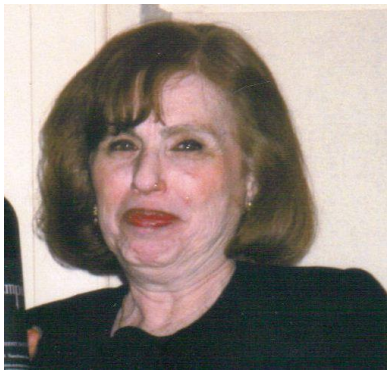
ILLINOIS ASSOCIATION OF BLOOD BANKS

The Phyllis Unger
Annual Case Studies Meeting

Wednesday, February 10, 2016



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. 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. Bystander Hemolysis in Sickle Cell Patients
   K. Gvozdjan, L. Sereseanu, S. A. Campbell- Lee

2. The Impact of RBC Blood Group DNA Phenotyping on Subsequent Antibody Studies
   G Ramsey, JG Zinni, K Hartman, RD Sumugod, PF Lindholm

3. Multiple Complications and Massive Hemolysis in a Patient with Sickle Cell Disease
   Phillip J. DeChristopher, MD, PhD and Megann Wojciechowski, RN

4. Daratumumab and Red Cell Serologic Testing: A Single Institution Experience
   C. Joseph-Douglas, B. Baron, A. Treml, G. Wool

5. Two Patients Taking Daratumumab (Darzalex)
   Jim Perkins, MD

6. Transfusion Related Anaphylactoid Reaction/ Anaphylaxis
   J. Alvarez Argote 1, A. Treml 1, G. Wool 1

7. Transfusion Reactions in Two Patients Associated with the Same Platelet Apheresis Unit
   C. Aronson, J. Hamilton, R. Millslagle, J. Walsh, M. Papari

8. Mystery of Nocella
   Bhavesh Delvadia, MLS (ASCP)SBB and Dr. Bryant Barbara, MD, MT(ASCP)SBB

9. Genotyping to Resolve RhD Variant Phenotypes in Pregnant Women: Rh “Pluses and Minuses”
   G Ramsey, JG Zinni, K Hartman, RD Sumugod, PF Lindholm

Title: Bystander Hemolysis in Sickle Cell Patients

Authors: K. Gvozdjan, L. Sereseanu, S. A. Campbell-Lee

Affiliations: University of Illinois Hospital & Health Sciences System, Department of Pathology, Chicago, IL

INTRODUCTION: Hyperhemolysis in sickle cell patients involves allogeneic and autologous red blood cell destruction. Although various pathogenic mechanisms and successful therapeutic options have been proposed, standardized laboratory and treatment approaches are still lacking. Our aim was to retrospectively study potential immunologic predisposition (alloantibodies), relevant hematologic parameters, specific treatment, and outcome of eight cases of bystander hemolysis in sickle cell patients treated at our institution from 2007 through 2015.

METHODS: Laboratory and treatment data were accessed through electronic medical records. Transfusion history was obtained from our hospital's blood bank electronic database.

Results

	Patient	Previous Alloantibodies	New Antibodies	Time (Days)	Nadir Hb (g/dL)	Retic (thous/μL)	LDH (U/L)	Treatment	Outcome
1[a]	40 F	C, E, K, S ,Fy^a, Jk^b	none	17	5.0	622.9	555	supportive	Favorable
1[b]	42 F	C, E, K, S, Fy^a, Jk^b, Js^a, M	none	10	4.6	357.1	714	steroids, IVIG, DPO	Favorable
2	36 M	D, C, E, K, M, S, Le^b	none	8	2.1	190.6	1406	steroids, Rituximab	Favorable
3	21 M	C, E, S, Jk^b	none	6	2.9	85.5	6813	steroids, EPO	Fatal
4	19 M	C, E	none	5	5.5	141.5	1440	steroids	Favorable
5	55 F	e, C, K, Fy^a, Fy^b, Kp^a, Lu^a, Co^b, Le^b, *	none	9	5.0	213.4	541	steroids	Favorable
6	42 F	E, K Fy^a, Jk^b, M	none	10	5.1	175.2	683	steroids	Favorable
7	21 F	E, K, Js^b, S	none	4	4.2	335.4	699	steroids, IVIG, EPO	Favorable

