Title: A delayed and an acute hemolytic transfusion reaction mediated by probable partial anti-c in a patient with multiple Rh variant alleles and the corresponding alloantibodies of anti-D, -C, -E, and -hr^{s}.

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Abstract: The Rh system is the most complex of the human blood groups. Of the 55 antigens that have been characterized, the system's principle antigens D, C, E, c, and e are responsible for the majority of clinically significant Rh antibodies. In the last few years, advancements in molecular testing have provided a wealth of information on the genetic diversity of the Rh locus. More than 275 RHD and 50 RHCE alleles have been documented. Nucleotide changes can result in qualitative and quantitative changes in Rh antigen expression. [1] Persons who express a partial D on their red blood cells can be immunized to make anti-D. Similarly, people who are homozygous or hemizygous for alleles encoding partial c and/or partial e can also produce alloantibodies. [2] This case presents a patient with *RHD*DAR* alleles in conjunction with two partial *RHCE*ce* alleles with the corresponding antibodies to anti-D, -C, -E, and probable -hr^{s}. Further transfusion of D-C-E-K- red blood cells resulted in a suspected acute hemolytic transfusion reaction and the subsequent identification of partial anti-c. Monocyte monolayer assay testing suggests clinical significance with a range 29.5%-38.5% reactive monocytes.

Background:

The Rh system is a very polymorphic and immunogenic blood group system that is second only to the ABO system in its clinical significance for blood transfusion. Antibodies to antigens in the Rh system have been implicated in hemolytic disease of the newborn as well as acute and delayed hemolytic transfusion reactions. In addition to the common antigens of the Rh system (D, C, c, E, e) there are 55 distinct antigens that comprise the Rh blood group system[1]. The Rh locus consists of a pair of adjacent highly homologous genes, *RHD* and *RHCE*, that respectively encode for the D antigen and the CE antigen. The antigenic diversity present in this blood group, is attributed to a variety of molecular mechanisms including nucleotide substitutions and gene conversion[3]. These molecular mechanisms lead to the creation of unique protein sequences with resultant unique antigens and an increase in the number of antigens in the Rh system relative to other blood systems. Further adding to the complexity, variant *RhD* and *RHCE* alleles can encode qualitative and quantitative changes in the Rh antigens.[4] Individuals with such variants when exposed to the conventional antigen during transfusion will recognize those epitopes that they lack as foreign and an immune response will be elicited. Routine serologic RBC phenotyping does not differentiate between conventional and variant Rh antigens. Within the Rh blood group system, partial D, C and e antigen variants and their corresponding alloantibodies have been well described. Variants to c antigens are much less frequent as compared to variant forms of the other major Rh antigens. [5] When variant c antigens do occur it is believed to be the result of a Gly96Ser transmembrane amino acid change which destroys RH-26 and results in a weakened c expression. [6] Westhoff et al postulate that the less frequent occurrence of c variants in comparison with other Rh antigens is that the positions of the two proline residues involved in c expression form a stable structure

that is resistant to the changes that often occur with Rhce.[7] Alloanti-c in a c+ individual was first reported by Moulds and colleagues in 1982 with a number of additional cases reported since that time.[8,9,10,11]

We report the case of the identification of a partial c antigen with alloanti-c -in a 72-year-old multi-transfused African American female patient with RHD*DAR alleles and two partial RHCE*ce alleles.

Case Report: The patient is a 72- year- old African American female with a past medical history of multiple sclerosis, congestive heart failure and recurrent gastrointestinal bleeding secondary to diverticulosis. The patient presented to the emergency room with acute gastrointestinal bleeding and received fluid support and two units of PRBCs. She was admitted to the hospital for further evaluation and treatment. A colonoscopy was performed which revealed a diverticular bleed in the ascending colon that was successfully cauterized. During this time, her hemoglobin was observed to fall to 4.9 g/dl and a hematocrit of 16% and she was emergently transfused four units of PRBCs. Initial serological results indicated the patient to be group A weak D positive with a positive antibody screen. The patient's previous serological history included identification of anti-D, anti-C, anti-E, warm autoantibody, and cold autoantibody. The initial antibody screening was performed by the hospital, and the presence of additional red cell antibodies was suspected. The patient's sample was referred to the Immunohematology Reference Lab (American Red Cross, Charlotte, NC) for further serologic evaluation and antibody identification.

