The differentiation of delayed hemolytic and delayed serologic transfusion reactions: incidence and predictors of hemolysis

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Background: After differentiation of the entities of clinically detectable delayed hemolytic (DHTR) and delayed serologic transfusion reactions (DSTR), previous investigators calculated a DHTR:DSTR incidence ratio of 18:72 from a retrospective review of patients with serologic evidence of DHTR or DSTR. There are no published data on factors that may influence the occurrence of DHTR versus DSTR in a given patient.
Study Design and Methods: Retrospective review was conducted of 292 patients at the Mayo Clinic who, between 1980 and 1992, received a clinical diagnosis of DHTR or DSTR concurrently with a serologic diagnosis. Red cell alloantibody specificity, the activity of the patient's reticuloendothelial system, and concurrent immunosuppression were evaluated as potential predictors of the occurrence of DHTR versus DSTR in different patients.
Results: The incidence of DHTR or DSTR was 1 in 1899 allogeneic red cell units transfused, with a DHTR:DSTR ratio of 36:64. Alloantibody specificity was the only variable that affected the occurrence of DHTR versus DSTR at the clinical level, with the anti-Jk^a and anti-Fy^a specificities, as well as multiple coexisting specificities, significantly associated with detectable hemolysis ($p<0.05$).
Conclusion: Clinically detectable DHTRs are found to occur more commonly than previously believed when the clinical and serologic diagnoses are made concurrently and appropriate work-ups for hemolysis are ordered. The association of certain alloantibody specificities with detectable DHTRs may have implications for clinical transfusion practice. **TRANSFUSION** 1995;35:26–32.

Abbreviations: DHTR = delayed hemolytic transfusion reaction; DSTR = delayed serologic transfusion reaction; ICD = immune complex disease; RBC(s) = red cell(s); RES = reticuloendothelial system.

THE DEFINITION OF A delayed hemolytic transfusion reaction (DHTR) is accelerated destruction of transfused red cells (RBCs) after an interval during which the recipient mounts an immune response to an antigen carried by the transfused cells.[1] Using "serologic" diagnostic criteria (i.e., a newly detected alloantibody in eluate and/or posttransfusion serum studies after a negative antibody screen in the pretransfusion serum), Taswell et al.[2] reported a DHTR incidence of 1 in 1500 RBC units transfused at the Mayo Clinic between 1978 and 1980. The introduction of more sensitive methods of antibody detection and a greater awareness of the occurrence of asymptomatic DHTRs probably accounted for the increase in the incidence of diagnosed DHTRs at that institution from 1 in 11,650 units[3] to 1 in 1,500 units[2] over a 17-year period.[2-4]

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Many DHTRs are clinically asymptomatic, but absence of the signs and symptoms of hemolysis does not exclude relatively minor increases in the rate of RBC destruction.[1] Taswell et al.[2] differentiated, in patients meeting the serologic criteria for a diagnosis of DHTR, between DHTRs with *detectable hemolysis* of the transfused RBCs (now referred to as DHTRs) and asymptomatic episodes of RBC sensitization without hemolysis. Ness et al.[5] later coined the term delayed serologic transfusion reaction (DSTR) for these "silent" DHTRs, and they reported that only 6 (18%) of 34 patients who had fulfilled the serologic criteria for DHTR or DSTR could be assigned a clinical diagnosis of DHTR. All six of those patients (who were retrospectively evaluated for signs and symptoms of hemolysis[5]) had experienced a decrease in hemoglobin level after a transfusion.[1] Only one had had fever, and one had reduced urine output; elevated serum bilirubin or creatinine levels had each been recorded in two patients.[5]

The assiduousness with which signs and symptoms of hemolysis are sought in routine clinical practice varies, and anemia, fever, jaundice, and renal failure are com-

mon occurrences in tertiary-care hospitals. Therefore, the distinction between a clinically detectable DHTR and an asymptomatic episode of DSTR is not made with consistency.[1] In addition, published data on factors that may influence the occurrence of DHTR versus DSTR in a given patient are extremely sparse. On the basis of a preliminary review of the Mayo Clinic experience from 1978 through 1984, Pineda et al. reported that Kidd and Duffy system antibodies were significantly more likely to be associated with DHTR than with DSTR.[6]

