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The Importance of Antibody Detection and Identification in the Chronically Transfused Patient

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H&O What types of patients require maintenance therapy with chronic blood transfusions?

SS In my hospital in Washington, DC, the list begins with sickle cell disease, as an example of an inherited anemia, and myelodysplastic syndrome, as an example of an acquired anemia. There is also a geographic aspect to the answer. If my hospital were in certain regions of Asia or Southern Europe, the list would begin with thalassemia, the most common chronic anemia requiring frequent transfusions in those areas.

H&O What are the advantages to this approach?

SS Most physicians try to limit the number of transfusions of red blood cells to the minimum number necessary to maintain a patient's lifestyle. For most patients with chronic anemia, the advantage of chronic transfusions is that they keep people active who otherwise might be too weak to enjoy their lives. Persons with sickle cell anemia have unique requirements for transfusions of red blood cells. In these persons, chronic transfusions of red blood cells may be required repeatedly to prevent new or recurrent strokes, chronic pain, pulmonary hypertension, acute chest syndrome, and anemia associated with cardiac or renal failure. The goal of chronic transfusions in these situations is to dilute the patient's own red blood cells (nearly 100% Hb SS) to 30–50% Hb S, which will prevent spontaneous sickling and thromboses. It is esti-

ated that 90% of patients with sickle cell anemia require transfusions of red blood cells to prevent complications of their disease.¹

H&O What are the most frequent risks and/or complications for patients who are chronically transfused?

SS In addition to the risks conventionally associated with blood transfusions (transfusion-related acute lung injury; allergic, febrile, and hemolytic reactions; and transfusion-transmitted infections), persons receiving chronic transfusions of red blood cells have the additional risks of iron overload (hemosiderosis, hemochromatosis)² and alloimmunization to blood group antigens.³

H&O What is the importance of antibody detection and identification, particularly in patients with sickle cell disease and myelodysplastic syndrome?

SS Persons with sickle cell disease experience lifelong hemolysis. Their ability to sustain a fragile, compensated chronic hemolytic anemia depends on the ability of their bone marrow to maintain continuous and vigorous hematopoiesis. Any event that suppresses hematopoiesis—for example, an infection or folic acid deficiency—can precipitate an acute, severe anemia requiring an urgent transfusion of red blood cells. Unless advance preparations have been made in the transfusion service, life-threatening delays may occur while the transfusion service identifies a newly formed alloantibody and the community blood center locates units of donated red blood cells with a serologically compatible phenotype. To avoid such logistical crises, all patients with diagnoses likely to require chronic transfusions—and, in particular, persons with sickle cell disease—should have an extended red blood cell phenotype performed for antigens most likely to be implicated

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(D, C, E, c, e, K, S, s, Jk^a, Jk^b, Fy^a, Fy^b). The extended phenotype should be performed before chronic transfusions have been initiated because once donors' red blood cells have been transfused and mixed with the patient's own red blood cells, phenotyping is considerably more difficult. If a chronically transfused patient develops a new antibody, the transfusion service can review the patient's phenotype and identify which alloantibodies that patient can form (patient's red cells do not express the antigen) and which ones the patient cannot form (patient's red cells express the antigen). This shortcut in the conventional laboratory protocol for antibody identification can save critical hours. At our hospital, we support 2 adult patients with sickle cell disease and multiple alloantibodies whose transfusions require red blood cells with exceedingly rare blood group phenotypes. In addition to having their extended blood group phenotypes readily available, we maintain a small inventory of serologically matched frozen red blood cells for the occasional need for an urgent transfusion for each of these persons. Although the cause of anemia in myelodysplastic syndrome is different, the sample prearrangements with the transfusion service apply.

H&O What are the most commonly used antibody screening techniques?

SS Traditionally, transfusion services screened patients' sera in test tubes using pooled or extensively phenotyped individual reagent red blood cells (screen and identification panels). In recent years, more sensitive methods using gel columns, microplates, or solid-phase microplates have been developed and automated. Today, most large- and medium-sized transfusion services screen and identify patients' alloantibodies using plasma in automated analyzers, often with neural networks capable of interpreting agglutination patterns, identifying antibodies according to internal programs, and delivering the results to the laboratory's information system via an electronic interface. Currently, some advanced transfusion services augment serologic screens for blood group antibodies using molecular analysis to determine the patient's potential for forming antibodies by genotyping. Chronically transfused persons with sickle cell disease may have requirements for compatible red blood cells that cannot be resolved by conventional serologic methods. Genotyping, particularly within the complex Rh blood group system, offers an additional and more precise approach to identifying alloantibodies in such patients.^{4,5}

H&O How often should patients undergo screening?

