A retrospective analysis of the value of monocyte monolayer assay results for predicting the clinical significance of blood group alloantibodies

Patricia A. Arndt and George Garratty

BACKGROUND: Cellular assays (e.g., monocyte monolayer assays [MMAs]) have been used to predict the clinical significance of red blood cell (RBC) alloantibodies.
STUDY DESIGN AND METHODS: Twenty years of MMA data were retrospectively analyzed to 1) determine the optimal cut point (by correlating MMA results from 46 patients with RBC survival study results and/or laboratory and clinical signs of hemolytic transfusion reactions [HTRs] when incompatible blood was transfused), and 2) determine what percentage of 251 unusual alloantibodies (most to high-incidence antigens) were predicted to be clinically significant.
RESULTS: Two MMA cut points (5% and 20%) were chosen using a receiver-operating characteristics curve. No patients with MMA results less than or equal to 5 percent had clinical signs of a reaction; one-third of patients with MMA results 5.1 to 20 percent versus two-thirds with results greater than 20 percent had clinical signs of a HTR after transfusion of incompatible blood. Using 5-percent or 20-percent cut points, 173 (69%) or 97 (39%) of 251 unusual alloantibodies gave positive MMAs, respectively.
CONCLUSION: A negative MMA (≤5%) indicates that incompatible blood can be given without risk of an overt HTR but does not guarantee normal long-term survival of those RBCs. Most unusual alloantibodies are predicted to cause shortened RBC survival, but transfusion of incompatible blood may not result in any clinical or laboratory signs of a HTR. We have used the MMA for approximately 20 years, instead of a 1-hour chromium-51 RBC survival, to aid in the decision to transfuse RBCs incompatible with antibodies to high-incidence antigens.

Alloantibodies to red blood cell (RBC) antigens are usually categorized into three groups as far as clinical significance is concerned: those that are usually clinically significant (e.g., antibodies to antigens in the Rh, Kell, Kidd, and Duffy systems), those that are usually clinically insignificant (e.g., antibodies that do not react at 37°C), and those that have variable clinical significance (e.g., anti-Yt^a, -Ge, -Lu^b).[1,2] Because blood lacking the putative antigens for these latter specificities ("antigen negative") is rare and should not be wasted, it is important to try to determine which patients require "antigen-negative" blood to avoid an acute hemolytic transfusion reaction (HTR). RBC survival studies using radioisotopes are one option, but they are difficult to get performed and most nuclear medicine departments have little experience with RBC survival studies. As alternatives, cellular assays (e.g., the monocyte monolayer assay [MMA] and the chemiluminescence test), have been developed and used successfully by various investigators for this purpose.[3-9]

Two retrospective evaluations of data, generated over 20 years of using a MMA to predict the clinical significance of these types of alloantibodies, were carried out. One evaluation (Study I) determined the optimal MMA cutoff point based on clinical data (i.e., response to transfusion of incompatible blood). The other evaluation (Study II) determined the percentage of unusual alloantibodies

ABBREVIATIONS: HTR = hemolytic transfusion reaction; MMA = monocyte monolayer assay; ROC = receiver-operating characteristics.

From the Research Department, American Red Cross Blood Services, Southern California Region, Los Angeles, California.

Address reprint requests to: Patricia A. Arndt, MS, MT(ASCP)SBB, Senior Research Associate, American Red Cross Blood Services, 1130 S. Vermont Ave., Los Angeles, CA 90006; e-mail: arndtp@usa.redcross.org.

Received for publication December 18, 2003; revision received April 2, 2004, and accepted April 26, 2004.

TRANSFUSION 2004;44:1273-1281.

(mostly antibodies to high-incidence antigens) that appeared to be clinically significant by MMA; the immunoglobulin G (IgG) subclass of some of these antibodies was determined.

MATERIALS AND METHODS

Samples: Study I

The retrospective study to determine the appropriate MMA cutoff value involved analysis of results from 46 patients with unusual alloantibodies who had received incompatible blood. Twelve patients had received chromium-51 (^{51}Cr)-labeled aliquots, 29 patients had been transfused with one or more whole units of incompatible blood, and five patients had received both a ^{51}Cr-labeled aliquot and one or more units of blood. The antibodies studied were 11 anti-Yt^a; 6 anti-Ge; 4 anti-Lan; 4 Lutheran antibodies; two each anti-At^a, -Hy, and –JMH; one each anti-Co^b, -Cr^a, -Di^b, -Do^b, -Gy^a, -hr^B, -In^b, -Jo^a, -Js^b, -K11, -MiIII, -U, -Yk^a, and -Yt^b; and one unidentified alloantibody. An anti-Yt^a from one patient provided two MMA data points: one data point was associated with transfusion of incompatible blood and the second with a ^{51}Cr survival study (see Table 1).

