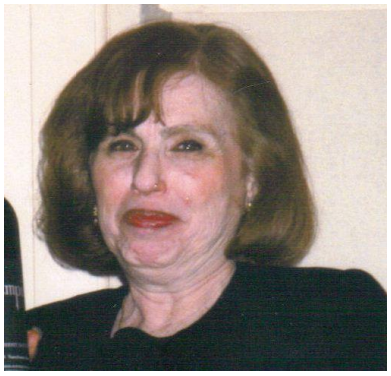
ILLINOIS ASSOCIATION OF BLOOD BANKS

The Phyllis Unger
Annual Case Studies Meeting

Thursday, February 13, 2020



This case studies meeting is dedicated to Phyllis Unger, in memory of all the wonderful case studies she presented to students, technicians, technologists, residents, fellows and physicians. Phyllis was a dedicated blood banker who spent much of her time teaching and educating whether she was at Michael Reese, University of Illinois or LifeSource (Vitalant, Inc.). She was always willing to answer questions or test a sample if you sent it to her. She inspired many blood bankers and gave them the desire to look further into a problem. Phyllis was the first Medical Technologist to be President of the ILABB. Prior to this only a physician could hold the office. She wrote many papers and contributed to a few books including "Blood Group System: MN and Gerbich." She had many things outside of blood banking that she enjoyed as well such as travel, music and bridge. Even these things helped give her blood banking perspective. She was known for never saying anything bad about anyone and always finding the best in them. We hope that this annual meeting will serve as a lasting memory to the knowledge she shared with all who came in contact with her over the years. Thank you Phyllis.

Agenda

Social hour: 6-7pm

Presentations will begin promptly at 7pm.

1. Utility Of Long-Term Plasma Exchange For Post-Transplant Warm Autoimmune Hemolytic Anemia, A Case Report
   Amin, N., Hultman, M., Su, L., Wool, G.

2. Complicated case of delayed hemolytic transfusion reaction, with systemic and technical challenges
   Amin, N., Madden, P., Hultman, M., Musa, G., Wool, G., Su, L.

3. Resolving Weak D Serological Typing Discrepancies using RHD Genotyping
   Christina Barriteau MD MPH, Paul F. Lindholm MD, Glenn Ramsey MD

4. Two Fatal Complications Associated with Acute Epstein-Barr-Virus (EBV) - Associated Infectious Mononucleosis (IM)
   Phillip J. DeChristopher, MD, PhD, Kristina Gvozdjan, MD, Lavinia Sereseanu, MT(ASCP), Dianna Rodheim, MT(ASCP)

5. Another ABO Discrepancy—Why it's not a Group O
   Christine Howard-Menk, MS, MT(ASCP)SBB; Mona Papari, MD; Jason Crane, DO

6. Identification of Anti-Hr0 (Rh17) in a Multigravida Never Transfused RH: 1, -2, -3, 4, -5 Female
   JG Zinni, B Patel, MA Keith, MM Hinrichs, M Kwan, C Geurkink

Title: Utility of Long-Term Plasma Exchange for Post-Transplant Warm Autoimmune Hemolytic Anemia, a Case Report

Authors: Amin, N., Hultman, M., Su, L., Wool, G.