SS The answer depends on how often the patient is transfused with red blood cells. Some chronically transfused

persons with sickle cell disease are antibody responders who make multiple antibodies early in life and continue to form antibodies for the remainder of their lives. Others, who have received at least as many transfusions, are non-antibody responders and make fewer antibodies, even after more transfusions. We reviewed alloantibodies formed in 351 persons with sickle cell disease in Washington, DC, who were transfused with 8,939 units of ABO/D-matched (only) red blood cells during a 12-year study period.⁶ Although 102 (29.1%) recipients formed 1 or more alloantibodies, 70.9% of those transfused did not form any alloantibodies. Some blood group antibodies, for example, anti-Jk^a and anti-Jk^b, are short-lived. If a timely post-transfusion antibody screen is not performed on plasma from an antibody responder who is Jk(a-) or Jk(b-), the patient may return for another transfusion after the time when the antibody is detectable and experience a delayed hemolytic transfusion reaction. The standard practice is to perform an antibody screen whenever a transfusion of red blood cells is ordered. Although this approach, admittedly, misses the detection of some newly formed and transient alloantibodies, it seems justified by our data that more than 70% of persons with sickle cell anemia who are transfused with conventional ABO/D-matched red blood cells do not form alloantibodies. That number is reduced even more if the recipients are receiving any form of phenotype-matched red blood cells.

H&O What is the difference between clinically significant alloantibodies and clinically insignificant alloantibodies?

SS The definition of a "clinically significant alloantibody" is one that is expected to shorten the survival of transfused red blood cells if they express the corresponding (cognate) antigen. Reagent red blood cells are selected for screen and identification panels for transfusion services because they express antigens that correspond to antibodies that are considered to be clinically significant (for example, C, D, E, K). However, since reagent screen cells are otherwise normal human red blood cells, they also express antigens that correspond to antibodies that are considered to be clinically insignificant (for example, Le^a, Le^b, M, N, P₁). Therefore, in the course of a standard pre-transfusion antibody screen and identification, a transfusion service technologist may report detecting anti-E (which would be considered clinically significant) and anti-P₁ (which would be considered clinically insignificant). In this situation, the medical director of the transfusion service should select E- red blood cells for transfusion and ignore the anti-P₁. Some newer automated blood bank analyzers pre-warm patients' plasma and reagent red blood cells to

37°C before testing.⁷ Since most clinically insignificant blood group antibodies do not react at body temperature, they will not be detected, bypassing time-consuming efforts traditionally used in the process of detecting irrelevant clinically insignificant antibodies.⁸

H&O What are the management options for chronically transfused patients with antibodies?

SS Since transfusing red blood cells is the only realistic option for managing these patients, a practical transfusion strategy should be one intended to decrease the likelihood of alloantibody formation. There are 2 principal choices: matching for D, C, c, E, e, K, S, Fy^a, and Jk^a (extended phenotype matching) or for D, C, c, E, e, and K (limited phenotype matching).⁶ Proponents of extended phenotype matching argue that since the patients form alloantibodies only to blood group antigens that are not present on their own red blood cells, these patients can receive transfusions without the complication of having to wait while antibodies are identified and serologically compatible units of red blood cells are located. I do not favor this strategy because it creates the problem of finding these difficult-to-locate blood units every time red blood cells are needed for a transfusion—and most patients will never form an antibody and never require such special units. Applying this strategy to a person with the phenotype Ro [C- E- K- S- Jk(b-) Fy(a-)] would mean that the same problem we would be trying to prevent—locating such difficult-to-find units because the patient has anti-C, -E, -K, -S, -Jk^b and -Fy^a—would be the exact problem the transfusion service would have every time the patient needs a transfusion. A survey of 1,182 hospital transfusion services in North America revealed that most match transfusions for non-alloimmunized patients with sickle cell disease for ABO and D, only.⁹ It is true that if extended phenotype matching was implemented for this patient, 70.9% of antibodies that are likely to be formed would be prevented.⁶ The problem is that this phenotype is present in only 0.6% of random donors. I favor “limited phenotype matching,” that is, matching for D, C, c, E, e, and K. In this situation, 53.3% of all antibodies likely to be formed by the same patient (described above) would be prevented. The C-, E-, K- phenotype is present in 13.6% of random blood donors and, therefore, serologically matched red blood cells can be located relatively efficiently.

Before closing, I would like to share an additional observation on this subject. On occasion in our hospital, we have had to transfuse Rh(D)-positive red blood cells to an Rh(D)-negative patient undergoing a liver or multi-organ transplant. I have had the opportunity to perform an antibody screen on approximately 15 of these patients months-to-years later when they returned to the clinic or the hospital. Not one has formed anti-D. Presumably, the immunosuppressive regimens for preventing rejection of organ transplants are also highly effective for preventing primary alloimmunization to the highly immunogenic Rh(D) blood group antigen. There is a certain logic to support this observation. Transfusions of red blood cells are, in effect, transplantations of the erythrocyte organ, and alloantibodies are the recipient's immune response to “foreign” antigens on the transplanted “tissue.” An immunosuppressive protocol designed to maximally reduce rejection of an allogeneic organ transplant would, logically, also reduce the immune response to transplanted red blood cells. This observation raises the possibility of preventing antibody formation in chronically transfused patients by simultaneous immunosuppression. Perhaps there is a resident or fellow in training who is reading this interview and will pursue this observation in his or her medical center.

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