Results associated with 11 of these antibodies were published in the study by Nance et al.,[7] and 8 were included in the study by Hadley et al.[9] In addition, information on 13 antibodies were published as case reports.[10-22] Information about clinical or laboratory signs of a reaction to transfusion of incompatible blood were obtained from the cited case reports[10-22] and/or the submitting institutions. Because there was no systematic checklist or review of the patients' medical records, it is possible that mild cases of hemolysis could have been missed. Overt cases of hemolysis (those that we are trying to avoid by performing the MMA) should not have been missed. Clinical signs of a transfusion reaction included jaundice, fever, chills, change in blood pressure, back pain, vomiting, tachypnea, and hemoglobinuria. Laboratory signs included a falling hemoglobin (Hb)/hematocrit (Hct) level, rising bilirubin or lactate dehydrogenase, falling haptoglobin, hemoglobinemia, and/or the presence of bilirubin in the urine.

Samples: Study II

Samples used to determine the percentage of unusual alloantibodies with a positive MMA were from 251 patients who required transfusion and/or were pregnant (these included 45 antibodies from Study I). Antibody specificities included 73 anti-Yt^a, 27 anti-Ge, 19 anti-Lu^b, 14 anti-Jr^a, and other antibodies listed in Table 2. In the majority of these cases, we did not have clinical follow-up to know if the patient ever received "antigen positive" blood, and if so, how the transfusion was tolerated. After the cutpoint was selected from Study I, MMAs were performed on eight patients who received antigen positive blood and who had some clinical or laboratory follow-up. Two of these patients were recently reported.[23]

MMA: Studies I and II

The MMA was performed as previously described.[4,7] Briefly, mononuclear cells from normal volunteer donors were separated by centrifugation over a Ficoll-sodium diatrizoate density gradient (Ficoll-Paque, Amersham Biosciences, Uppsala, Sweden). The mononuclear cells were washed with phosphate-buffered saline, suspended in culture media (RPMI Medium 1640, Gibco/Invitrogen Life Technologies, Carlsbad, CA) containing 5-percent fetal calf serum (Gibco/Invitrogen), and added to 8-well tissue culture chamber slides (Nalge Nunc, Naperville, IL). After a 1-hour incubation at 37°C (in ambient air), the supernatant containing nonadherent lymphocytes was removed via pipette, and sensitized RBCs (patient's serum [or plasma when serum was not available] + antigen-positive or antigen-negative RBCs ± fresh normal serum as a source of complement; incubated 60 min at 37°C with no additive; washed) were added. After another 1-hour incubation at 37°C (in ambient air), the nonadherent RBCs were removed, first by removal of the supernatant via pipette and then by dipping the microscope slides in phosphate-buffered saline.

TABLE 1. Examples of alloantibodies studied to determine the appropriate cutpoint for the MMA in Study I

Anti-	IAT	MMA result (% reactivity)	Response to transfusion of incompatible RBCs
Di^b	2$^1/_2$+	5.5	• Transfused three Di(b+) units; no clinical reaction but 6 days later Hb level dropped, bilirubin increased.[22]
Yt^a	1$^1/_2$+	10.8	• ^{51}Cr study = 100% @1 hr, 95% @24 hr; T_{50}Cr = 14 days (normal ≅ 28-32 days). • Transfused 15 least incompatible units; no signs of clinical reaction; bilirubin and lactate dehydrogenase values were unchanged.
Yt^a	1$^1/_2$+	0	• Transfused two Yt(a+) units two months after first MMA; no clinical reaction noted.[7]
	1+	16	• MMA repeated 5 months after transfusion of Yt(a+) RBCs; ^{51}Cr study = 80% @1 hr, <5% @24 hr; T_{50}Cr = 5 hr.[7]
Lu8	1$^1/_2$+	12-65	• Transfused three incompatible units → immediate transfusion reaction (temperature and blood pressure increased), Hb level dropped, bilirubin increased, urine bilirubin reported as "positive."[16]