Affiliations: University of Chicago, Department of Pathology, Chicago, IL

Case: We present a 3-year-old female patient with history of severe transplant-related WAIHA who has been treated with long term Therapeutic plasma exchange (TPE).
The patient was diagnosed with severe idiopathic aplastic anemia at age 13 months and received MUD-HSCT at age 18 months. 6 months after the HSCT, the patient's course was complicated by severe hemolytic anemia (hemoglobin 2.4g/dl, haptoglobin<20, absolute retic. count (300 x10*3/ul) and positive DAT with both IgG and C3). The patient was treated with steroids, IVIG, and Rituximab without any cure or significant improvement. She was further treated with immunosuppressive drugs (Cyclosporine) and underwent splenectomy but continued to have hemolysis, and required numerous transfusions.
The patient's blood type was A-positive, blood bank history was significant for alloantibodies, cold and warm autoantibody of broad specificities which made antibody screening challenging and often weren't able to rule out presence of antibodies despite utilizing PEG and LISS methods. Direct antiglobulin test (DAT) evolved over time to strong positive (3+ using anti-IgG in gel, 3+ using anti-C3 in tube). Allo-adsorption performed in reference lab revealed anti-C and anti-Le(a). The patient has been provided least incompatible units, C and Le(a) negative, genotypically matched blood for E, Fy(a), Jk(a) antigens to prevent further alloimmunization. Considering the refractoriness of the patient's WAIHA, our apheresis service was consulted for long term TPE. During the past 2 years the patient has received more than 100 sessions of TPE, both in the inpatient and outpatient settings, with varying frequencies of every other week, once per week to twice a week and as needed for acute severe hemolysis. We investigated the efficacy of TPE in these settings.
The patient was chemotherapy conditioned and receiving twice per week TPE in preparation for a second BMT for the last 3 months. She suffered from multiple bacterial and viral infections during the chemotherapy conditioning and eventually passed away at age 47 months.

Results: We investigated whether the intensity of TPE treatment correlated with LDH and reticulocyte level, or with transfusion requirements.

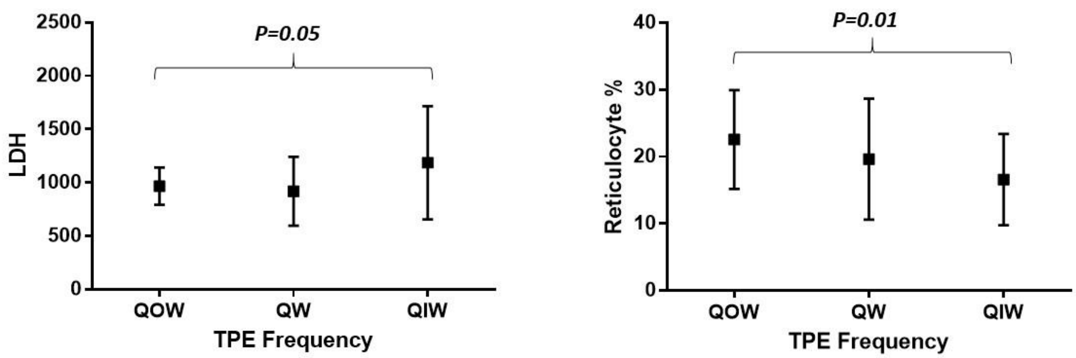


Figure 1: LDH and reticulocyte levels, by treatment category (mean, SD). Statistical analysis by ANOVA.

Mean LDH level trended towards being increased by more intensive TPE therapy, which approached significance. In contract, there was a significant trend towards reduced median percent reticulocytes with more intensive TPE therapy (Figure1). Transfusion burden clearly did not decrease with increased TPE frequency (Table1), but this is likely confounded by the patient's worsened clinical status.

Conclusion: Although it is difficult to make a conclusion on the effectiveness of TPE in treatment of WAIHA based on a single case study, our findings show that increasing TPE frequency did not result in a lower transfusion requirement. When this patient received more intensive TPE, LDH trended higher and percent reticulocytes trended lower. These findings are likely confounded by disease flares, multiple ongoing infections, and comorbidities which confound lab data and make it difficult to draw a firm conclusion.
Further studies of long-term treatment of WAIHA with larger number of patients are needed to evaluate the effectiveness and frequency of TPE in patients with refractory AIHA.
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Title: Complicated case of delayed hemolytic transfusion reaction, with systemic and technical challenges

Authors: Amin, N., Hultman, M., Su, L., Wool, G.