Garratty[7] related the pathogenicity of RBC alloantibodies to specific qualitative and quantitative characteristics of the antibody, some attributes of the target antigen, and the activity of the patient's reticuloendothelial system (RES). Of all such characteristics, antibody specificity and thermal range (which are routinely determined at the blood bank laboratory) are the most important.[7] The activity of a patient's RES[8-10] can be inferred from his or her diagnosis, medical history, and treatments received. Other antibody and target antigen characteristics[7] are demonstrable only by research techniques, so that the pertinent data are not available in a retrospective review of the clinical behavior of previously detected RBC alloantibodies.

The purposes of the present study were to determine specific incidence figures for clinically detectable DHTRs and for episodes of DSTR and to identify variables that might have influenced the occurrence of DHTR versus DSTR in patients in whom a serologic diagnosis of DHTR or DSTR had been made. Three sets of potentially relevant factors (i.e., alloantibody specificity,[7] activity of the patient's RES,[8-10] and concurrent immunosuppression[11-14]) were retrospectively evaluated in a review of all diagnoses of DHTR or DSTR made at the Mayo Clinic between 1980 and 1992.

Materials and Methods

Starting in 1980, a procedure for the diagnosis of DHTR versus DSTR was implemented at the Mayo Clinic Blood Bank. Initial serologic diagnosis was based on techniques conforming to the standards of the American Association of Blood Banks, as detailed in the *Technical Manual*.[15-17] Antibody screening procedures involved enzymatic analysis (two-stage papain) and testing in saline at 22°C (immediate-spin), in albumin at 37°C, and in anti-human globulin serum. Antibody screening was performed with two RBC samples (bearing complementary antigen phenotypes) in a 2-percent suspension (in Alsevers RBC preservative) with 3 drops of serum. All patients received crossmatch-compatible blood that was tested in the same phases as RBC antibody screening, with the exception of enzymatic testing. We performed antibody identification with commercial cell panels in the same phases as antibody screening, including enzyme treatment. Direct antiglobulin testing with polyspecific and monospecific reagents was performed on samples obtained before and after transfusion. RBC antibodies were eluted by an acid-stromal technique. Our serologic diagnostic criteria for DHTR and DSTR were reported previously.[2-4]

All diagnoses of DHTR versus DSTR were working clinical diagnoses, intended for patient management and made while the patient was still in the hospital or, occasionally, shortly after discharge. After every serologic diagnosis of DHTR or DSTR, the transfusion medicine resident contacted the clinical service to request an appropriate work-up for hemolysis and to inquire about pertinent signs and symptoms. Relevant laboratory values over a period of 3 to 21 days after transfusion[1] were recorded. Each case was discussed in a daily conference of the blood transfusion service medical staff and in turn was discussed with the staff of the clinical service, as follow-up continued. Alternative explanations for noted laboratory abnormalities and/or clinical signs and symptoms were examined with reference to each patient's primary diagnosis, comorbidities, and medical history. As customary for all medical decisions intended for patient management, refutation or acceptance of these alternative explanations was based on medical judgment and consensus among colleagues. Detection of any preset minimum number of signs and symptoms of hemolysis was not required for a diagnosis of DHTR.

If a patient had experienced a posttransfusion decrease in the blood hemoglobin level (by at least 1 g/dL from the immediate pretransfusion level), absent an obvious, alternative explanation for the decrease, a diagnosis of DHTR could be made. However, after review of both clinical and laboratory data, and if alternative explanations existed, we required *at least* one more sign or symptom of hemolysis for that diagnosis, as outlined in our previous studies.[2-4] The following were evaluated: an elevation in the serum indirect bilirubin or in the serum creatinine, or a reduction in the serum haptoglobin (by at least 50%), as compared to the pretransfusion level; hemosiderinuria, hemoglobinuria, or hemoglobinemia; and otherwise unexplained fever or decreased urine output. The positive predictive value of signs and symptoms of hemolysis varies with the particular clinical situation; accordingly, the number of hemolytic signs and symptoms that contributed to a diagnosis of DHTR in our study population varied from patient to patient.