TABLE 2. MMA results of 251 unusual alloantibodies

System	Anti-	Number tested	Number (%) positive (>5% reactivity)	Number of positives with strength of reactivity 5.1-20%	>20%
Cartwright	Yt^a	73*	47 (64)	28	19
	Yt^b	2	2 (100)	1	1
Chido/Rodgers	Ch	4	4 (100)	2	2
Colton	Co^a	4*	2 (50)	0	2
	Co^b	1	1 (100)	1	0
Cromer	Cr^a	5	5 (100)	2	3
	Es^a	1	1 (100)	0	1
Diego	Di^a	1	1 (100)	0	1
	Di^b	4	4 (100)	1	3
	Wr^a	4*	2 (50)	2	0
Dombrock	Do^a	1	0 (0)	0	0
	Do^b	3	1 (33)	0	1
	Gy^a	6*	4 (67)	2	2
	Hy	8†	5 (63)	5	0
	Jo^a	5	4 (80)	3	1
Er	Er^a	1	0 (0)	0	0
Gerbich	Ge	27*	16 (59)	8	8
Globoside	P	1	1 (100)	0	1
	PP$_1$P^k	2	2 (100)	0	2
Hh	H	1	1 (100)	1	0
HLA	Bg^a (HLA-B7)	1	1 (100)	0	1
Indian	In^b	3	2 (67)	2	0
John Milton Hagen	JMH	4	2 (50)	1	1
Kell	Js^b	3	1 (33)	0	1
	Kp^b	4*	3 (75)	2	1
	K11	1	1 (100)	0	1
	Ku	1	1 (100)	0	1
Kidd	Jk3	1	1 (100)	0	1
Knops	Yk^a	3	1 (33)	1	0
	Kn^a	3*	2 (67)	2	0
	Kn/McC	5*	2 (40)	1	1
Landsteiner-Wiener	LW	1	1 (100)	0	1
Lutheran	Lu^a	2	2 (100)	0	2
	Lu^b	19	15 (79)	2	13
	Lu3	3	2 (67)	1	1
	Lu8	1	1 (100)	1	0
	Lu12	1	1 (100)	1	0
MNS	“MiIII”	1	1 (100)	0	1
	U	3	3 (100)	0	3
Rh	hr^B	1	1 (100)	0	1
	Rh29	1	1 (100)	0	1
	Go^a	1	1 (100)	1	0
Scianna	Sc1	1	1 (100)	0	1
Xg	Xg^a	3	3 (100)	1	2
Independent	AnWj	1	1 (100)	1	0
	At^a	3	3 (100)	0	3
	Jr^a	14*	5 (36)	3	2
	Lan	7	6 (86)	0	6
	Vel	5	5 (83)	0	5
Total		251	173 (69)	76	97

* One antibody of this specificity (two anti-Jr^a) was not tested in the presence of fresh normal serum (as a source of complement) and gave negative results by MMA.

† One antibody of this specificity was not tested without the addition of fresh normal serum (as a source of complement) and gave negative results by MMA.

The slides were stained with a Wright-Giemsa stain and observed microscopically. Two hundred to 600 monocytes were counted, and the percentage of reactive monocytes (i.e., monocytes with RBCs adhering and/or phagocytized) was determined. A cutoff of 3-percent monocyte reactivity, based on reactivity of unsensitized RBCs in the MMA (mean + 3 standard deviations < 3%), had been used for many years to determine a positive versus negative assay. The objective of Study I was to determine if this cutoff was still appropriate and, if not, what the new cutoff value should be (based on clinical data rather than results with unsensitized RBCs).

Positive and negative controls were performed with each MMA. The positive control consisted of a 1 in 500

dilution of an IgG1 + IgG3 anti-D (kindly provided by Professor C. P. Engelfriet, the Netherlands) that reacted 3+ by antiglobulin test and gave greater than 10-percent reactivity by MMA. If the positive control reacted less than 10 percent, any negative results were considered invalid. Negative controls included patient's serum plus antigen-negative RBCs, test RBCs without patient's serum added, and a monocyte control where no RBCs were added to the monolayer.

For almost all patients' samples, the MMA test was performed twice: with and without fresh normal serum as a source of complement added during the RBC sensitization step. The higher of these two results was used as the final result. For some patients, multiple samples (i.e., from different dates) were tested by MMA. In Study I, the MMA result from the sample that correlated temporally with the RBC survival study and/or transfusion of incompatible blood was used. A pretransfusion sample was not always available (e.g., for 9 out of 29 patients). The use of post-transfusion samples in these nine cases could have resulted in possible errors in this study, if the antibody changed its characteristics after transfusion.[24,25]

In Study II, the highest MMA result was used. For some patients, there was no significant difference seen in the MMA results of different samples. In others, the antibody appeared to change its characteristics (e.g., after transfusion of incompatible blood) and the MMA result changed from negative to positive or became more strongly positive. Sometimes the MMA result changed from positive to negative (e.g., if the tested sample was drawn soon after transfusion of incompatible blood and most of the free serum antibody was adsorbed onto the transfused RBCs).

