issit PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific 1998.

4. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfusion Medicine Reviews, Vol21, No1 2007: 58-71.

5. Petz. LD, Garratty G. Immune Hemolytic Anemias 2nd ed. Philadelphia, PA: Churchill Livingstone, 2004.

6. BD Vacutainer® CPT TubesTM Cell Preparation Tube with Sodium Citrate [package insert]. Franklin Lakes, NJ: Becton, Dickinson and Company; 06/2015.

7. Moulds JJ, Billingsley KL, Kalvelage M. Monocyte monolayer assay (MMA) [standard operating procedure}. Shreveport, LA: LifeShare Blood Center; 11/2016.

8. Arndt PA, Garratty G. A retrospective analysis of the value of monocyte monolayer assay results for predicting the clinical significance of blood group alloantibodies. Transfusion. 2004 Sep;44(9):1273-81

2021 | **ORAL & POSTER** Abstracts

The following Abstracts were submitted for review by the South Central Association of Blood Banks Program Committee chairs and were selected in either ORAL or POSTER categories for the 2021 Abstract Journal.

Abstract #	Type	Abstract Title	Abstract Authors
	ORAL	Correlation of antihuman globulin test tube strength and clinical significance of antibodies by MMA	Sol Haberman Award Winner: Luis A. Pena Marquez
#1	ORAL	The Effect of COVID-19 on Donation Habits of Donors	Dr. Richard Gammon, MD, OneBlood, SMT Medical Direction, Orlando, Alicia Bellido Prichard MBA MT (ASCP)SBB , OneBlood, Inc., St. Petersburg,, Michael Gannett, MLS(ASCP)SBB, OneBlood, Inc., Orlando IRL, and Mr. Boyan Yordanov, OneBlood, St. Petersburg
#2	POSTER	Liquid Plasma - Improving Outcomes and Reducing Costs	Julia Maxwell, Methodist Health System - LeeAnn Walker, University of Texas Medical Branch
#3	ORAL	CMV Screening of Group Specific Orders - Good Stewardship of the Blood Supply	Tara Francis, OneBlood, IRL, Orlando, Florida; Alexander Delk, OneBlood, IRL, Fort Lauderdale, Florida; Richard R. Gammon, OneBlood, Scientific, Medical, Technical Direction, Orlando
#4	ORAL	Monitoring HLA Matched Platelet Requests - Good Stewardship of the Blood Supply	Richard Gammon, Michael Gannett - OneBlood, Orlando
#5	POSTER	Use of Recombinant Antigen to Aid in Identification of a High Incidence Antibody	Michelle Conceicao, OneBlood; Alexander Delk, OneBlood; Richard Gammon, OneBlood

T/S # 1 - ORAL

Title: The Effect of COVID-19 on Donation Habits of Donors

Author(s): Richard Gammon, MD, OneBlood, SMT Medical Direction, Orlando, Alicia Bellido Prichard MBA MT (ASCP) SBB , OneBlood, Inc., St. Petersburg, Michael Gannett, MLS(ASCP)SBB, OneBlood, Inc., Orlando IRL, Orlando, and Mr. Boyan Yordanov, OneBlood, Inc., St. Petersburg,

Background/Case Studies:

Blood centers (BC) rely on collections at schools and businesses. Shelter-in-place orders issued in 2020 due to COVID-19 closed these facilities. Additional donor campaigns were conducted to ensure adequate blood supplies during the pandemic. Testing for antibodies to SARS-CoV-2 for all allogenic donations was implemented 05/18/20. This study was to determine how COVID-19 affected the donation habits of donors.

Study Design/Methods:

Three time periods were reviewed [May to June of 2018 vs. 2019 (control), May to June 2019 vs.2020 (study group)]. The following were reviewed: first-time, repeat, and lapsed donors (no donation > 2 years), gender, age, ethnicity and ABO blood groups.

Results/Findings:

There were 264,593 donations. In 2020 there was a significant increase in total donations [2019-20 p=<.0001, 2018-19 p = 0.683] and by gender [2019-20 M p= 0.004, F p= <0.0001, 2018-19 M p=0.716, F p= 0.657]. In 2019-20 for the three largest ethnicities there was a significant decrease in Hispanic (p= 0.001) and African American (p <0.0001) and a significant increase in Caucasian (p<0.0001) donations. This was nonsignificant in 2018-19 [African American p=0.415, Hispanic p=0.620, Caucasian p = 0.685].