[a] Patient 1, first episode of hyperhemolysis

[b] Patient 1, second episode of hyperhemolysis (2 years 8 months after the first episode)

*Antibody against unidentified low frequency antigen

DPO, Darbepoetin Alfa

EPO, Epoetin Alfa

IVIG, Intravenous immunoglobulin

CONCLUSION: All of the patients had more than one alloantibody prior to transfusion (median 5, range 2-9 alloantibodies) yet received antigen matched RBC prior to the episode. Anti-E was the most frequently identified alloantibody (6/7 patients). Laboratory findings indicated a spectrum of disease severity, with nadir hemoglobin (Hb) and absolute reticulocyte (Abs. retic) counts (mean ± SD) 4.3 ± 1.2 g/dL, range 2.1-5.5 g/dL, and 265.2 ± 171.2 thousand/μL, range 85.5 – 622.9 thousand/μL, respectively. Patient 1 required only supportive care during her first

episode of hyperhemolysis, while patient 3 suffered a fatal outcome despite receiving corticosteroid and erythropoietin treatment. This may be explained by the high degree of hemolysis (LDH 6813 U/L) occurring shortly after transfusion in patient 3, and without adequate increase in red blood cell production (Abs. retic 85.5 thousand/μL). Although patients 4 and 7 also presented acutely, their hemoglobin levels and reticulocyte counts were appreciably higher than noted for patient 3. Further studies are needed in order to identify specific risk factors and optimal management of hyperhemolyzing sickle cell patients.

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Title: The Impact of RBC Blood Group DNA Phenotyping on Subsequent Antibody Studies

Authors: G Ramsey, JG Zinni, K Hartman, RD Sumugod, PF Lindholm

Affiliation: Northwestern University and Northwestern Memorial Hospital, Chicago, IL

BACKGROUND: RBC blood group DNA phenotyping in patients with RBC antibodies not only confirms initial alloantibody identifications but also limits the possibilities for future alloantibodies to those antigens for which the patient is negative. We are investigating the impact of DNA phenotyping on the workload needed for testing subsequent specimens.

METHODS: In our transfusion service, we detected RBC antibodies with solid-phase red cell adherence (Capture-R™, Immucor, Norcross, GA), supplemented by polyethylene-glycol and low-ionic-strength-saline testing. In all patients with RBC antibody problems and no prior full phenotyping, we performed DNA typing by multiplex PCR with oligonucleotide extension (HEA BeadChip™ during the study period, Immucor BioArray, Warren, NJ). Our initial antibody identification used 3 homozygous reagent RBCs to rule out antibodies to each major non-ABO antigen (DCcEe, K, Fy^a, Fy^b, Jk^a, Jk^b, MNSs). Subsequently we ruled out antibodies with one homozygous reagent cell per antigen for which the patient was negative, unless additional reactivity was found. (Anti-K was sought with Kk RBCs.) We retrospectively reviewed antibody files for patients with genotyping performed 1.5 years previously, providing time for follow-up. For complexity assessment, the number of antibodies at the time of each workup was noted, including autoantibodies and nonspecific reactivity. The total number of reagent RBCs used in screening and panels was obtained for each follow-up study. Means were compared statistically with Student's t-test, seeking two-tailed $p<0.05$ (GraphPad).

RESULTS: We examined antibody files of 58 consecutive patients in a 2-month period for whom DNA phenotyping was performed as above. Ten (17%) had received RBCs within the previous 3 months, which would have interfered with serological phenotyping. Twenty patients (34%) had 55 subsequent workups after their DNA phenotyping was obtained (mean 2.75, median 1, range 1-11). Fifty-five percent of these workups were done in the presence of one antibody, 29% with two, and 16% with three. The median number of reagent RBCs used in these workups was 7, including screening cells (mean 11.4, mode 5, range 3-34). The number of antibodies in the workup did not affect the median number of reagent RBCs needed. For comparison, 9 of these 58 patients (16%) had 13 subsequent antibody workups performed before DNA
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phenotyping was completed. In these workups, the median reagent RBCs used was 13 (mean 14.1, range 6-23) (p=0.24).