Results: Initial serological testing performed by the IRL indicated the patient was Group A weak D positive with a negative direct antiglobulin test. Serologic phenotype testing was performed and the results were C-,E-,c+,e+ ,K-, Fy(a-b-), Jk(a+b+) S+s-. The patient's plasma exhibited pan-reactivity with D negative phenotypically similar cells in the presence of a negative autocontrol. This reactivity suggested the presence of an additional antibody (ies) unrelated to the previously identified allo-anti-D, -C, and -E. Plasma studies showed 1+ reactivity using D negative phenotypically similar cells with LISS-IAT and 3+ reactivity at PEG-IAT. The reactivity was resistant to ficin treatment as well as 0.2M dithiothreitol. Given the patient's history of warm and cold autoantibodies, adsorption studies were performed at 37C using ZZAP treated autologous cells. No reduction in strength was observed with the autoadsorbed plasma. Allogeneic adsorptions were performed using papain treated R_o cells. Clinically significant antibodies to common red cell blood group antigens were excluded in the allo-adsorbed plasma. An acid elute was prepared from the adsorbing cells to ascertain the identity of the pan-reactivity. The acid eluate demonstrated an anti-e like antibody. The eluate was reactive with 3 sources of D- e+ cells and negative with 3 sources of D- e+ variant cells. The facility was contacted to report the anti-e like reactivity and molecular genotype testing was recommended for further characterization. No compatible units negative for D, C, E, e, and K were available for immediate transfusion. Therefore, after consultation with the facility units negative for D, C, E, and K were recommended in the event emergent transfusion was needed. Unfortunately, there was insufficient time to perform a monocyte

monolayer assay (MMA) prior to transfusion of incompatible blood. An MMA can be performed to help assess the clinical significance of an antibody when antigen negative units are rare and not likely to be readily available. Given the patient's clinical situation as a GI bleed, and low Hgb the facility transfused two crossmatch incompatible units negative for D-C-E- and K over a 48-hour time period. No adverse reactions were noted after transfusion of e+ units in the presence of the anti-e like antibody.

Molecular testing for RHD and RHCE was requested for the patient sample. Molecular results indicated the patient to have:

Probable RHD genotype:	*RHD*DAR* (hemizygous or homozygous)
Probable RHCE genotype:	*RHCE*ceEK/ RHCE*ceAR*
Predicted phenotype:	Partial D+ C- E- partial c+ partial e+ VS- $V+^{w}$ hr^{s}-

The molecular results confirmed suspected serological reactivity. RHD*DAR alleles are associated with D typing discrepancies and the production of allo-anti-D. In addition, the patient had two partial *RHCE*ce* alleles associated with the hr^{s}- phenotype and the production of allo anti-e and/or anti- hr^{s}. The facility was contacted to report the additional probable anti-hr^{s} reactivity in the presence of previously identified anti-D, -C- and E. Consultation was made with the American Rare Donor Program and no genotyped matched units were available for further transfusion needs. Despite the presence of the probable anti-hr^{s}, the patient tolerated the previous 2 units well, with no adverse effects. The patient's Hgb increased to 7.7 g/dl with a Hct of 23% and she was discharged.

Five days post initial transfusion the patient was readmitted to the hospital for a suspected delayed hemolytic transfusion reaction. Patient presented with dark urine, Hgb of 6.3 g/dL, total bilirubin 2.6 mg/dL, LDH 1034 IU, and a haptoglobin <15 mg/dL. The patient then received two additional units (D-C-E-K-). Upon completion of transfusion of the second unit the patient began to display symptoms of a possible acute hemolytic transfusion reaction. The patient's temperature increased from 98F to 99.5F with accompanying symptoms of rigors, dyspnea, and severe back pain. Blood pressure increased from 149/68 mm/Hg to 166/94mm/Hg with an increase in pulse from 85 BPM to 93 BPM. Oxygen saturation remained unchanged at 99%. The facility began an initial transfusion reaction investigation. The post transfusion sample was severely hemolyzed, but the DAT remained negative. Gram stain was performed and was negative for any microorganisms. A current blood sample was drawn and sent to the local IRL for additional serological testing.