A diagnosis of DHTR versus DSTR was made by a staff consultant after sufficient time had elapsed and adequate laboratory results had been accumulated. A record of all diagnoses of DHTR and DSTR was maintained by one of the authors (RR). The present study is a retrospective review of the working clinical diagnoses of DHTR or DSTR that were made at the Mayo Clinic from 1980 through 1992: 296 such diagnoses were recorded during that period. A clinical distinction between DHTR and DSTR could not be made in four of these cases, and those patients were excluded from the present analysis.

We reviewed the blood bank and medical records of the remaining 292 patients and collected information on the following variables:

1) RBC alloantibody (or alloantibodies) implicated in DHTR or DSTR from serum and/or eluate studies;

2) patient's gender, date of birth, and discharge diagnosis at the time of the index episode of DHTR or DSTR (the index hospitalization);

3) history of a disease often associated with presence of immune complexes (immune complex disease [ICD], i.e., systemic lupus erythematosus, other collagen-vascular disease, necrotizing vasculitis, immune complex glomerulonephritis, or other ICD) before (or during) the index hospitalization;

4) receipt of cancer chemotherapy or immunosuppressive therapy (i.e., at least 50 mg/day of prednisone or equivalent or azathioprine, cyclophosphamide, methotrexate, cyclosporine, anti-lymphocyte globulin, or intravenous immunoglobulin) during the index hospitalization;

5) history of solid organ transplant, bone marrow transplant, or hematologic or other malignancy (excluding carcinomas in situ, papillary carcinoma of the thyroid, and nonmelanocytic carcinomas of the skin) before (or during) the index hospitalization;

6) history of massive transfusion, surgery under general anesthesia, or infection during the 3 months preceding the diagnosis of DHTR or DSTR; and

7) history of splenectomy.

The frequencies of DHTR and DSTR were compared by chi-square (or Fisher's exact) testing in men and women; among young (under 41), middle-aged (41-65), and senior (over 65) patients; in patients discharged by different clinical services; in patients with a history (as compared to no history) of ICDs, immunosuppressive therapy, cancer chemotherapy, malignancy, solid organ or bone marrow transplant, or splenectomy; and in patients undergoing massive transfusion or surgery under general anesthesia or having an infection within 3 months of the episode of DHTR or DSTR. The frequencies at which DHTR and DSTR occurred in the presence of particular RBC alloantibodies (as compared to all other RBC alloantibodies) were similarly compared by chi-square testing (with Yates' correction for continuity) or Fisher's exact testing (as appropriate). We used a software package[18] for all statistical analyses.

Results

Two hundred ninety-two patients were diagnosed with DHTR or DSTR over 13 years (1980-1992). There were 132 men (45%) and 160 women (55%). The mean age was 58.8 years (with a median of 62, and a 75th percentile of 72). The youngest patient was 5 years old, and there were four children under the age of 14. Two hundred ten (71.9%) patients experienced DHTR or DSTR in the course of a surgical admission, including hospitalizations for orthopedic (18.5%), gastrointestinal (18.5%), cardiac (7.9%), vascular (8.2%), urologic (5.5%), thoracic (4.5%), neurologic (2.4%), gynecologic (2.1%), or other (4.3%) surgical procedures. Eighty-two patients (28.1%) were diagnosed with DHTR or DSTR during a medical admission to the oncologic (9.9%), gastrointestinal (8.2%), hematologic/nononcologic (3.4%), renal (1.7%), pulmonary (1.0%), cardiac (0.3%), or other medical (3.6%) service.

A diagnosis of DHTR was made in 104 patients (35.6%) and DSTR was diagnosed in 188 (64.4%). Overall, between 1980 and 1992, 562,124 units of allogeneic RBCs were transfused at our institution, and 296 cases of DHTR or DSTR were detected. The observed incidence of DHTR or DSTR was 1 in 1899 allogeneic RBC units transfused (95% CI of 1 in 1892 to 1 in 2165).[19] The incidence of DHTR was 1 in 5405 units transfused (95% CI of 1 in 4426 to 1 in 6918), and the incidence of DSTR was 1 in 2990 units transfused (95% CI of 1 in 2476 to 1 in 3588).