DISCUSSION: We have previously reported that routine DNA blood group phenotyping reveals unexpected or novel findings in 6% of our patients with alloantibodies.[1-5] In this study we examined the impact of this technology on streamlining subsequent antibody workups, by focusing the testing on antigens for which the patient is negative. One-sixth of our patients were recently transfused, and one-third had follow-up testing guided by genotype. In this preliminary analysis, the median number of reagent RBCs needed for workups in previously genotyped patients was less than half a panel of selected reagent RBCs. Further analysis is in progress.

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Title: Multiple Complications and Massive Hemolysis in a Patient with Sickle Cell Disease

Authors: Phillip J. DeChristopher, MD, PhD and Megann Wojciechowski, RN

Affiliation: Loyola University Health System, Maywood, IL

BACKGROUND: RBC transfusions remain a major disease-altering therapy for patients with sickle cell disease (SCD), who suffer various SCD-related clinical crises. A double-edge sword, RBC transfusion is associated with many complications including, but not limited to, varying kinds and degrees of hemolysis.

CASE REPORT: A 33-y/o AAM was admitted with severe pains in his shoulders and knees, not relieved by PO dilaudid. Six days PTA he was discharged from Westlake Community Hospital for a separate sickle cell vasooclusive pain crisis and a community-acquired pneumonia, having received **2 units** of crossmatch-compatible RBCs. There was no current or historical evidence of prior rbc alloimmunization. Admitting H & H = 8.0 g/dL & 24.1%, antibody screens negative on hospital days (HD) 1 and 3, receiving **2 units** of RBC by simple transfusion. By HD-3, he developed an acute chest syndrome with respiratory failure and rapidly progressive AKI (creatinine 3.59).

Assessing multiorgan failure (MOF) syndrome, we urgently performed an RBC exchange transfusion (HD-4): Based on the patient's 6' 4" & 107 Kg size & TBV of 7.4 liters, we exchanged him with **14 units** of RBC's (LR, Hgb S-neg, electronic XM'ed); post-exchange Hgb A of 86.2%.
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Within 20 hours (HD-5), Hgb dropped from 10.9 to 5.3 g/dL, associated with acute spikes in potassium (7.4 mmol/L), LDH (6614 IU/L) and T. bilirubin (48.5 mg/dL). Repeat Hgb electrophoresis showed a 10% in Hgb A%. On HD-5 (48 hours post-exchange), the IAT was now strongly positive due to **alloanti-C and -Jk(b)**; the DAT was positive (PS, IgG & C3d) with alloanti-C and Jk(b) in the eluate. Hyperkalemia and AKI prompted start of HD, but progressive anuric ARF required HD for 25 days. A bone marrow biopsy showed extensive necrosis and hemophagocytosis. T. bilirubin, serum ferritin and LDH peaked on HD#6 (**59.9 mg/dL**), HD #7 (**71,231 ng/dL**) and HD#7 (**6859 IU/L**), respectively. All liver enzymes were 5 – 10 x ULN until HD #10. From HD-4 to -7, seven additional RBC's (C-, E-, K- & Jk(b)- & Hgb S-neg; LR, AHG XM-compatible) were simply transfused to maintain Hgb between 6.7 to 8.0 g/dL. Brain MRI showed innumerable punctate foci of microhemorrhages at the corticomedulary junction in B/L hemispheres and cerebellum. Treatment included IV steroids and IVIg. Six weeks post-event, **alloanti-E** became detectable. The patient survived but various "soft" neurologic symptoms, and pulmonary artery hypertension developed.

CONCLUSIONS: The admitting pain crisis could have been a *forme frust* of a DHTR from recent RBC transfusions (DHTRs and pain crises are difficult to distinguish clinically). The MOF syndrome started with acute respiratory and renal failures progressed to widespread injuries to the liver, bone marrow and CNS, with evidence for a DSTR not being apparent until after RBC exchange. Acute clinical hemolysis was likely intravascular [secondary to anti-Jk(b) & complement], extravascular and possibly hyperhemoltyic.