Results/Findings: *continued*

There was a significant increase in first-time (p<0.0001) and lapsed donors (p<0.0001) in 2019-20 compared with 2018-19 [first-time, p= 0.087, lapsed p=0.308] not seen in repeat donors [2018-19 p=0.730, 2019-20 p = 0.705]. The mean donor age increased significantly in 2019-20 (p=0.024) compared to 2018-19 (p=0.904). In 2019- 20 there was a significant decrease in the percentage of donors < 30 years [2019-20 16-20 p<0.0001, 21-30 p<0.0001 vs. 2018-19 16-20 p=0.053, 21-30 p= 0.464]. There was also a significant increase in all blood types in 2020 [19-20 all blood types p<0.0001, 18-19 O+ p=0.621, O- p=0.724, A+ p=0.774, A- p=0.986, B+ p=0.176, B- p=0.665, AB+ p=0.840, AB- p=0.395].

Conclusions:

Statistically significant changes occurred in the donation habits of donors in 2020 when compared to the control group. Additional donor campaigns and testing for SARS-CoV-2 antibodies may explain the increase in total donations as well as first-time and lapsed donors and among all blood types. The significant decrease in donors < age 30 years may be caused by the closure of educational facilities. Changes in the three largest ethnicities could not be explained.

Year	Males*	Females*	African American	Hispanic	Caucasian	First-Time	Repeat	Lapsed	16-20 years	21-29 years	Mean Age
2018	43,645	38,126	7,076	14,102	56,994	16,202	54,878	10,695	6,003	12,276	45.08
2019	42,961	37,333	6,780	13,672	55,927	15,216	53,888	11,192	4,858	11,830	45.84
2020	47,745	54,768	3,555	11,076	83,931	30,745	52,999	18,778	2,547	9,036	50.07

*15 gender not provided

T/S # 2 - POSTER

Title: Liquid Plasma – Improving Outcomes and Reducing Costs

Author(s): Julia Maxwell, Methodist Health System, Dallas, LeeAnn Walker, University of Texas Medical Branch, Galveston

Background:

The purpose of this study was to determine if the implementation of Liquid Plasma (LP) resulted in plasma availability for patients. This study also set out to determine if LP resulted in reduction in waste and cost savings to blood banks. Additionally a cost savings to patients was quantified.

Methods:

This study used retrospective chart review analyzing data from April 1, 2017 to May 31, 2018. Data included the types of products transfused (red blood cells, fresh frozen plasma or liquid plasma) and product wastage. One hundred and fifty four adult patients who had emergency issue or Massive Transfusion Protocol (MTP) orders were included, along with the number of products transfused for 24 hours after initial order. Discard reports from Transfusion Service operational data were used to determine product wastage. The total number of red blood cell and plasma products transfused, the plasma to red cell ratio, the time to plasma issue, and percentage of patients who received plasma was studied. Savings to patients by utilizing LP was evaluated along with product wastage and cost savings to blood bank and patients was quantified.

Results:

Liquid plasma reduced total transfusions for patients with an MTP and an emergency issue order. It also increased the plasma to red blood cell ratio in emergency issue only patients from 0.02:1 to 0.4:1 (p=0.016) and allowed 16% more patients to receive plasma (p<0.001). The time from order to plasma issue was sooner in the ALP group. Twenty three units of liquid plasma were transfused, resulting in a cost saving to the patients from $90 up to $360. Liquid plasma resulted in an average savings of $230 per patient. Before implementation of LP, plasma wastage was 113 units compared to 85 after LP (p<0.001). This resulted in a savings of $1,288 for the Transfusion Service.

Conclusion:

Having liquid plasma readily available resulted in more patients receiving plasma, an increase in plasma to RBC ratio, and fewer total products being transfused. This has been shown to contribute to more favorable outcomes. A savings to the Transfusion Service and the patient was recognized which is important in curtailing ever increasing health care costs.

T/S # 3- ORAL

Title: CMV Screening of Group Specific Orders- Good Stewardship of the Blood Supply

Author(s): Tara Francis, OneBlood-IRL Orlando; Alexander Delk, OneBlood- IRL Ft. Lauderdale; Richard R. Gammon, OneBlood-Orlando

Background:

In addition to antigen negative red blood cells (RBC), Immunohematology Reference Laboratories must provide RBC that are cytomegalovirus (CMV) negative. Due to the high percentage of CMV positive individuals, there is a challenge to find CMV negative, antigen negative RBC. The IRL selects predominantly group O donors to test for CMV and these RBC are sometimes needed to fill orders for non-group O patients. This study evaluated the number of units sent that were out-of-group to fulfill CMV negative requests.

