# Case report: passively acquired anti-D in a D+ pregnant patient

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A sample was submitted for serologic evaluation from a pregnant patient with immune thrombocytopenic purpura (ITP) for possible transfusion in the future because of a decreased platelet count. Anti-D and -E were identified in the patient's serum using several antibody identification techniques, and anti-D was recovered in an acid eluate prepared from the patient's red cells. It was discovered that WinRho™ had been administered to treat the ITP. This product has been licensed for treatment of nonsplenectomized D+ children and adults with ITP to increase the platelet count. Administration of anti-D to D+ individuals for treatment of ITP can cause a red cell anemia. ***Immunohematology*** **1999;15:69–70.**

**Key Words:** RhIG, WinRho™, ITP, immune blockage

The administration of Rh Immune globulin (RhIG) as antenatal prophylaxis has become accepted practice; therefore, it is not uncommon to detect anti-D in the serum of D– pregnant women during the third trimester and at delivery.

Since June 1995, $Rh_o$ (D) Immune Globulin Intravenous (Human), known as WinRho SDF™ (NABI, Boca Raton, FL), has been licensed by the U.S. Food and Drug Administration for treatment of D+ children with chronic or acute immune thrombocytopenic purpura (ITP), D+ adults with chronic ITP, and children and adults with ITP secondary to HIV infection, to increase platelet counts.[1] Anti-D, -C, -E, -G, -V, and -$Fy^a$ have been detected in the serum of D+ individuals after infusion of RhIG and also have been recovered in eluates prepared from their red blood cells (RBCs).[2]

A sample was submitted to our laboratory for serologic evaluation on a pregnant woman with a diagnosis of ITP. The workup was requested because it was thought that the patient may require future transfusion therapy. The list of medications included RhIG, Carafate, Solumedrol, Solucort, and Benadryl. The RhIG was identified as WinRho SDF™ and was used for the treatment of ITP.

## Case Report

Blood samples from a 16-year-old female were submitted to the reference laboratory for antibody identification. Her diagnosis was pregnancy and ITP. The request form indicated this to be the first pregnancy with a due date in approximately 8 weeks. There was no transfusion history recorded, and the assumption was the patient had not been transfused previously. The patient was receiving RhIG, Solumedrol, Solucort, and Benadryl during her pregnancy.

## Materials and Methods

Routine tests were performed with standard methods and licensed commercial reagents. ABO and Rh were determined with monoclonal anti-A, monoclonal anti-B (Gamma Biologicals, Houston, TX) and blended monoclonal-polyclonal anti-D antisera (Ortho-Clinical Diagnostics, Raritan, NJ). The Rh phenotype was determined using monoclonal anti-C, -E, -c, and -e (Ortho-Clinical Diagnostics). Initial antibody identification was performed using MTS ID-Micro Typing System™ (Ortho-Clinical Diagnostics). Standard tube test methods were performed using an in-house panel of reagent RBCs with known phenotypes (Blood Systems Laboratories, Scottsdale, AZ), a low-ionic-strength solution (LISS) technique (N-Hance, Gamma Biologicals), polyethylene glycol (PEG; Blood Systems Laboratories), and monospecific anti-IgG (Immucor, Inc., Norcross, GA). Direct antiglobulin testing (DAT) was performed using polyspecific antihuman globulin (AHG) containing anti-IgG and -C3d, monospecific anti-IgG, monospecific anti-C3b, -C3d (Immucor) and a 6% albumin control (Blood Systems Laboratories). An acid eluate prepared from the patient's IgG-coated RBCs (Elu-Kit II, Gamma Biologicals) was tested with a panel of reagent RBCs using the routine method listed in the reagent circular.

An autologous adsorption was performed to determine if the antibody detected was an allo- or an autoantibody. The patient's RBCs were treated with 0.2 M dithiothreitol and 1% ficin in pH 7.3 phosphate-buffered saline, incubated for 30 minutes at 36°C to 38°C, and washed to remove any residual reagent. One volume of serum was incubated for 30 minutes at 36°C to 38°C with two volumes of treated RBCs. The

adsorbed serum was harvested and tested with a LISS technique using an in-house panel of reagent RBCs.

## Results

The patient's RBCs typed as group A, D+C+E–c–e+. The initial antibody identification performed using the MTS ID-Micro Typing System™ appeared to be anti-D with weak reactions with two of three E+ cells. A subsequent panel performed using a LISS technique appeared to be anti-D. Antibody identification using PEG appeared to be anti-D and a weakly reactive anti-E. The DAT was strongly positive with polyspecific AHG and anti-IgG, and negative with anti-C3b, -C3d, and the 6% albumin control. An acid eluate prepared from the patient's RBCs reacted with the D+ cells and was nonreactive with D– cells. The autoadsorbed serum was nonreactive with D+ reagent RBCs.

## Discussion

A possible interpretation of the initial serologic findings was an autoimmune process with relative anti-D specificity. With the diagnosis of ITP, an autoimmune disorder, a warm-reactive autoagglutinin could be possible. A discussion with the submitting facility disclosed that the patient had received WinRho SDF™ to treat ITP. (Because the patient is D+, she would not be a candidate for antenatal RhIG prophylaxis.) According to the reagent circular, administration of WinRho SDF™ to nonsplenectomized D+ individuals has been shown to increase platelets in ITP patients usually within 1 to 2 days and peak within 7 to 14 days after initiation of therapy.[1] Although the duration of the response is variable, the average duration is approximately 30 days. A decrease in hemoglobin can be expected with this treatment.[1]

The rationale behind administration of intravenous anti-D is the blockade of Fc receptors of the mononuclear phagocytes located in the spleen by antibody-coated RBCs. It is also felt that the spleen is the site of antiplatelet antibody production. Splenectomy as a treatment for ITP would remove the site of platelet destruction and platelet antibody production. The decision to treat a patient with WinRho SDF™ or perform a splenectomy depends on the physician's evaluation of the disease state.

Often the diagnosis listed on a reference laboratory request form or in a hospital computer system is not the patient's primary disease. When anti-D is identified in a D+ patient, further inquiry is necessary to determine the patient's actual disease diagnosis, and if ITP is the diagnosis, it is necessary to determine if WinRho SDF™ has been administered as a treatment for ITP. Since this laboratory's encounter with this patient, we have identified anti-D in another D+ patient with ITP and consulted with other facilities that encountered these same serologic findings in two patients in less than 6 months. This situation will become more common as this form of treatment for ITP increases because of its efficacy, lower cost compared to intravenous immune globulin, and relatively few side effects (unpublished data, 1998, AABB/ARC Reference Laboratory Meeting, Peachtree, GA). The mean hemoglobin decrease 7 days after transfusion is 0.8 g/dL; therefore, transfusion has not been required because of this therapy.[3] Should a patient need a transfusion, the recommendation is to transfuse D– units when the patient needs oxygen-carrying capacity and D+ units when oxygen-carrying capacity is adequate.[4] This strategy will maintain the Fc blockade, which allows the platelet count to increase.

## References

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