Take-Home Lessons (re-learned):

- Histories *negative* for rbc alloimmunization are notoriously unreliable.
- Alloantibodies disappear over time.
- Vaso-occlusive pain crises mimic DHTR's
- Prophylactic "extended" phenotyping matching is the recommended rule.
- MOF syndrome probably best treated with RBC exchange transfusion

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Title: Daratumumab and Red Cell Serologic Testing: A Single Institution Experience

Authors: C. Joseph-Douglas, B. Baron, A. Treml, G. Wool

Affiliation: University of Chicago, Chicago, IL

BACKGROUND: Daratumumab (DARA) is a monoclonal antibody targeting CD38 and is currently undergoing trials for treatment of hematopoietic malignancies. CD38 is highly expressed on plasma cells, but is widely expressed in many cell types, including B-cells and RBCs. There have been several reports of DARA treatment causing a panagglutinin effect.

University of Chicago Medicine (UCM) was a site for a DARA Phase III trial for plasma cell myeloma. UCM is also a site for a DARA Phase II trial for B-cell lymphoma. Here we report
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our Blood Bank's experience with 10 patients with advanced stage plasma cell myeloma and 2 patients with advanced stage follicular lymphoma treated with DARA.

CASE REPORT: The first patient treated with DARA at UCM started treatment 3/2/2014. Since 9/2015, the protocol for drawing antibody screens (AS) and alerting UCM blood bank to impending DARA treatment has greatly improved. The patient's blood bank record is modified to include DARA treatment and their sample is either phenotyped or genotyped depending on recent transfusion. When a DARA patient has a positive AS, subsequent panels are done in tube and if clinically significant antibodies cannot be ruled out, the sample is sent for DTT-treated panel at IRL.

All patients receiving DARA had a T&S drawn at UCM before starting DARA and all AS were negative.

Of the 10 myeloma patients, only five had subsequent T&S sent to UCM blood bank after starting DARA treatment. Only four patients subsequently needed transfusion. Five patients were genotyped, one was phenotyped.
Three myeloma patients had positive AS after starting DARA and required transfusion support. Details of these three patients are reported in Table.

Table- DARA myeloma patients with positive AS

Patient #	Initial AS/DAT testing after starting DARA	DTT-treated panel performed	Subsequent AS/DAT	pRBC compatibility testing/transfusion after starting DARA	Clinical outcome
1	AS and DAT positive nine days after starting DARA	No	AS reverted to negative 16 days after starting DARA	2U transfused (1U EXM, 3U PEG compatible)	Patient deceased one month from starting DARA
2	AS positive and DAT negative 23 days after starting DARA	Yes, no underlying alloantibodies	AS consistently reverted to negative four months after starting DARA. All DAT negative.	17U transfused (14U EXM, 3U LISS compatible, 2U LISS incompatible)	DARA stopped due to disease progression
3	AS positive and DAT negative 7 weeks after starting DARA	Yes, no underlying alloantibodies	AS still positive three months after starting DARA. All DAT negative.	3U transfused (4U PEG compatible)	DARA stopped due to disease progression

Of the two lymphoma patients, neither had subsequent T&S sent to UCM blood bank after starting DARA treatment. Both patients were phenotyped.

Four myeloma patients and both lymphoma patients showed benefit and remain on DARA treatment.

CONCLUSION: DARA patients required significant coordination between UCM Blood Bank and the trial coordinators.

Of 12 total DARA patients, only five had subsequent T&S sent to UCM Blood Bank after starting DARA and only three had a positive AS. Only one patient had a positive DAT (with a negative eluate).

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Title: Two Patients Taking Daratumumab (Darzalex)

Authors: Jim Perkins, MD

Affiliations: NorthShore University HealthSystem Blood Banks

BACKGROUND: On 11/16/'15 the FDA approved daratumumab for patients with refractory multiple myeloma after at least three prior treatments. Daratumumab is an IgG, human monoclonal antibody directed against CD38, an antigen that's highly expressed on myeloma cells. Low level CD38 is expressed on lymphoid and myeloid cells and on RBCs. Because of the latter patients receiving daratumumab passively acquire a panagglutinin causing positive indirect antiglobulin tests in antibody detection, identification and crossmatching by all standard methods. Patients *may* have positive direct antiglobulin tests (DAT). These findings may last over 6 months after discontinuing the drug. Dithiothreitol (DTT) destroys CD38 allowing screening and identification of underlying alloantibodies (exception; Kell system antibodies). Daratumumab is associated cytopenias including anemia, although severe hemolysis hasn't been observed.