The prevalence of DHTR was higher when the implicated RBC alloantibody was anti-Jk^a ($p<0.0001$ before correction for multiple comparisons) or anti-Fy^a ($p = 0.0018$). These antibodies caused DHTR (as opposed to DSTR) in 65.3 and 57.1 percent, respectively, of the patients in whom they were detected. Anti-Jk^a and anti-Fy^a were the only antibodies implicated in a DHTR or DSTR case in 32 and 29 patients, respectively, and they caused DHTR in 59.4 and 55.2 percent, respectively, of these patients ($p = 0.0001$ for anti-Jk^a; $p = 0.0010$ for anti-Fy^a). The association of these antibodies with a higher prevalence of DHTR also was significant ($p<0.05$) after correction for multiple comparisons. Table 1 shows the episodes of DHTR versus DSTR that were associated with each RBC alloantibody in our series.

Table 1. *RBC alloantibodies causing DHTR versus DSTR*

Antibody	Number of examples	Episodes of DHTR		Episodes of DSTR	
		Number	Percent	Number	Percent
Anti-D	1	1	100	0	0
Anti-G	1	0	0	1	100
Anti-C	20	8	40	12	60
Anti-C^w	1	1	100	0	0
Anti-c	32	14	43.7	18	56.2
Anti-E	127	37	29.1	90	70.9
Anti-e	9	3	33.3	6	66.7
Anti-V	1	0	0	1	100
Anti-K	44	12	27.3	32	72.7
Anti-Kp^a	1	1	100	0	0
Anti-Js^a	1	0	0	1	100
Anti-Jk^a	49	32	65.3	17	37.4*
Anti-Jk^b	7	2	28.6	5	71.4
Anti-Fy^a	42	24	57.1	18	42.9*
Anti-Fy^b	6	3	50	3	50
Anti-M	2	0	0	2	100
Anti-S	4	3	75	1	25
Anti-Lu^b	1	1	100	0	0
Anti-Yt^a	2	1	50	1	50
Anti-A$_1$†	2	1	50	1	50
Anti-Co^b	1	0	0	1	100

* Significant difference; difference was also significant ($p<0.05$) after correction for multiple comparisons.

† Not passively infused.

Multiple RBC alloantibodies (i.e., more than one) were detected in 55 patients, with two antibodies implicated in 47 and three or more antibodies identified in 8. The prevalence of DHTR was higher when multiple antibodies were involved, with 35 cases of DHTR (63.6%) and 20 cases of DSTR (36.4%) diagnosed in these patients ($p<0.0001$ before correction for multiple comparisons and $p<0.05$ after). When antibodies individually linked to a higher prevalence of DHTR (i.e., anti-Jk^a and anti-Fy^a) were excluded from the analysis, multiple antibodies were detected in 26 patients, in whom 15 cases of DHTR (57.7%) and 11 cases of DSTR (42.3%) were diagnosed. The prevalence of DHTR was thus higher when multiple antibodies were involved, even in the absence of antibodies that showed an individual association with detectable hemolysis ($p = 0.0001$ before correction for multiple comparisons and $p<0.05$ after).

All calculations shown above and in Table 1 were based on data derived from serum and/or eluate studies implicating alloantibodies of particular specificities in episodes of DHTR or DSTR. The calculations included 24 antibodies that were detected in posttransfusion serum studies (after a negative antibody screen in the pretransfusion serum) but were not identified in eluates (in contrast to other *coexisting* antibodies). The specificities of these 24 antibodies were: anti-c, 4; anti-E, 9; anti-e, 1; anti-V, 1; anti-K, 5; anti-Js^a, 1; anti-Jk^a, 1; anti-Jk^b, 1; and anti-Fy^a, 1. DHTR (as opposed to DSTR) had been diagnosed in 16 of these cases, with implication of the following antibodies: anti-c, 3; anti-E, 6; anti-e, 1; anti-K, 3; anti-Jk^a, 1; anti-Jk^b, 1; and anti-Fy^a, 1. Exclusion of these 24 antibodies from the analysis did not alter the significance calculations shown above and in Table 1.

