Inaccurate Doses of Rh Immune Globulin After Rh-Incompatible Fetomaternal Hemorrhage

Survey of Laboratory Practice

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・Context.—Rh(D)-negative women with a large fetomaternal hemorrhage (FMH) from an Rh(D)-positive fetus are at risk for anti-D alloimmunization if they do not receive adequate Rh immune globulin (RhIG). Determination of the adequate RhIG dose for these women is a critical laboratory procedure for protecting their future Rh(D)-positive children.

Objective.—To determine how often laboratories recommended an inaccurate dose of RhIG for excess FMH.

Design.—Nearly 1600 laboratories using the College of American Pathologists’ proficiency testing for fetal red blood cell detection were surveyed to determine (1) their calculation method and (2) the number of RhIG doses recommended for a survey specimen, based on their measured percentage of fetal red blood cells. We surveyed nearly 1450 laboratories for their accuracy in determining RhIG dose, using 2 common calculation methods we provided.

Results.—The AABB Technical Manual method was used by 67% of responding laboratories. However, 20.7% of laboratories using this method would have recommended an inaccurate dose of RhIG—11.5% too much and 9.2% too little—for the level of FMH reported in the survey specimen. If all laboratories had used the common recommendation of 300 μg/30 mL of fetal blood present, 2% would have recommended RhIG doses too low for the volume of FMH they measured. In 3 of the 4 calculation exercises we provided, 20% to 30% of laboratories underestimated the necessary dose of RhIG.

Conclusions.—Based on our surveys, some mothers with excess FMH may be receiving inaccurate doses of RhIG. Laboratories performing quantification of FMH should review their procedures and training for calculating RhIG dosage.

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Rh immune globulin (RhIG) provides prophylaxis against alloimmunization to the D blood group antigen in Rh(D)-negative patients who are exposed to Rh(D)-positive red blood cells (RBCs) by pregnancy or transfusion. Rh(D)-negative women are given RhIG in the early third trimester and again after delivery of an Rh(D)-positive infant, and also during pregnancy for events that may have associated fetomaternal hemorrhage (FMH), such as abdominal trauma, vaginal bleeding, ectopic pregnancy, fetal death, and invasive obstetric procedures.1 The presence of passive anti-D suppresses the patient’s own immune response. The mechanism of action is still uncertain, but the removal of Rh(D)-positive RBCs from the circulation by the antibody is an important component.2

A total of 20 μg RhIG is sufficient to protect against 1 mL Rh(D)-positive RBCs. In North America, the most common obstetric RhIG dose formulation contains 1500 IU (often expressed as 300 μg), enough to abrogate the alloimmunization risk from 15 mL RBCs (or about 30 mL of fetal whole blood). However, FMH greater than this volume occurs in 3 in 1000 deliveries, requiring more than 1 dose of RhIG for adequate prophylaxis.3 Guidelines have varied on whether to test for excess FMH after all Rh(D)-incompatible deliveries or only for those with clinical risk events. However, many episodes of excess FMH are not associated with a clinical risk event.4 Obstetric guidelines in the United States and United Kingdom5,6 call for routine testing, in agreement with the specific laboratory accreditation requirement of the College of American Pathologists (CAP) for routine FMH testing (checklist item TRM.40790),7 and the general requirement of the AABB (formerly the American Association of Blood Banks) for ensuring that an adequate RhIG dose is given after all Rh(D)-incompatible deliveries (standard 5.20.3).8

For editorial comment, see p 343.

Most US laboratories perform a qualitative screen for excess FMH using the fetal rosette test, followed, if positive, by a Kleihauer-Betke-type stain of the maternal blood smear to count the percentage of RBCs with fetal hemoglobin (HbF). Flow cytometry for RBCs containing HbF or Rh(D) antigen is used by about 4% of laboratories performing fetal RBC quantitation.9 The percentage of RBCs containing HbF present in the mother’s circulation is used to calculate the volume of Rh(D)-positive RBCs present,
whether more than 1 dose of RhIG is needed and, if so, the total number of doses. These dose calculations usually start with the assumptions of (1) an arbitrary maternal blood volume of 5000 mL and (2) coverage of 15 mL fetal RBCs or 30 mL fetal whole blood per 300-μg dose of RhIG. The AABB Technical Manual is more conservative than these assumptions "because of the inherent wide estimate of the test."11 To the remainder of the division of the fetal blood volume by 30 is 0.5 or greater, then the number of doses is rounded up to the next integer, and another dose is added (eg, 50 mL/30 = 1.7, so give 2 + 1 = 3 doses).

The purpose of our survey was to probe the accuracy of laboratory practice for determining the number of doses of RhIG that would be given after excess FMH and, in particular, to determine the frequency of potentially inaccurate doses of RhIG that would be recommended in this situation.

MATERIALS AND METHODS

The CAP proficiency testing program offers a survey for fetal RBC detection to laboratories that perform this test. Twice each year, 2 specimens are provided in each survey. Participants can screen the specimens with a fetal rosette test and also determine a flow cytometric technique. They also report the number of doses of RhIG that they would recommend giving to the mother based on their quantitation. Each dose in the survey and in our study questions is assumed to contain the common US formulation of 300 μg RhIG.