On 1/15/'16 the AABB alerted members to problems performing compatibility testing in patients taking daratumumab. We identified two patients that received the drug and initiated standard immunohematologic testing.

CASE 1: AR was diagnosed with myeloma in 2008 at age 48 after an MRI performed for chronic back pain demonstrated a bone lesion. Biopsy revealed a plasmacytoma, and bone marrow biopsy disclosed myeloma. No serum paraprotein was detected, but the urine contained lambda light chains. She responded to 3 cycles of bortezomib (Velcade, a proteasome inhibiter) and dexamethasone, and underwent high dose melphalan with autologous HPC rescue in 3/'09 and 11/'12. Between and after the transplants she received varying doses of multiple agents including carfilzomib (Kyprolis, a proteasome inhibiter) cyclophosphamide, dexamethasone, and pomalidomide. She also required periodic IVIg treatment for hypogammaglobulinemia with multiple infections. Nonetheless her myeloma progressed, and daratumumab was added on 12/22/'15. Her serum light chain level has dropped sharply.

On 12/21/'15 and all previous occasions AR's antibody screen was negative. Repeat screening on a 1/25/'16 specimen was positive by 'gel' technique, and all cells in a panel reacted 1+ .
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The plasma failed to react with the same cells treated with DTT, however. The gel DAT was negative. Her extended blood group phenotype is D+ C+ c+ E+ e+ , K+ k+ , Jk(a+ b+), Fy(a-b+), M-N+ S+ s+ .

CASE 2: TA was diagnosed with multiple myeloma at age 72 in 10/'11 when she presented with pneumococcal pneumonia and sepsis and was found to have anemia, a serum paraprotein, and skull lesions. She was started on bortezomib, lenolidomide (Revlimid), and dexamethasone. Progressive disease in mid-2014 and again in mid-2015 prompted changes in her regimen to include cyclophosphamide and carfilzomib, and pomalidomide and carfilzomib respectively. The latter regimen didn't interrupt the progression, and on 1/19/'16 she received a single dose of daratumumab.

Throughout her course TA has had anti-D in her serum and a positive DAT with a non-reactive eluate, and these abnormalities were present on 1/19/'16. However, a specimen from 1/25/'16 contained a 1+ panagglutinin (all D-neg cells reacting) in gel. DTT treatment of the D-neg panel cells eliminated the gel reactivity, but didn't diminish the reaction with a D-pos cell. The DAT and eluate were unchanged.

CONCLUSION: These cases demonstrate the expected immunohematologic findings due to daratumumab. Implications for transfusion will be discussed.

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Title: Transfusion Related Anaphlactoid Reaction/ Anaphylaxis

Authors: J. Alvarez Argote 1, A. Treml 1, G. Wool 1

Affiliation: University of Chicago, Chicago, IL

BACKGROUND: Transfusion related anaphylactoid reaction/anaphylaxis (TRA) is a rare diagnosis, presenting in about 1:20,000 transfusions, yet it is responsible for about 6% of transfusion-related fatalities. It occurs more frequently with plasma-rich products (plasma or platelets). TRA typically presents early during the transfusion. Symptoms may include urticaria, angioedema, hypotension, arrhythmias, dyspnea, wheezing, stridor, nausea, vomiting, diarrhea or abdominal cramping. However, the mix of these symptoms may vary, making the diagnosis difficult in certain situations. Herein we describe an atypical case of TRA which was diagnosed after a thorough work up.