Study Design and Methods:

Requests for CMV negative and antigen negative RBC were divided into two periods. Period 1 (January 1, 2019 - February 29, 2020) before intervention and Period 2 (March 1, 2020-May 31, 2020) post-intervention (to increase the amount of group B donors tested for CMV by 20%) were evaluated. ABO Rh units requested were compared to ABO Rh specific units provided.

Results:

Period 1: 537 CMV negative RBC units were provided. 99/188(52.66%) group B positive requests were fulfilled using O RBCs. A total of 39/504 (7.74%) group O/D negative units were sent to fill D positive orders.

Period 2: 119 CMV negative RBC units were provided. 18/51 (35%) group B positive requests were fulfilled using O RBCs. Only 2/113 (1.77%) Group O/D negative units were sent to fill D positive orders.

Discussion:

36.4% of CMV negative antigen orders during both periods were requested for B positive patients and 49.0% orders were filled with group O RBCs. To decrease unnecessary group O usage for non-group O patients, the IRL practice has changed to increase CMV testing for group B donors. Since the change in algorithm, there was a 17.66% decrease group O usage for group B patients allowing for better stewardship of the group O blood supply.

Period 1

Patient ABO	Unit Group*					Total units sent	Number and Percentage of Group/Rh specific units sent		Number and Percentage of Out of Group and/or Rh units sent		Number and Percentage of Group O's sent	
	O+	O-	A+	A-	B+							
A-	0	1	0	8	0	9	8	88.89%	1	11.11%	1	11.11 %
A+	14	1	51	2	0	68	51	75%	17	25%	15	22.06 %
AB-	0	1	0	2	0	3	0	0	3	100%	1	33.33 %
AB+	7	0	5	17	12	41	0	0	41	100%	7	17.07 %
B-	0	1	0	0	0	1	0	0	1	100%	1	100%
B+	77	22	0	0	89	188	89	47.34%	99	52.66%	99	52.66 %
O-	0	20	0	0	0	20	20	100%	0	0%	20	100%
O+	191	16	0	0	0	207	191	92.27%	16	7.73%	191	100%
Total	**289**	**62**	**56**	**29**	**101**	**537**	**359**	**66.8%**	178	33.14%	335	62.38 %

*No Group B-, AB+ and AB- units were distributed

Period 2

Patient ABO/Rh	Unit Group*					Total units sent	Number and Percentage of Group/Rh specific units sent		Number and Percentage of Out of Group and/or Rh units sent		Number and Percentage of Group O's sent	
	O+	O-	A+	A-	B+							
A-		3				3	0	0%	3	100%	3	100%
A+	2		7	3		12	7	58%	5	42%	2	17%
B+	17	1			33	51	33	65%	18	35%	18	35%
O-		3				3	3	100%	0	0%	3	100%
O+	31					31	31	100%	0	0%	31	100%
AB+	6	1	6	2	4	19	0	0%	19	100%	7	37%
Total	**56**	**8**	**13**	**5**	**37**	**119**	74	62.18%	45	37.82%	64	53.78%

* No Group B-, AB+, or AB- RBC units distributed.

T/S # 4 - ORAL

Title: Monitoring HLA Matched Platelet Requests – Good Stewardship of the Blood Supply

Author(s): Richard Gammon,MD., Michael Gannett: OneBlood, Inc. Orlando

Background/Case Studies:

Physicians order HLA matched platelets to treat platelet refractoriness. While an unusual order would be brought to the medical director, in most cases, the immunohematology reference laboratory (IRL) provides compatible units with the only restriction being availability. Recently the IRL requested the platelet count accompany the order. This study reviewed correlation between HLA matched product requests and platelet count.

Study Design and Methods:

This retrospective study reviewed all requests for HLA compatible platelets the IRL received from 02/01/20 - 04/30/20. If no history, low resolution genotyping determined HLA type. Items reviewed included platelet count, product orders, demographics and diagnoses.