Diagnoses of DHTR versus DSTR did not differ in frequency in patients discharged from different surgical and medical services (data not shown). Of 210 surgical patients, 79 (37.6%) were diagnosed with DHTR and 131 (62.4%) with DSTR. Of

82 medical patients, 25 (30.5%) experienced a DHTR, and 57 (69.5%) manifested only DSTR (p = 0.2528). Similarly, diagnoses of DHTR versus DSTR did not differ in frequency in men and women; in patients from different age groups; in patients with diseases often associated with immune complexes, and in patients with malignancies, splenectomy, and immunologic perturbations (as compared to patients without these conditions); or in immunosuppressed (as compared to immunocompetent) patients (Table 2). It is noted that two of four children under 14 years of age were diagnosed with DHTR and two with DSTR (p = 0.5452).

The patient characteristics listed in Table 2 did not show a significant association with a reduced frequency of DHTR (as opposed to DSTR) in the 90 patients who had an antibody linked to an increased DHTR prevalence (i.e., anti-Jk^a or anti-Fy^a) (data not shown). Therefore, a patient's failure to manifest the clinical syndrome of DHTR in the presence of anti-Jk^a and/or anti-Fy^a could not be attributed to a limited capacity of his or her RES to phagocytose sensitized donor RBCs[8-10] or to a concurrent state of immunosuppression.[11-14]

Discussion

The incidence of DHTR or DSTR at the Mayo Clinic from 1980 through 1992 was 1 in 1899 transfused units of allogeneic RBCs, a figure similar to that reported from our institution between 1978 and 1980[2] and to that observed at the Johns Hopkins Medical Center in 1986 and 1987 (1/1605 units[5]). The true current incidence of DHTR or DSTR remains unknown, as none of the three studies was designed as a prospective investigation with systematic follow-up of *all* recipients of RBC transfusion and testing of serial posttransfusion samples. For this reason, the agreement between the three studies might be due to a similar underreporting bias at the two institutions. For example, DHTR or DSTR episodes that follow outpatient transfusions (or transfusions given to inpatients shortly before their discharge from the hospital) may have been missed in all three investigations. However, the concordance between the three studies does suggest that the true current incidence of *detected* DHTR or DSTR should be between 1 in 1500 and 1 in 2000 allogeneic RBC units transfused at a tertiary-care medical center.

The ratio of DHTR to DSTR was 36 to 64 in our study, as compared to 18 to 82 in the Johns Hopkins study.[5] A superficial inspection of these figures might lead one to assume that clinically detectable DHTRs occurred with a higher frequency in our study, and that the difference might be due to the uncertainty associated with the definition of the outcome variable (i.e., delayed hemolysis

Table 2. *Attributes of patients presenting with DHTR versus DSTR**

Attribute	Number of patients	Episodes of DHTR		Episodes of DSTR	
		Number	Percent	Number	Percent
Age					
Young (under 41)	55	20	36.4	35	63.6
Middle-aged (41-65)	115	36	31.3	79	68.7
Senior (over 65)	122	48	39.3	74	60.7
Gender					
Male	132	46	34.8	86	65.2
Female	160	58	36.2	102	63.8
ICD (current)†‡	34	9	26.5	25	73.5
Immunosuppression (current)					
Immunosuppressive therapy§	21	7	33.3	14	66.7
Solid organ transplant	7	3	42.9	4	57.1
Bone marrow transplant	0	0	0	0	0
Malignancy (current)					
Cancer chemotherapy	13	4	30.8	9	69.2
Hematologic malignancy	28	8	28.6	20	71.4
Other malignancy‖	58	22	37.9	36	62.1
Immunologic perturbations¶					
Massive transfusion§	21	8	38.1	13	61.9
Surgery/anesthesia‖	222	81	36.5	141	63.5
Infection‖	93	36	38.7	57	61.3
Splenectomy§	17	6	35.3	11	64.7

* No significant difference was detected.

† Disease active during index hospitalization; disease activity (i.e., illness severity) was not graded.

‡ The study had a 75-percent power to detect a decrease of 60 percent in the incidence of DHTR in patients with currently active ICD, as compared to patients without active ICD.