CASE REPORT: A 66 year-old woman with Philadelphia chromosome-positive acute B-lymphoblastic leukemia was admitted for autologous stem cell transplant. The patient subsequently had evidence of pneumonia. A bronchoscopy was planned and the patient was ordered transfused with one U apheresis platelets due to a platelet count of 23,000 /uL. Upon completion of transfusion, the patient experienced hypotension, crampy abdominal pain, and dyspnea. The blood pressure dropped from 109/60 mmHg pre- to 58/37 mmHg post-transfusion, the heart rate increased from 101 /min pre- to 119 /min post-transfusion, and her Oxygen saturation dropped from 94% on room air pre- to 80% on room air and 95% on 3L nasal
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cannula post-transfusion. Her temperature remained normal. There was no wheezing, stridor, skin rash, or hives. 45 minutes later the patient developed nausea, vomiting, and diarrhea. Chest X ray showed atelectasis at the bases but no new infiltrates. The patient received 2 liters of saline and her blood pressure normalized; no epinephrine was given.
Blood bank workup for clerical errors and hemolysis was negative. Bacterial cultures of the empty platelet bag as well as the split product were negative. The patient had previously received 5 U red cells and 2 U apheresis platelets from our blood bank before this reaction, without reported reactions. The patient has a previously reported contact allergy to chlorhexidine.
Immunoglobulin levels were measured and all were mildly reduced, including total IgA (93 mg/dL). No anti-IgA antibodies were detected by ELISA. Haptoglobin was elevated (255 mg/dL). Total serum tryptase level 46 minutes after transfusion was elevated (18.2 ng/mL, reference range <11.5 ng/mL), and subsequent level a day later was normal (6.1 ng/mL). At this point, TRA was the most likely diagnosis, although symptoms due to underlying disease (pneumonia and immune suppression) could not be entirely ruled out. Therefore, we recommended transfusing platelets with supernatant removed, without changes to transfused pRBC products.

CONCLUSION: This is an atypical presentation for TRA, given the lack of wheezing and skin findings, but the Hemovigilance criteria for a definitive allergic transfusion reaction (by hypotension and respiratory distress) were met. Moreover, 9.5% of allergic transfusion reactions lack skin manifestations. The transient tryptase elevation supported the diagnosis. Nonetheless we did not find a specific etiology for the reaction since anti-IgA antibodies were ruled out and the patient has no evidence of haptoglobin deficiency. The patient had received previous blood products prior to this reaction, without complications. Although management becomes difficult, we also suggested minimizing transfusion of plasma products.

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Title: Transfusion Reactions in Two Patients Associated with the Same Platelet Apheresis Unit

Authors: C. Aronson, J. Hamilton, R. Millslagle, J. Walsh, M. Papari

Affiliation: ACL Laboratories, Rosemont, IL

BACKGROUND: A 59 year-old male patient with pancreatic cancer was transfused a unit of apheresis platelets prior to a planned surgical procedure. After transfusion of ~100 ml of the unit the patient experienced tachycardia, facial flushing, abdominal, back, and chest pain, shortness of breath with wheezing and an increase in temperature from 35.9 C to 37.0 C; a transfusion reaction investigation was initiated. About 10 minutes prior to this patient being transfused, a different patient at another hospital within our system received transfusion of an apheresis platelet, which was part of the same collection as the platelet unit involved in the transfusion reaction. The second patient is a 74 year-old male with myelodysplastic syndrome and was receiving a platelet transfusion prior to chemotherapy in the outpatient infusion center. Most of the platelet unit had been transfused (170 of 194 mL) when the patient started experiencing rigors, tachycardia and hypertension, but no fever. The patient was treated with
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Demerol, and was admitted to the hospital through the Emergency Department due to persistence of symptoms. The Transfusion Service was not notified as it was felt that the reaction was associated with a contaminated port rather than the transfusion of the platelet product.

CASE REPORT: Blood cultures were collected on both patients as part of a possible sepsis workup. Procalcitonin and Lactic Acid testing were found to be elevated in both patients. Because the transfusing nurse of the first patient felt that this reaction was a possible bacterial contamination, she contacted infectious control to have the product container sent for microbiology testing. Transfusion Service (TS) policy is to only culture if there is an increase in temperature of 2 degrees C or greater or if requested. Due to the request for additional testing, the TS Supervisor looked into the computer system to see if there were any other associated components within the hospital system. It was then that it was discovered that the platelet co-component had been transfused at a different site and that this patient had symptoms of a possible transfusion reaction. The Supervisor contacted the site Medical Director so that the clinician could be notified of the potential of bacterial contamination of the platelet product. The container from the outpatient transfusion was not returned to the TS so no culture was possible. Gram stain for the blood cultures on both patients showed gram positive cocci in clusters. Unit culture and blood cultures from both patients were all positive for Staphylococcus aureus. The blood center was notified of the transfusion reactions; genetic testing performed on the 3 positive cultures showed that the microorganism was genetically identical. Another part of the triple apheresis platelet was transfused at another hospital, on day 3 after collection without evidence of any transfusion reaction. Both units transfused at our facilities were transfused on day 5 after collection. Our system does not currently perform enhanced bacterial detection of platelet products at the TS. A sample from the parent platelet unit was tested by the blood center bacterial screening test and found to be negative.