Affiliations: University of Chicago, Department of Pathology, Chicago, IL

We present a 69-year-old female patient with past medical history of Hereditary Hemorrhagic Telangiectasia (HHT), recurrent episodes of epistaxis and GI bleeding, and significant previous transfusion history who presented to the ED with complaints of epistaxis, severe
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lightheadedness, fatigue and dyspnea on exertion. The patient's hemoglobin was 5.5 g/dl. She had no prior blood bank history at our institution and her antibody history was unknown to us. Her blood type was O-positive and screen was negative. The patient was issued two units of electronically cross-matched blood, transfused in the ED, and was sent home.
Four weeks later, she again presented to the ED with complaints of dyspnea, fatigue and lightheadedness. Laboratory work up revealed hemoglobin of 3.9 g/dl, undetectable haptoglobin (<20 mg/dl), elevated LDH (324 U/L), high reticulocyte percent (4.5% relative, 113x10^3/uL absolute). Direct antiglobulin test was positive, 1+ with anti-IgG reagent in gel, and negative with anti-C3 reagent in tube. Elution revealed anti-C.

Further review of the patient's outside transfusion history (available electronically via our EMR) revealed that the patient had a history of anti-C and anti-E and antibody of undetermined specificity at a different hospital. The patient subsequently received 8 units of C and E antigen negative, cross match compatible blood over the next 4 days.
However, the patient continued to show signs of mild hemolysis and required 3 more pRBC transfusions. Managerial review of antibody panel indicated the possibility of a new Fy(a) antibody which was initially ruled out by a single negative homozygous screen cell (per UCMC SOP). Testing additional cells confirmed the presence of anti-Fy(a). Retrospective phenotyping of 10 recently transfused pRBC units revealed that 6 of them were Fy(a) antigen positive. The patient's genotyping revealed that patient was negative for Fy(a) antigen.
After the patient began to receive pRBC negative for C, E and Fy(a), her anemia and symptoms improved.

This case revealed several process and technical deficiencies. Upon presentation, the initial antibody screen was negative. In that setting, it is our process that outside hospital transfusion histories are not sought out. It is well known, however, that a negative screen cannot rule out evanescent antibodies and a delayed hemolytic transfusion reaction could result.
Ruling out antibodies based on a single negative homozygote cell (our current process) optimizes work-up time and cost, but can be deceiving in weak or difficult to detect antibodies. Our process is consistent with the Technical manual which recommends antibody exclusion based on a nonreactive double-dose antigen-positive red cell.

This case illustrates the necessity of robust processes for performing, interpreting, and reviewing immunohematology testing. Our current processes may benefit from reconsideration and improvement. Blood bank staff may benefit from additional training to acquire skills to detect weak or difficult to detect antibodies. Also, it can be challenging to balance workflow concerns and the need for effective communication between transfusion services; achieving such a balance can help prevent hemolytic reactions in multi-transfused patients.

Title: Resolving Weak D Serological Typing Discrepancies using RHD Genotyping

Authors: Christina Barriteau MD MPH, Paul F. Lindholm MD, Glenn Ramsey MD

Affiliation: Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Background: The D antigen is part of the Rh blood group system and is one of the most immunogenic antigens. Over 100 weak D variants have been identified. Determining the type of weak D variant has clinical implications including the risk for D alloimmunization which is relevant for individuals requiring blood transfusion and women of child bearing potential. Serological tests cannot determine the type of weak D variant and RHD genotyping methods are necessary. Research on the serological characteristics of weak D variants in automated solid phase testing is limited. Our study describes characteristics of weak D variants in automated solid phase testing and the use of RHD genotyping to resolve D typing discrepancies.

Methods: We conducted a retrospective analysis of RHD genotyping in adult patients with serological weak D phenotypes at Northwestern Memorial Hospital from January 2019 to December 2019. Patients were classified as serological weak D phenotype if automated solid phase or manual testing in test tube resulted in ≤ 2+ reaction in any or all phases. Cases with serological weak D phenotypes were reviewed by blood bank physicians to determine eligibility for RHD genotyping based on likelihood of transfusion or women of child bearing age. RHD genotyping was performed with the Immucor BioArray RHD BeadChip, which can detect >70 weak or partial RHD variants. Descriptive analysis and summary statistics were tabulated.