§ The study had a 75-percent power to detect a decrease of 72 percent in the incidence of DHTR in splenectomized patients or patients currently receiving immunosuppressive therapy or massive transfusion, as compared to patients not subject to each of these interventions.

‖ The study had a 75-percent power to detect a decrease of 50 percent in the incidence of DHTR in surgical patients or patients currently afflicted with a (hematologic or other) malignancy or experiencing a recent infection, as compared to medical patients or patients currently free of malignant disease or infection.

¶ Occurring during a 3-month period preceding the episode of DHTR or DSTR.

after transfusion) and/or different blood bank administrative systems (as outlined above), which may have facilitated more detailed and more immediate work-ups for hemolysis on the Mayo Clinic patients. However, examination of the 95-percent CIs for the figures reported by each study reveals that there was no difference between our study and the investigation of Ness et al.[5] in either the absolute incidence rate of DHTR or the frequency of DHTR as a percentage of all serologic episodes of DHTR or DSTR.

More specifically, Ness et al. reported a DHTR incidence of 1 in 9,094 (6/54,562) transfused RBC units. The 95-percent CI for this incidence rate (1/4,198-1/24,734 units[19]) completely contained the 95-percent CI for the DHTR incidence calculated from our study (1/4,426-1/6,918). Ness et al.[5] similarly, reported that DHTR occurred in 18 percent (6/34) of all serologic episodes of DHTR or DSTR. The 95-percent CI for this proportion (5.2-34.4%[19]) overlapped that for the 36-percent proportion calculated from our study (30.9-41.7%). Therefore, the two studies calculated similar figures for the absolute incidence of clinically detectable DHTRs as well as for the proportion of clinically detectable DHTRs among all serologic episodes of DHTR or DSTR.

Even though our findings did not differ to a significant extent from those of Ness et al.,[5] the general reader may well observe that clinically detectable DHTRs occurred more commonly than previously believed,[5] in a setting where the clinical and serologic diagnoses were made concurrently and appropriate work-ups for hemolysis were ordered. Our sample was 10 times as large as that of Ness et al.[5] in terms of the number of RBC units transfused during the study period, and 8 times as large in terms of the number of registered serologic episodes of DHTR or DSTR. The larger sample size naturally resulted in calculation of a more stable CI for the DHTR:DSTR ratio in our study (see above). It can thus be assumed (with 95% confidence) that the true DHTR:DSTR ratio in a tertiary-care hospital population is between 31:69 and 42:58. This represents a substantial (albeit not significant) change from the hitherto prevailing belief regarding the relative frequencies of DHTR and DSTR.

Frank et al.[10] observed a large difference in the length of survival of strongly sensitized RBCs injected in patients with systemic lupus erythematosus and of those injected in normal controls. They postulated that the better survival of cells injected in patients from the former group was due to blockade of the Fc receptors on the RES macrophages by DNA and anti-DNA complexes.[10] In reviewing those findings, Garratty commented, "It is not hard to imagine that this phenomenon might occur with a wide range of immune complexes, in varying degrees, in many patients. It seems that this might be a common explanation for different RBC survivals and clinical reactions when incompatible blood is transfused."[7(p150)]

Both Garratty and Frank et al. were discussing[7] and observing[10] instances of *acute* extravascular destruction of strongly sensitized allogeneic RBCs, but such circumstances may not apply in our current research setting. However, on the basis of those findings, we hypothesized that the blockade of a patient's RES by immune complexes could similarly account (at least some of the time) for the occurrence of DSTR versus DHTR in different patients. Despite the fact that our study had sufficient statistical power to detect a difference in the rate of hemolysis as large as that reported by Frank et al.,[10] the clinical experience at our institution over the last 13 years (Table 2) did not support this hypothesis. In a similar vein, we did not observe a lower incidence of DHTR (relative to DSTR) in patients who had previously undergone splenectomy.