CONCLUSION: A transfusion reaction due to bacterial contamination of platelets occurred in two patients. Although the source of bacterial contamination cannot be identified with certainty, it is assumed that the contamination occurred at either the time of collection or during processing of the parent product. This case illustrates the use of microbiology genetic testing in establishing common origin of a bacterial contaminant, and confirms the higher risk of bacterial contamination of platelets with increased storage time.

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Title: Mystery of Nocella

Authors: Bhavesh Delvadia, MLS (ASCP)SBB and Dr. Bryant Barbara, MD, MT(ASCP)SBB

Affiliation: Heartland Blood Centers, Aurora IL

BACKGROUND & CASE DESCRIPTION: An 88-year old Caucasian man was admitted to the hospital with unclassified anemia. His hemoglobin level was 5.7 g/dL. Two units of RBCs were
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ordered by his oncologist. Initial type and screen performed at the hospital found that his blood type was O Negative and two of three screening cells positive in the indirect antiglobulin phase (IAT). This patient had a history of cold auto antibody (non-specific) from another hospital. The antibody workup was performed. On initial panel, there was non-specific reactivity observed with few panel cells, however, no specific antibody pattern was identified. The sample was referred to our immunohematology reference lab (IRL) for further evaluation.

Testing at our IRL revealed the presence of an antibody reactive at IAT with almost all cells. Given the patient's history of a cold auto antibody, pre-warm IAT was performed and all cells on panel were negative. The Direct Antiglobulin Test (DAT) was negative for both IgG and C3. Cold autoantibody was reported to hospital. We recommended the hospital give crossmatch compatible RBCs using pre-warm method for crossmatch. Based on this recommendation, the patient was transfused with two crossmatch compatible RBCs using pre-warm technique. The following day, the patient's hemoglobin was noted to be 8.6 g/dL, however after two days, the patient's hemoglobin dropped to 5.8 g/dL and the bilirubin value was elevated. Two units of RBCs were ordered again by his oncologist. The sample was referred to our IRL for antibody identification.

Because of the patient's known history of cold autoantibody, we approached in workup as previously performed. However, this time the pre-warm technique did not work, so Rest adsorption was performed. This failed to remove the antibody reactivity as well. The Direct Antiglobulin Test (DAT) was performed and found to be negative for both IgG and C3 even after the recent 2 unit transfusion. On extensive review of the antibody workup, it was noted that the patient's plasma was reacting very weakly with all but a few panel cells. We decided to perform phenotyping on patient's pretransfusion red cells. The patient typed as D-C-E-c+e+, K+k-, Fy(a-b+), Jk(a+b+), M+N+S+s+, Le(a-b+), P1+. Since the patient was determined to be Cellano (k) negative, we re-examined the antibody reactivity on all our panel cells. We found all K-k+ and K+k+ cells were negative, and all but two homozygous k (Cellano) were positive. Interestingly, these two negative K-k+ cells were positive for Kpa. It appeared that the negative reactions with homozygous k (Cellano) cells were due to the presence of the Kpa antigen on those cells. DTT panel was performed to confirm the presence of anti-k. All other clinically significant antibodies were ruled out using selected cells. One units of k negative RBCs were issued to patient for transfusion. No complications were reported post-transfusion.