RBC survival studies: Study I

RBC survival studies using ^{51}Cr radioisotope were performed by the institutions submitting the samples for an MMA. Results were expressed as the percentage of cells surviving at 1 hour and/or 24 hours, and/or as the time taken for RBC survival to fall to 50 percent of its original value ($T_{50}Cr$).

IgG subclassing: Study II

IgG subclassing was performed by a capillary method as previously described.[26] Briefly, equal volumes of a 1 in 10 dilution of anti-IgG1, -IgG2, -IgG3, -IgG4 (Central Laboratory of the Netherlands Red Cross, Amsterdam, the Netherlands), or 6-percent albumin and 50-percent (vol/vol) sensitized RBCs were drawn into 0.4 × 90-mm capillary tubes (Diagnostic Technology, Great Neck, NY). Filled capillary tubes were inverted, and the end of the capillary containing antiserum was inserted into a clay holder at a 45° angle. Reactions (agglutination) were read with a hand lens, and results were documented after each of three 15-minute "trips" at room temperature of the RBCs through the antisera in the capillary tubes. Results were not included if the 6-percent albumin control showed agglutination or if none of the subclassing antisera were reactive.

Statistical analysis: Study I

The cutpoints for the MMA were chosen based on the analysis of a receiver-operating characteristics (ROC) curve (Fig. 1).[27-29] In general, the more sensitive a test is (able to detect true positives), the less specific it becomes (resulting in a number of false positives) and vice versa. This inverse relationship has been characterized as a "trade off" between sensitivity and specificity.[29] This relationship can be displayed graphically on an ROC curve by showing how sensitivity varies with specificity. On the ROC curve, the y-axis represents sensitivity (the proportion of true positives) and the x-axis represents 1-specificity (the proportion of false positives) for different cut points. Sensitivity and specificity (based on true positives, true negatives, false positives, and false negatives) were determined as described by Galen and Gambino.[30] Sensitivity equals the number of true positives divided by the sum of true positives and false negatives, specificity equals true negatives divided by the sum of false positives

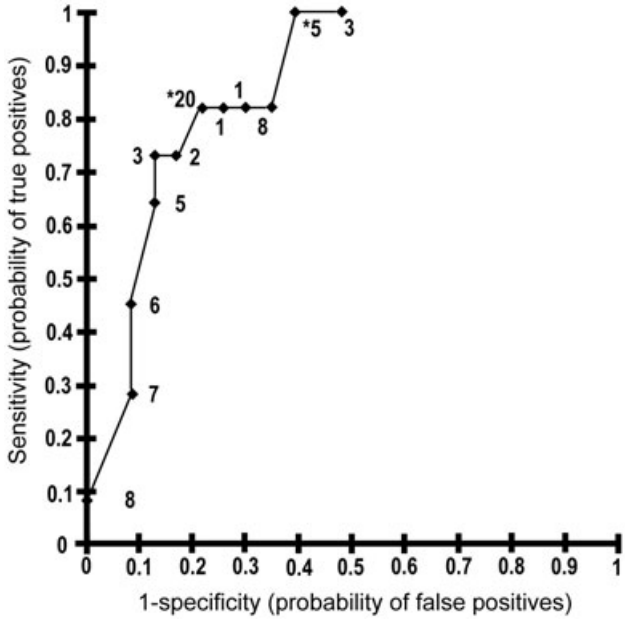

Fig. 1. ROC curve based on MMA results of 34 antibodies and the presence or absence of a clinical reaction to transfusion of incompatible blood. The curve shows 1-specificity versus sensitivity for different MMA cut points. Two MMA cut points, 5 percent and 20 percent (indicated by asterisks), were selected based on this ROC curve.

and true negatives, and 1-specificity equals false positives divided by the sum of false positives and true negatives.

In general, the best cutoff for balancing sensitivity and specificity of a test is the one represented by the point on the ROC curve closest to the upper left-hand corner (the most true positives and the least false positives). But the upper left-hand cut point is best only if the patient might suffer equally from a false-positive or a false-negative labeling. If the alloantibody is directed against a high-incidence antigen, a false-positive MMA would lead to unnecessary use of rare antigen-negative blood (which would then not be available for another patient with a clinically significant antibody). A false-negative MMA would result in a patient having an increased chance of a clinical transfusion reaction if the patient received transfusion of incompatible blood.