Results: RHD genotyping was performed on 41 patients, 6 males and 35 females. The mean patient age was 45.1 years old (range 19 to 84) and 25 women were of child bearing age defined as age <50 years old. Fifty-four percent of the cohort was Caucasian, 32% African American, 5% Hispanic and 10% unknown. The RHD BeadChip detected four RHD variants: weak D type 1 (12%), weak D type 3 (24%), weak D type 4.0 or 4.3 (32%) and DAR (12%). The D psi D-negative allele was detected heterozygously in some cases. Weak D type 4.0 or 4.3 and DAR were classified as partial D based on International Society of Blood Transfusion nomenclature. Seven of 41 (17%) weak D patients were unable to be classified by the RHD BeadChip and were categorized as D negative. D variants displayed heterogeneity in automated solid phase testing (Table 1). RHD genotyping resulted in D status change in 18/41 (44%) of patients, with 14/41 (34%) converting from D negative to D positive and 4/41 (10%) converting from D positive to D negative.

Conclusion: RHD genotyping is a useful tool to resolve serological weak D discrepancies. Partial D variants were more common in African Americans and were associated with a strong reaction in D4 automated solid phase testing. RHD genotyping resulted in a change in D phenotype in 44% of patients, which has clinical implications including use of Rh immune globulin and D negative red blood cell transfusions.

Table 1: Most common characteristics of *RHD* variants

RHD variant (n)	Automated Solid phase D4 IgM	Automated Solid phase D5 IgM	Manual Immediate spin Gammaclone IgM	Most common DCE phenotype	Most common race/ethnicity
Type 1 (5)	0	0	0-w+*	R1r	Caucasian
Type 3 (10)	?	?	2+	R1r	Caucasian
Type 4.0 or 4.3 (14)	3+	?	2+	Ro	African American
DAR (2)	?-3	0-?	1-3+	Ro	African American
DAR/D psi (3)	?	0	0	Ro	African American
Unidentified (7)	3+	?	2+	R1r	Caucasian

Most common is defined as the most common result in the cohort

?: indeterminate results in solid phase testing

*Type 1: 4+ antiglobulin, solid phase or manual IgG

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Title: Two Fatal Complications Associated with Acute Epstein-Barr-Virus (EBV) - Associated Infectious Mononucleosis (IM)

Authors: Phillip J. DeChristopher, MD, PhD, Kristina Gvozdjan, MD, Lavinia Sereseanu, MT(ASCP), Dianna Rodheim, MT(ASCP)

Affiliation: Loyola University Health System, Maywood, IL

Background: EBV (also known as HHV-4) is a ds-DNA virus, a member of the family of herpesviruses. It has a limited tropism for T and B lymphocytes and certain epithelial cells. Primary acute EBV viral infections target the oropharynx where mucosal epithelial cells and B cells become productively infected. Primary, acute EBV-associated infections, known as IM, cause fever, pharyngitis, URT infections and cervical lymphadenopathy. Incubation periods vary from 30 to 50 days. Latent EBV infections are associated with a number of malignancies. Humans are the only source of EBV.
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Case #1: A 24-y/o female was admitted with a 1-month h/o fevers, sore throat, throat swelling and night sweats. PE showed Temp of 103oF, HR 130's-40 and bulky lymphadenopathy. She was admitted for w/u of either an unidentified infection or a malignancy. EBV viremia, demonstrated by PCR (11,267 copies / mL) and cervical LN biopsy showed lymphadenitis, strongly positive for EBV, were diagnostic. On hospital Day-4, SOB, chest tightness and sinus tachycardia developed. Day-5: Altered mental status, a respiratory code called, PEA arrest x 2, requiring ACLS and pressor support. Acute renal and liver injury, thrombocytopenia and coagulopathy developed. The only T & S showed an IgG warm autoantibody in the plasma and eluate, associated with laboratory and clinical evidence of massive hemolysis (Hgb nadir at 4.6 g/dL) with complement activation. Cardiogenic shock, worsening pressor requirements and acidosis led to her demise on Day 6. Autopsy showed hepatosplenomegaly and diffuse lymphadenopathy.