Results/Findings:

554 patients received 794 components (401 females, 114 males and 39 not stated), mean platelet count was 20,670/uL (1,000-291,000/uL). For males mean count was 42,790/uL (6,000 -73,890) and for females 15,580/uL (4,000-31,750). The mean platelet count at which products were ordered varied by decade with highest for ages 50-59 at 44,160/uL and lowest for ages 80-89 at 4,000/uL. The highest mean counts at which orders were placed for both genders was seen for ages 10-19 at 68,000/uL(males) and 31,750/uL (females). Lowest counts differed by gender and age: for males ages 30-39 at 6,000/uL and for females ages 80-89 at 4,000/uL. Patients age > 50 received the greatest number of HLA matched platelets products: ages 60-69 - 175 components (males 27 and females 136). Almost half of all of platelets went to those thrombocytopenic: (Table) 0-9,999/uL, 259 products (32.6%), 10,000-19,999/uL, 97 products (12.2%). Patient blood types were: O+ 221 (39.9%), O- 44 (7.9%), A+ 127, (22.9%), A- 20 (3.6%), B+ 35 (6.5%), B- 20 (3.6%), AB+ 19 (3.4%), AB- 1 (0.2%), no type 67 (12.0%). Top diagnoses (ICD 10 codes) were: acute leukemia 183(33.0%), hematology oncology 65 (11.7%), myelodysplastic syndrome 41 (7.4%), anemia 36 (6.5%) and bone marrow transplant 16 (2.9%). Mean number of platelets ordered was 1.43 (1-5).

Conclusions:

The majority of HLA compatible platelet orders occurred in older patients with thrombocytopenia and acute leukemia. Some requests had platelet counts > 100,000/uL. Being able to monitor orders would allow for better platelet stewardship. Outliers such as a high platelet count could be forwarded to the medical director to determine justification.

T/S # 5 - POSTER

Title: Use of Recombinant Antigen to Aid in Identification of a High Incidence Antibody

Author(s): Michelle Conceicao, Alexander Delk, OneBlood- IRL Ft. Lauderdale;
Richard R. Gammon, OneBlood-Orlando

Background/Case Studies:

A 71-year-old Caucasian female had a negative antibody screen and was transfused three units of red blood cells (RBCs) within a four-day period at a local hospital. Twenty-four days later the patient was readmitted to the same hospital where another type and screen was performed. The type and screen now showed the patient had a positive antibody screen. The referring facility attempted to identify the antibody by running reagent red cell panels using solid phase methodology, polyethylene glycol (PEG) and 22% Albumin tube techniques. The antibody was reactive with all cells tested and the facility was unable to identify any antibody specificity; therefore, a sample was sent to the Immunohematology Reference Laboratory (IRL) for further evaluation. Initial testing indicated the antibody might be directed against a high prevalence Scianna antigen. Little data is known of the clinical significance of anti-Sc1; however, in this case the decision was made to use recombinant Sc1 blood group antigen to prove the antibody had Scianna blood group specificity.

Study Design/Methods:

The IRL's serologic testing was performed using tube techniques and red cell treatments including direct antiglobulin test (DAT), PEG, 0.2M dithiothreitol (DTT), papain, alloadsorption, and neutralization using recombinant Sc1 blood group antigen (rBGASc1). Red cell genotyping was performed using the HEA BeadChip.

Results/Findings:

The antibody reacted 1+ at immediate spin (IS) phase and 3+ at PEG-indirect antiglobulin testing (IAT) with all reagent red cells tested. The DAT and autologous control were negative. The antibody reactivity strength remained unchanged after 0.2M DTT treatment followed by papain treatment. Due to this reactivity pattern an antibody to a high prevalence antigen was suspected. HEA BeadChip tested concurrently revealed the patient was Sc:-1,2. The Sc:-1 phenotype was also confirmed serologically using anti-Sc-1 from the IRL's rare antisera library. The IRL had very limited amount of Sc:-1 cells therefore, the (rBGASc1) was used to neutralize the antibody proving it was Scianna-specific. Two sources of Sc:-1 frozen RBCs were thawed and tested, both yielding negative IS and PEG-IAT results. Additional underlying common alloantibodies were excluded using the rare thawed cells and adsorbed sera.

Conclusion:

This case demonstrated how a variety of techniques used by the IRL including HEA BeadChip, red cell treatments, adsorption, neutralization and frozen rare RBCs were utilized concurrently to solve a difficult antibody identification case. The use of (rBGASc1) as a screening tool ensured correct blood group specificity before rare RBCs were thawed.

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