RESULTS

Study I: retrospective study to determine the appropriate MMA cut point

Examples of results (MMA and response to transfusion of incompatible blood) for four antibodies are given in Table 1. Fig. 2 shows the MMA (% reactivity) results plotted against the presence or absence of clinical signs or only laboratory signs of a transfusion reaction. Fig. 1 shows the ROC curve that was generated based on the presence or absence of a clinical reaction to transfusion using 12 different cut points (3%, 5%, 8%, 10%, 15%, 20%, 25%, 35%, 50%, 60%, 70%, 80%) of monocyte reactivity. The y-axis represents sensitivity (e.g., positive MMA results and clinical reactions occurred) and the x-axis represents 1-specificity (e.g., positive MMA results and no clinical reactions). As examples, a 3-percent MMA cut point would provide 100-percent sensitivity (no false negatives) but only 52-percent specificity (a high number of false positives). In contrast, an 80-percent MMA cut point would provide 100-percent specificity (no false positives) but only 9-percent sensitivity (a high number of false negatives).

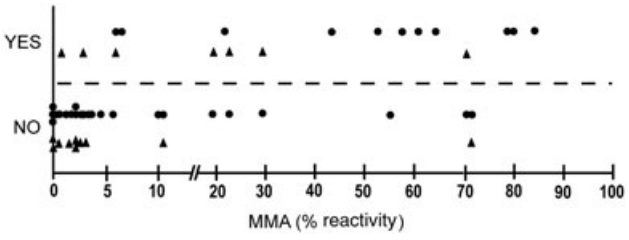

Fig. 2. MMA results (% reactivity of monocytes) versus presence (YES) or absence (NO) of a clinical reaction (●) (34 antibodies) or only laboratory signs of a reaction (▲) (17 antibodies). Clinical signs of a reaction included jaundice, fever, chills, blood pressure change, back pain, vomiting, tachypnea, and hemoglobinuria. Laboratory signs included decreased Hb/Hct level, increased bilirubin, increased lactate dehydrogenase, decreased haptoglobin, hemoglobinemia, and presence of bilirubin in urine.

Figure 3 shows the ROC curves that were generated based on ^{51}Cr survival study results at 1 hour and 24 hours. The overall accuracy of a test is conveyed by the area under the ROC curve.[29] Thus, based on the ROC curves in Fig. 3, the MMA appears to have better accuracy when compared to the 1-hour ^{51}Cr results than the 24-hour ^{51}Cr results. The best cut point on the curve comparing MMA versus the 1-hour ^{51}Cr survival study results was 20 percent because it gave a sensitivity of 100 percent (no false negatives) and a higher specificity than the 3- to 15-percent cut points (the least number of false positives).

Two cut points for the MMA were selected based on the ROC curve that compared MMA results with clinical reactions: 5 percent because it gave a sensitivity of 100 percent (no false negatives) and a higher specificity than the 3-percent cut point (61% vs. 52%) and 20 percent because it gave the best balance between true positives and false positives. Table 3 shows the results seen using the 5-percent and 20-percent cut points compared to ^{51}Cr results and/or the results of transfusing units of incompatible blood. Interpretation of the results in Table 3 depends on how one defines clinical "significance" (Table 4). The

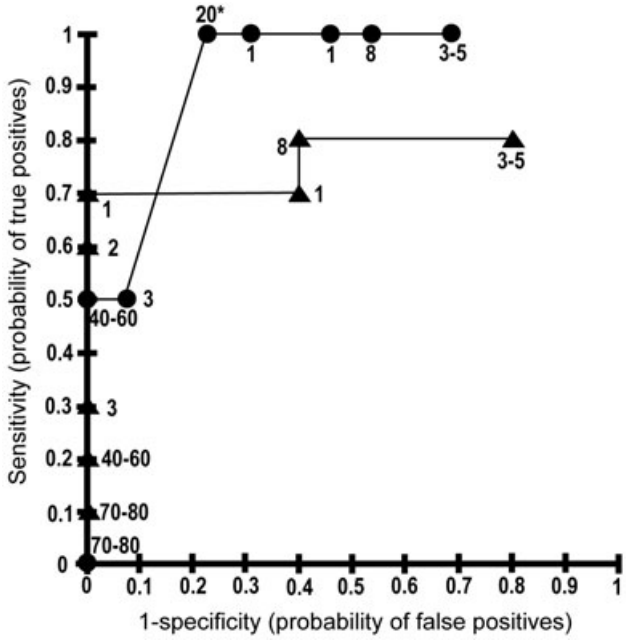

Fig. 3. ROC curves based on MMA results of 17 antibodies and normal (>70%) versus abnormal (<70%) results of 1-hour (●) or 24-hour (▲) ^{51}Cr survival studies. The curves show 1-specificity versus sensitivity for different MMA cut points. The best MMA cut point, based on the 1-hour ^{51}Cr survival study results, was 20 percent (indicated by an asterisk).