Case #2: An 18-y/o male hockey athlete with no PMH was found unconscious on the bathroom floor at home. CT at OSH showed a splenic rupture and a massive hemoperitoneum. Transferred to our Level 1 Trauma Center, he was admitted comatose, intubated / ventilated in hemorrhagic shock (P 53, BP 73/48) with temp of 31.7oC and pH 6.84. Emergent OR Ex Lap, MTP initiated, found to have a ruptured spleen. He remained hemodynamically unstable requiring 4 vasopressors throughout, developed an intraoperative cardiac arrest and was returned to sinus rhythm. Despite continual maximal medical and pressor support and ongoing MTP, he had a terminal cardiac arrest, pronounced dead less than 6 hours after admission.

RESULTS / METHODS: Case 1: Standard reagent cells and antisera were used. Automated gel testing used the Ortho Vision instrument. Immucor Elukit was used for elution. Complement DAT testing used tube methods and anti-C3b/d. Transfusion support in the last 10-12 hours of life included 3 units of plasma, 3 apheresis platelets, 1 RBC (XM-incompatible by emergency release). Ante-mortem plasma showed a Coomb's-reactive autoantibody of broad thermal amplitude, reactive from 37oC down to 4oC, where 3 cord rbc's were agglutinated (1+ to 2+) and 3 adult cells were non-reactive, suggesting little-i preference.
Case #2: In the OR, the EBL was 20 liters. 7 cycles of the MTP invoked, 5 cycles issued: 17 units of RBC, 15 units of thawed plasma, 3 adult doses of platelets were transfused. Surgical splenectomy specimen, weighing 608 grams (4 x normal), had multiple capsular lacerations. Splenic tissue showed IHC-positive EBV within lymphocyte nuclei.

Conclusions: We present 2 lethal complications associated with EBV infections. The EBV-associated, acutely severe autoimmune hemolysis (late in onset and clinically unappreciated) leading to death, is distinctly rare outcome. Although EBV-associated splenic ruptures have been reported, atraumatic, spontaneous fatal cases are very uncommon.

Title: Another ABO Discrepancy—Why it's not a Group O

Authors: Christine Howard-Menk, MS, MT(ASCP)SBB; Mona Papari, MD; Jason Crane, DO

Affiliations: Vitalant-IL, IRL, Rosemont, IL

Background: A donor sample was referred back to the IRL for ABO/Rh resolution after being flagged as being discrepant by the testing facility. This was the second donation from a 17 year-old female donor, of probable Asian descent. The donor history had no indication of any health issue, previous transfusion or bone marrow transplant,. Both donations were typed by the Olympus PK7300 instrument as group O by forward typing, and as group B by reverse typing. The first donation was resolved as group O, Rh positive. The second donation was not resolved by the testing facility and returned to Vitalant-IRL for further testing.

Methods: Gel and tube methods were utilized in the resolution of this sample at our facility, in addition to molecular testing.

Results: Initial testing was performed using Gel. Donor had a negative DAT, and a negative antibody screen. Donor forward tested as a group O, and reversed as a Group B. The donor typed as Rh positive.

Using Tube at immediate spin (IS) and room temperature (RT) the sample forwards as O, and reverses as B; the auto control is negative. At 4C, the sample appears like an O on reverse, but auto control and group O screen cells are positive indicating a cold auto agglutinin is present and results at 4C cannot be interpreted.

Ficin treatment of donor and reverse cells was also performed and testing was repeated at IS, RT and 4C using tube. At 4C, donor cells versus anti-B and anti-A, B were microscopically positive. Anti-A was negative, indicating that this may be a subgroup of B. However, the addition of ficin testing increased the strength of the cold auto agglutinin; therefore interpretation of the sample at 4C could not be done.

Removal of the cold auto agglutinin was performed so that testing could be interpreted at 4C.

At x4 cold autoabsorbed plasma, reverse typing still looked like a B at IS and RT. At 4C, B cells were positive (3+), however residual cold agglutinin was detected using group O cells (+). An additional cold autoabsorbtion (x5) was performed to ensure 4C anti-A and anti-B reactions were not due to this residual cold agglutinin. Reverse typing at 4C looks like a group O (reacting with A, A2 and B cells), however the isoagglutinin reactivity was not consistent, as expected. Because this donor had two discrepant ABO types, it was determined that ABO genetic sequencing would be beneficial.