The DAT on both the pre and post transfusion samples was negative. Due to the recent transfusion and suspected transfusion reaction, an eluate was prepared despite the negative DAT. Expecting the eluate to contain the previously identified probably anti-hr^{s}, the eluate was tested against a panel of e+ and e- cells. However, all cells were reactive 4+ at PEG-IAT, and the patient's EGA treated autologous cells were negative as was the last wash. All cells tested with the eluate were positive except for Rh null cells. Two Rh null cells were tested and found to be negative with the patient's neat eluate. Additionally, one Rh:-46 cell was available for testing and was significantly weaker in reactivity and displayed only a 1+ reaction. This cell was negative for c and had a weakened expression of e. Given that the patient's

molecular results indicated an altered expression of c, the possibility of anti-c or anti-Hr was suspected. The eluate was tested against a panel of hr^s- cells to investigate the presence of anti-Hr, however, all cells were 3+ reactive with the neat eluate. The eluate was adsorbed using R1R1 cells. Anti-c was demonstrated in the adsorbed eluate at PEG-IAT. Additionally, the previously identified anti-hr^s was also shown to be demonstrating in the neat eluate. Plasma studies were also performed on the post transfusion sample. The plasma was reactive 4+ with two phenotypically similar cells. The autocontrol remained negative. Adsorption studies were performed using aliquots of R1R1 cells. The adsorbed plasma demonstrated the presence of anti-c. To further confirm the anti-c specificity an additional aliquot of the plasma was adsorbed with R2R2 cells. An acid eluate was then prepared from the adsorption cells. This eluate reacted with D-c+ E- cells and was non-reactive with Rh null cells. Clinically significant antibodies to other major blood group antigens were excluded in the allogeneic adsorbed eluate and plasma.

The presence of anti-c in conjunction with Anti-D, anti-C, anti-E, and probable anti-hr^s presented significant challenges in the possibility of locating any compatible blood for this patient. A monocyte monolayer assay was performed in an attempt to assess the clinical significance of the alloantibody to the partial c antigen. Two random sources of group O, D-, C-, E-, K-, and autogeneic cells were used. Results are listed in Table 1. Since the patient had antibodies to both c and e variant antigens as well as D, C, and E antigens, phenotypically similar cells were chosen for the MMA test. Cells that could further distinguish the significance of the reactivity with regard to the partial anti-c or probable anti-hr^s were not available. MMA testing using genotypically similar red cells would have been ideal, but none are currently available in the United States.

Discussion: We report the serologic and molecular findings on a 72-year-old multi-transfused African American female patient. The presence of anti-c in conjunction with anti-D, anti-C, anti-E, and probable anti-hr^s posed significant challenges in the identification and provision of compatible blood for this patient. The MMA results, the patient's clinical status, and post transfusion laboratory and serological results indicate that the alloanti-c to the partial -c antigen is clinically significant. Individuals of African descent have a much higher incidence of variant Rh antigens as compared to individuals of European descent, the latter constituting the largest percentage of blood donors by ethnicity.[12] The high prevalence of variant antigens in African-Americans patients thereby increases the risk of alloimmunization when these patients are exposed to the conventional antigen during transfusion.[13,14] The detection of the presence of a variant antigen, identification of the corresponding alloantibody, determination of its clinical significance and the logistics of finding compatible blood can be quite challenging. Rh null cells were recommended for any future transfusions, until such time that a possible genotype match could be identified for further compatibility testing and evaluation. This case demonstrates the increasingly vital role of molecular testing in the overall clinical/laboratory assessment of alloimmunized patients with variant red cell antigens and for subsequent transfusion recommendations and guidance.

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Tables:

Table 1: MMA Results

	Coated Test RBCs IgG DAT	% Reactive Monocytes
Random # 1 D-C-E-K- Fy(a-b-) s-	4+	30.3% with fresh complement
Random #1 D-C-E-K- Fy(a-b-) s-	4+	34.0% without fresh complement
Random #2 D-C-E-K-	4+	38.5% with fresh complement
Random # 2 D-C-E-K-	4+	29.5% without fresh complement
Auto cells	Negative	0.3% without fresh complement

"The normal range for the MMA is 0%-3% reactive monocytes. Values above 3% suggest that the antibody may cause accelerated clearance of antigen-positive red blood cells."

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