TABLE 3. Results of response to transfusion of incompatible blood (whole units and/or ^{51}Cr-labeled aliquots) using two MMA cutpoints: 5 percent and 20 percent

MMA* % reactivity	Number	1-hr ^{51}Cr >70% survival (number/total [%])	24-hr ^{51}Cr >70% survival (number/total [%])	T_{50}Cr normal survival (number [%])	Transfusion reaction? Clinical signs (number [%])	Transfusion reaction? Lab signs only* (number [%])
0-5	18	4/4 (100)	1/3 (33)	0/1 (0)	0/14 (0)	2/10 (20)
5.1-20	10	6/6 (100)	4/6 (67)	0/5 (0)	2/6 (33)	2/3 (67)
>20	19	3/5 (60)	0/6 (0)	0/6 (0)	9/14 (64)	3/4 (75)
Total	47	13/15 (87)	5/15 (33)	0/12 (0)	11/34 (32)	7/17 (41)

* These data refer to those patients with no clinical signs of a reaction who had laboratory data available. For example, of the 10 patients with MMA results of 5.1 to 20 percent, six had information about clinical signs. Of those six, two had clinical signs of a transfusion reaction, and four did not. Of the four that did not have clinical signs of a reaction, three had laboratory data available. Of those three with laboratory data, two had lab signs of a transfusion reaction, and one did not.

TABLE 4. Interpretation of MMA results based on definition of "significance"

A. If "significance" = abnormal RBC survival:
- MMA may not predict abnormal T_{50}Cr.
- Most antibodies (reacting by antiglobulin test) to high-incidence antigens would be classified as clinically significant.

B. If "significance" = laboratory signs, without clinical signs, of a reaction:

If MMA =	Then:
≤5%	20% had only laboratory signs of a reaction.
5.1-20%	67% had only laboratory signs.
>20%	75% had only laboratory signs.

C. If "significance" = a clinically obvious reaction:

If MMA =	Then:
≤5%	Incompatible blood could be given with little risk.
5.1-20%	33% of patients had clinical signs of a reaction.
>20%	64% of patients had clinical signs of reaction.

following statement is currently included when reporting our MMA results: "only results greater than 5 percent are associated with clinically obvious transfusion reactions in patients who receive incompatible RBCs (one-third of patients with MMA results 5.1-20% vs. two-thirds of patients with MMA results greater than 20% had clinical signs of a transfusion reaction when they were transfused incompatible RBCs). An MMA result of less than 5 percent does not, however, guarantee normal long-term survival of incompatible RBCs."

Study II: retrospective study to determine the percentage of unusual alloantibodies with a positive MMA

The results of studying 251 unusual alloantibodies by MMA are shown in Table 2. A total of 173 alloantibodies (69%) gave MMA results greater than 5-percent reactivity, and 97 (38%) gave MMA results greater than 20 percent. If a 3-percent cut point had been used, there would have been 13 more positive results. Only four specificities had a reasonably large number (n = 14-73) of samples tested; these were anti-Yt^a, anti-Ge, anti-Lu^b, and anti-Jr^a. The majority (59-79%) of the first three specificities were positive by the MMA using the 5-percent cut-point, whereas only about one-third of anti-Jr^a were positive. Other specificities had only eight or fewer examples tested; therefore, it is more difficult to state (based on these data) whether those specificities can be considered as usually being clinically significant or not.

Ten antibodies that gave negative MMA results were not tested in the presence of fresh normal serum; one antibody that gave a negative result was only tested in the presence of fresh normal serum (see Table 2). We had previously shown that some antibodies only give positive MMA results in the presence of fresh normal serum (as a source of complement) and vice versa.[31] Thus, these 11 results could possibly be false negatives (especially the five results with anti-Co^a, anti-Ge, anti-Jr^a (2), and anti-Yt^a, specificities, which are known to bind complement).

IgG subclassing results of 90 antibodies are shown in Table 5. Most of these antibodies were reactive with only one subclassing antisera; a few were reactive with more than one subclassing antisera. Most antibodies, including anti-Yt^a (previously reported to be primarily IgG4[32]), had an IgG1 component; the exceptions were anti-Di^b (3/4 were IgG3) and anti-JMH (5/7 were IgG4).

After the 5-percent cut point was selected in Study I, 10 samples from 8 patients, with anti-Yt^a(3), -Jr^a(3), -Ge3, and -Lu^b, who were transfused antigen-positive blood and had some follow-up, were studied. Three samples had negative MMAs; none were associated with a clinical reaction or laboratory signs of a reaction after transfusion. Of seven positive MMA results, two were associated with clinical signs of a reaction, three were associated with laboratory signs only, and two appeared to be false positives.