DISCUSSION: This patient has very weak example of anti-k showing dosage, and reacting only with homozygous cells; however the patient's plasma was negative against two homozygous k cells which were positive for the Kpa antigen. From the time that Kpa was first reported, it was apparent that the presence of Kpa in a Kell complex haplotype results in altered expression of other Kell system antigens encoded by that haplotype, i.e.: a cis position effect.[1] Allen et al. reported that on K-k+ Kp(a+b-) red cells the k antigen was sufficiently depressed that its presence could have been overlooked had not a potent anti-k been used.[2] In the same study, the expression of k on K-k+ Kp(a+b+) red cells was seen to be weaker than that on K-k+ Kp(a-b+) samples. Later studies also reported the suppression effect of Kpa in cis to other genes

encoding Kell system antigens, including k and Jsb.[3] The effect appears to result from reduced amount of the Kell glycoprotein (produced by the Kpa allele) inserted in the RBC membrane.[4]

In this case, anti-k was overlooked and ruled out by the K-k+ Kp(a+b+) cells, but the patient's antigen typing was supportive for presence of anti-k in the patient plasma. The Cellano antigen was first identified in 1949 when an antibody was found to react with the antithetical allele to K. This newly identified antigen was named Cellano by rearranging the last name of the original antibody producer, Nocella.[5]

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Title: Genotyping to Resolve RhD Variant Phenotypes in Pregnant Women: Rh "Pluses and Minuses

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BACKGROUD: Weak-D typing is not required in pregnant women. However, those known to have weak D phenotypes traditionally were not deemed candidates for Rh immune globulin (RhIG). Patients with partial D phenotypes, with some exceptions, often have normal RhD+ typings until anti-D appears. However, the proliferation of monoclonal anti-D typing methods, sensitive antibody detection measures, and RhD genotyping have blurred the distinction of weak "versus" partial D.[1]

METHODS: We performed RhD typing in automated or manual techniques (Immucor, Norcross, GA). RhD variant genotyping was referred to American Red Cross (Philadelphia, PA).

CASE REPORTS:

Case 1: A 36-year-old Caucasian woman in the 31st week of her third pregnancy presented with uterine contractions after mild trauma in a car accident. Her obstetrician's (OB's) outside laboratory had typed her as RhD+, but she reported conflicting RhD typing in testing elsewhere. To evaluate her need for RhIG, a blood type was requested. In view of the history, manual RhD
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tube testing (Gamma-clone) with antiglobulin phase was performed. Her RBCs typed negative at immediate-spin and positive in antiglobulin phase. We reported the patient's blood type as RhD-negative, weak-D positive. The OB's chart note said, "I have had at least 15 conversations with patient today regarding her blood type." We recommended RhIG treatment if clinically indicated, which was given, and RhD-variant genotyping to resolve her D typing. Her contractions subsided and she was discharged.

Case 2. A 29-year-old Caucasian woman in the 13th week of her first pregnancy had her RhD type reported as weak D by an outside laboratory to her OB, who then requested RhD typing from us ("this is too confusing"). In automated testing, two anti-D reagents gave discordant results (Series 4-negative, Series 5-positive), and the manual anti-D typing (Gamma-clone) was 2+ at immediate-spin. We reported her RhD type as RhD-negative, weak-D positive, and sought RhD genotyping.

RESULTS: Case 1's probable Rh genotype was *RHD*01W.1* (weak D type 1)/*RHD*01N.01* (RHD deletion). Patients with weak D type 1 are not at risk for allo-anti-D. We advised the OB to manage her as RhD+. At 37 weeks, she delivered a girl with normal RhD+ phenotype. RhIG was not given. For Case 2, RhD microarray genotyping (RHD Molecular BeadChip Test, Immucor BioArray, Warren, NJ) predicted a partial-D phenotype with either DCS-1 or DFV, two similar variants. Exon 5 sequencing identified *RhD*DCS1.* We advised the patient's OB to manage her as RhD-negative.

DISCUSSION: The AABB Technical Manual's advice on giving RhIG to pregnant weak-D-phenotype women has evolved from "not receive" (2011) to "controversial" (2014). An AABB/CAP/ACOG work group recently advised RhD genotyping for pregnant women with low-strength (≤2+ in initial testing, reactive at antiglobulin) or discordant D phenotype.[2] Our two sample cases illustrate the value to OBs and patients of RhD genotyping diagnosis. Patients with the common weak D types 1, 2 and 3 are not considered at risk for anti-D. RhD genotyping can be cost-effective for identifying a large portion of D-variant women who are immunologically RhD+ and do not need RhIG management.[3]

REFERENCES:

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2. Sandler SG, Flegel WA, Westhoff CM, et al. Transfusion 2015;55:680-9
3. Kacker S, Vassallo R, Keller MA, et al. Transfusion 2015;55:2095-103

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