Donor specimen was sent for ABO DNA sequencing and results indicated this donor is ABO*genotype: *B(28+2_3insT)/01. This donor is a Group $B_{very\ weak}$ The donor has a probable anti-B at 4C. The anti-B in the donor plasma was not reactive when prewarmed testing was used.

Conclusions: Molecular sequencing proves useful in identifying ABO subgroups, but serological knowledge of ABOs is still beneficial in recognizing the possibility that the ABO type may not be what it looks like.

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Title: Identification of Anti-Hr0 (Rh17) in a Multigravida Never Transfused RH: 1, -2, -3, 4, -5 Female

Authors: JG Zinni, B Patel, MA Keith, MM Hinrichs, M Kwan, C Geurkink

Affiliation: American Red Cross, Heart of America

Background: Hr0 is a highly prevalent antigen of the Rh system present in >99% of the population. The clinical significance of anti-Hr0 is considered to be variable with hemolysis ranging from mild to severe including HDFN. In an available literature search, of 18 women with a total of 26 pregnancies, 23 (88%) resulted in severe HDFN requiring transfusions.
Abbreviations: HDFN: hemolytic disease of the fetus or newborn; IS: immediate spin; ALB: albumin; PEG: polyethylene glycol; AGT: antiglobulin test; IAT: indirect antiglobulin test; SNP: single nucleotide polymorphism

Case Report: A 49 y/o Hispanic female with a history of no transfusions and five previous pregnancies presented for laparoscopic surgery regarding a renal mass. She had given birth to multiple children in Mexico with elevated bilirubin and severe jaundice. The patient presented one year later to a different facility with a leiomyoma of the uterus. Blood grouping, antibody identification, and crossmatched units were requested on both occasions.

Serological Results: The patient typed blood group O RhD+ without discrepancy. The initial selected cell panels reacted strongly with all non-chemically and chemically treated cells tested. Negative results were obtained when performing the direct antiglobulin test with polyspecific anti-human globulin and autocontrol IAT with monoclonal anti-IgG. An Rh phenotype was performed with IgM monoclonal antibody revealing C- c+ E- e- results. The E antigen typing was confirmed with human derived polyclonal anti-E. Further antigen typing revealed K-, Fy(a-b+), Jk(a+b+), M+,S+,s+ results. To investigate the presence of an antibody to a high incidence antigen in the Rh system, the patient's plasma was tested against rare hrB-, hrS-, D-- cells from group O donors at PEG/IAT. The D-- (also K+, and Fy(a+b-)) cells were the only donor cells that did not react at PEG-IAT. R1R1 and rr alloadsorbed plasma x3 were non-reactive when tested with the respective adsorbing cells at PEG-IAT, but the R2R2 alloadsorbed plasma x3 was reactive with the R2R2 alloadsorbing cells. The anti-Hr0 demonstrated reactivity at RT, 37C-ALB, ALB-IgG-AGT,
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PEG-IgG-AGT phases. Antibodies to all other common antigens were ruled out in the IS, RT, 37C-ALB, ALB-IgG-AGT and PEG-AGT phases.

Molecular Results: Initial and repeat SNP testing was performed at the American Red Cross National Reference Lab in Philadelphia. Both performances reported a low signal for the RHCE markers 676 G>C, 773 G>C, and 1006 G>T. These three markers are used in the prediction of RH5, RH3, RH20, and RH10.

Conclusion: Anti- Hr0 in the alloadsorbed x3 plasma reacting strongly with R2R2 cells may be supportive of the e and Hr0 antigenic mimicry. The three markers producing a low signal in the RHCE is indicative of variant or null alleles within the RHCE gene. The frequency of finding blood of the Hr0 Rh:-17 phenotype is <1 in 1000 ABO and Rh(D) compatible random blood donors. Autologous donations were collected prior to the first surgery, but not transfused and subsequently frozen.

NOTES