DISCUSSION

Analysis of our MMA results using an ROC curve helped us to set a new appropriate cut point (5%). Our previous

TABLE 5. IgG subclassing results for 90 unusual alloantibodies

System	Anti-	Number tested	Number reactive with only one subclassing antiserum (number reactive with more than one subclassing antiserum) IgG1	IgG2	IgG3	IgG4
Cartwright	Yt^a	30	27 (0)	0 (0)	0 (0)	3 (0)
Colton	Co^a	1	1 (0)	0 (0)	0 (0)	0 (0)
	Co^b	1	1 (0)	0 (0)	0 (0)	0 (0)
Diego	Di^a	1	0 (0)	0 (0)	1 (0)	0 (0)
	Di^b	4	1 (0)	0 (0)	3 (0)	0 (0)
	Wr^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Dombrock	Gy^a	5	2 (1)	1 (0)	0 (0)	1 (1)
	Hy	3	2 (0)	0 (0)	0 (0)	1 (0)
	Jo^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Gerbich	Ge	14	11 (3)	0 (2)	0 (3)	0 (1)
Globoside	P	1	0 (1)	0 (0)	0 (1)	0 (0)
Indian	In^b	2	2 (0)	0 (0)	0 (0)	0 (0)
John Milton Hagen	JMH*	2	1 (0)	0 (0)	0 (0)	1 (0)
Kell	Js^b	1	1 (0)	0 (0)	0 (0)	0 (0)
	Kp^b	3	3 (0)	0 (0)	0 (0)	0 (0)
Knops	Yk^a	1	0 (0)	0 (0)	0 (0)	1 (0)
Lutheran	Lu^b	4	3 (1)	0 (1)	0 (1)	0 (0)
	Lu3	2	0 (2)	0 (1)	0 (1)	0 (1)
	Lu8	1	1 (0)	0 (0)	0 (0)	0 (0)
MNS	U	3	1 (2)	0 (1)	0 (2)	0 (2)
Rh	Rh29	1	1 (0)	0 (0)	0 (0)	0 (0)
	Go^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Independent	At^a	2	1 (1)	0 (0)	0 (1)	0 (1)
	Lan	3	0 (2)	0 (0)	1 (2)	0 (0)
	Vel	2	1 (1)	0 (0)	0 (1)	0 (0)
Total		90	63 (14)	1 (5)	5 (12)	7 (6)

* Results on five additional anti-JMH (not tested by MMA): 1 = IgG1, 4 = IgG4.

cut point of 3 percent had been based on the mean + 3 standard deviations of results with normal, unsensitized RBCs, not on data about clinical response to transfusion of incompatible RBCs.[7] The 3-percent cut point, like the 5-percent cut point, gave 100-percent sensitivity, but the 3-percent cut point gave a slightly increased number of false positives as compared to the 5-percent cut point. Thus, the 5-percent cut point was felt to be a better choice.

Most of the 251 unusual alloantibodies, the majority of which were to high-incidence antigens, tested by MMA, gave results that indicated that they had the potential to be clinically significant (e.g., 79% of anti-Lu^b, 64% of anti-Yt^a, and 59% of anti-Ge gave MMA results that were >5% reactivity). The results with anti-Jr^a were surprising because only 5 of 14 (36%) antibodies gave a positive MMA; the literature would suggest that this antibody is usually associated with severe transfusion reactions, but perhaps these are the only case reports that get published. Our result with the one example of anti-Bg^a (indirect antiglobulin test [IAT] = 2 + and MMA = 61% reactivity vs. a pool of commercial Bg[a+] RBCs) is of special interest because Bg antibodies are antibodies to HLA, and recent publications drew attention to the possibility that HLA antibodies may be capable of causing HTRs.[33]

Interpretation of MMA results depends upon the desired outcome of the transfusion. None of the 12 long-term (T_{50}Cr) RBC survival study results in patients with antibodies to high-incidence antigens were normal (Tables 3 and 6); MMA results generally correlated well with the T_{50}Cr results in these 12 patients. Abnormal RBC survival was associated with some antibodies that immunohematologists usually consider benign; our results correlate with data published by Baldwin et al.[6] These investigators studied five patients with antibodies to high-incidence antigens. MMA and 1-hour ^{51}Cr RBC survival

TABLE 6. T_{50}Cr results of 12 antibodies from Study I. Results are given in order of increasing MMA % reactivity