Corticosteroids have been shown to interfere with the sequestration of antibody-coated RBCs in laboratory animals[11,12] and to effect a small diminution in the rate of clearance of D-sensitized RBCs in patients with rheumatoid arthritis.[13] They also slow RBC destruction in patients with autoimmune hemolytic anemia.[14] On the basis of these observations, we hypothesized that the concurrent receipt of immunosuppressive drugs (or the degree of immunosuppression necessary for a solid organ or bone marrow transplant) might explain some of the variation in the occurrence of DSTR versus DHTR in different patients. We further considered the concurrent systemic receipt of chemotherapeutic agents for the treatment of cancer, a history of hematologic or other malignancy, and such controversial immunologic perturbations as recent surgery under general anesthesia, massive transfusion,[20] and infection. None of these variables was associated with a decrease in the incidence of DHTR (relative to DSTR), and the hypothesis that immunosuppression might thwart the appearance of detectable hemolysis was not supported by our experience (Table 2).

It must be emphasized, however, that, as noted in the footnote to Table 2, our study had sufficient statistical power to detect only a *large* reduction (50-72%) in the incidence of DHTR in patients with RES blockade or immunosuppression. Although an effect similar in size to the shortened RBC survival reported by Frank et al.[10] would most likely be detectable with confidence, no conclusion can be reached from our data as to the existence of a more modest association between RES blockade or immunosuppression and a reduced likelihood of detectable DHTR. Small associations are unlikely to be demonstrated in studies performed at single institutions. For example, under similar sampling and confidence conditions as in the present study, 877 patients (with a DHTR:DSTR ratio of 36:64) would have to be enrolled

to establish a 34-percent reduction (38% vs. 25% difference) in the incidence of DHTR in patients with and without currently active ICD.[21]

The specificity of the implicated RBC alloantibody emerged as the only predictor of the occurrence of DHTR versus DSTR at the clinical level. Anti-Jk^a and anti-Fy^a were significantly more likely ($p<0.05$) to be associated with DHTR than with DSTR. DHTR and DSTR occurred, respectively, in 65.3 percent and 34.7 percent of patients with a Jk^a antibody and in 57.1 percent and 42.9 percent of patients with an Fy^a antibody, as compared to 29.6 percent and 70.4 percent and 32 and 68 percent, respectively, of patients with other antibodies. These results could have been predicted via extrapolation from the findings of experimental reports on the in vivo behavior of complement-binding IgG antibodies (such as anti-Jk^a and anti-Fy^a)[22] to the setting of delayed hemolysis. Acute extravascular hemolysis due to such antibodies is substantially more brisk and rapid than RBC clearance by non-complement-binding IgG antibodies.[22] Along these lines, anti-Jk^a and anti-Fy^a would be expected to cause detectable DHTR, while Rh system (and other antibodies that do not fix complement in vivo) would be associated with episodes of DSTR.

The association of the Kidd and Duffy system antibody specificities with clinically detectable DHTRs may justify modification of our transfusion policies in patients who are likely candidates for multiple transfusions over an extended time, such as those with thalassemias and hemoglobinopathies.[6] More specifically, in an effort to prevent delayed hemolytic episodes after transfusion, it might be advisable to match for the Jk^a and Fy^a antigenic determinants, in addition to ABO and D. Matching for the Kidd system antigens[23] or even providing blood that is phenotypically identical for antigens other than ABO and D[24,25] has been advocated by others, for the purpose of preventing alloimmunization to RBC antigens. However, if the incidence of alloimmunization is more related to differences in individual immune responses than to the overall number of transfusions,[26,27] it might not be medically indicated or cost-effective to provide antigen-matched blood to patients who chronically receive multiple transfusions to prevent alloimmunization,[28] while the argument for matching donor blood for Jk^a and Fy^a to prevent DHTRs would still be applicable.

In conclusion, the prevalence of clinically detectable DHTR appears to have been found to be higher than previously believed[5] when working clinical diagnoses of DHTR versus DSTR are reached shortly after the serologic diagnosis of DHTR or DSTR has been made. At least to the extent that the statistical power of our study allowed us to determine, there does not seem to be an association between the occurrence of detectable DHTR (as opposed to DSTR) and the activity of the patient's RES or the presence of a state of immunosuppression. The specificity of the implicated RBC alloantibody appears to be the only predictor of hemolysis when the serologic ingredients for a DHTR are present.

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