Anti-	IgG IAT	MMA (% reactivity)	^{51}Cr survival study 1 hr (%)	24 hr (%)	T_{50}Cr*
In^b	1+	2.1	97	62	<4 days
Co^b	2+	6.3	95	80	4 days
Yt^b	1$^1/_2$+	7.2	100	93	21 days
Lu12	1+	8.8	85	50	1 day
Yt^a	1$^1/_2$+	10.8	100	95	14 days
Ge	3$^1/_2$+	19	71	4	<1 day
Yt^a	1+	23	80	<5	5 hr
JMH	2+	24.7	95	41	<1 day
Lan	1+	26.5	NA	NA	10 min
At^a	3+	35	95	18	<1 day
Lan	1$^1/_2$+	66.5	16	7	<1 hr
At^a	3+	84	NA	1.7	35 min

* Normal range = 25 to 37 days.[34]
NA = not available.

studies suggested the patients could be transfused with incompatible blood, but long-term T_{50}Cr studies showed that three of four antibodies usually considered clinically insignificant (anti-McC^a [2], -JMH [1]) caused shortened RBC survival. Nevertheless, when transfused with incompatible units of RBCs, all five patients had the expected rise in Hct and showed no signs of an HTR.

The above data illustrates the problems in deciding on a definition of a clinically significant antibody. A "clinically significant" antibody to some means one that causes decreased RBC survival, and others are satisfied that the transfused incompatible RBCs do not cause an observable reaction (e.g., laboratory signs and/or clinical signs). The AABB Standards defines a clinically significant antibody as one that causes decreased RBC survival.[35] We believe that this definition is too conservative. For patients with relatively normal marrow function (i.e., most transfused patients), it may not be important for transfused RBCs to have perfectly normal survival, but it may be advisable to use "antigen-negative" blood for patients whose marrow is not functioning normally (i.e., optimal RBC survival is important). A pretransfusion estimation of clinical significance (e.g., MMA or 1-hr ^{51}Cr survival study) will have little value if normal RBC survival and no signs of an HTR (clinical or laboratory) are required; our results show that antigen-negative RBCs will have to be transfused. This approach is expensive and wastes blood of rare phenotypes that is needed for patients with alloantibodies to high-incidence antigens that are known to have an abnormal 1-hour or 24-hour ^{51}Cr RBC survival or MMA result.

A positive MMA will sometimes not be associated with signs of an HTR (45% [9/20] with MMA >5% had no clinical signs, and 29% [2/7] of these had no laboratory signs of a transfusion reaction) even if RBC survival (T_{50}Cr) is abnormal. Nevertheless, antigen-negative units should be obtained in these cases if possible. The physician should be counseled (using the data shown here, summarized in Table 3) so that the risk of waiting for the expensive, rare, antigen-negative blood can be balanced with the clinical significance of the antibody. It is important that the clinician be made aware of the various interpretations of the term "clinically significant" (i.e., ranging from abnormal RBC survival to a clinically obvious reaction; see Table 4).

Guidelines of the UK National Blood Service,[36] based on the review by Daniels et al.[37] recommend serologically least incompatible RBCs for patients with anti-Ch/Rg, -JMH, -Er^a, and for Gerbich and Knops antibodies. They also recommend serologically least incompatible RBCs for patients with anti-Yt^a, -Gy^a, -Hy, -Jo^a, -In^b, -Lan, -At^a, -Jr^a, and for Cromer and Lutheran (i.e., antibodies to high-incidence antigens in the Lutheran system, except Lu^b and Lu3) antibodies, but antigen-negative RBCs for strong examples of any of those antibodies. A more conservative opinion was expressed by Seltsam et al.,[38] who recently reviewed transfusion support in Germany, Switzerland, and Austria of patients with antibodies to high-incidence antigens. Twenty-three (41%) of 56 patients with antibodies to high-incidence antigens were transfused antigen-incompatible units; five (22%) delayed HTRs were reported based on laboratory signs only but none of the five patients were reported to have "negative effects" due to the hemolysis. Seltsam et al.[38] concluded that transfusion support had been unsatisfactory for the 23 patients who received incompatible blood.

In conclusion, for approximately 20 years we have used the MMA, instead of a 1-hour ^{51}Cr RBC survival, to aid in the decision to transfuse RBCs incompatible with antibodies to high-incidence antigens. We believe that this approach is safe and preferable to the more conservative approach of searching for rare "antigen-negative" units for all patients with alloantibodies to high-incidence antigens. If the MMA or equivalent assays (e.g., chemiluminescence test or ^{51}Cr RBC survival studies) are not available or time does not allow, then we would support the UK Guidelines[36] as a compromise approach.

ACKNOWLEDGMENTS

We would like to acknowledge Sandra Nance MS, MT(ASCP)SBB, for performing many of the MMAs in this study.

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