The CAP Transfusion Medicine Resource Committee wished to determine whether participating laboratories recommended sufficient RhIG for the volume of FMH they reported. We added supplementary questions about each laboratory’s FMH test method and RhIG dose calculation method to proficiency survey 2006 HBF-B. Using the results for specimen HBF-03 in that survey, 2 comparisons were made of FMH results to recommended RhIG doses. First, we examined the laboratories that used the AABB Technical Manual method for their calculation of RhIG dose. We extracted each laboratory’s reported percentage of fetal RBCs in the sample, calculated the RhIG dose for that percentage according to the Technical Manual method, and then compared the Technical Manual calculation with the number of doses which that laboratory had recommended in the survey’s results. The second comparison of FMH to RhIG dose was performed using the formula that each 300-μg dose of RhIG protects against 30 mL fetal whole blood in an assumed maternal blood volume of 5000 mL. We analyzed results with this method for all laboratories with available data, regardless of their own calculation technique.

In the next proficiency survey, 2007 HBF-A, we asked 4 supplementary questions to test the accuracy of RhIG dosage calculations by the participants.11 Two questions supplied the Technical Manual method to be applied, and 2 questions provided a detailed calculation method based on 30 mL fetal blood per dose in a 5000-mL maternal blood volume.

When the proficiency testing results were returned to the participating laboratories, the participant summaries included a discussion of the aggregate responses to the supplemental questions. Participants, therefore, had the opportunity to review the 2006 results before receiving the 2007 questions. The second survey’s introduction to the supplemental questions also referred to the previous results.

The replies from responding participant laboratories were compiled and analyzed as presented in “Results.” Statistical comparisons were performed using Pearson χ² test, or when values were less than 5, Fisher exact probability test as noted (2007 edition, VassarStats, Poughkeepsie, NY), with P < .05 considered significant.

RESULTS

In the first survey, 2 supplemental questions were added. Participants were asked for their routine method of quantifying fetal RBCs in postpartum maternal blood. Among 1393 laboratories responding, 73.8% used the Kleihauer-Betke or a similar HbF staining method, 3.8% used flow cytometry, 11.5% responded “other,” and 10.9% did not use the test for this purpose. They also were asked to choose their primary source for calculation of the number of doses (vials) of RhIG needed to be administered to treat the FMH. The AABB Technical Manual was cited for 66.8% of responding laboratories, the RhIG manufacturer’s directions by 10.7%, other published textbook or journal recommendations by 5.1%, and “other” by 17.4% (n = 1412). The answers to these 2 questions were correlated by laboratory to determine whether the test method affected the calculation method. The Kleihauer-Betke users followed the AABB Technical Manual in a similar proportion (70.4%) to the laboratories using flow cytometry (76.4%).

Specimen HBF-03 in this challenge had a target value of 1.4% for HbF staining methods and 1.0% for flow cytometry methods. (Flow cytometry routinely yields values averaging lower than manually counted staining.) The participating laboratories demonstrated good test performance with this specimen. The mean percentages of HbF reported were 1.28% to 1.34% (SD = 0.46–0.50) for the 2 most commonly used HbF staining reagents and 1.06% (SD = 0.19) for the flow cytometric results. (The results from the other specimen in this survey, HBF-04, were invalidated by the committee for aberrant results due to a problem in specimen production.)

Among 783 responding laboratories using the Technical Manual’s calculation method for specimen HBF-03, 72 (9.2%) recommended a dose of RhIG that was too low for the percentage of fetal RBCs they reported (Table 1). A total of 66 laboratory recommendations were 1 dose too low, 2 were 2 doses too low, and 4 were 3 or more doses too low. Laboratories reporting a higher level of FMH (≥21.1% fetal RBCs) were more likely to recommend too little RhIG, compared with those measuring a lower level of FMH (15.4% vs 8.5%, P = .046). Another 90 laboratories (11.5%) recommended too many doses for the volume of fetal RBCs they had measured.

The flow cytometry laboratories using the Technical Manual calculation had a much narrower range of results for the percentage of fetal hemoglobin (0.6%–1.4%) than the manual hemoglobin-staining laboratories (0.1%–12.0%), as reflected in the SDs above. However, 4 (15.4%) of 26 flow cytometry laboratories recommended doses that were 1 dose too low for their result, and 4 (15.4%) recommended too many doses. Their underdosage rate was statistically similar to that of the laboratories using manual staining (9.0%; P = .21 by 1-tailed Fisher test).

The second comparison of FMH to RhIG dose was performed by using the formula that each 300-μg dose of RhIG protects against 30 mL fetal whole blood, in an assumed maternal blood volume of 5000 mL. We analyzed results with this method for all 1200 laboratories with available data, regardless of their own calculation technique. Twenty-five laboratories (2.1%), all using manual staining, would have given too little RhIG for their measured hemorrhage based on this method.

We asked whether erroneous calculations contributed to the underdosings we observed. In the second survey, we
Results and discussions are provided to the participating laboratories with the goals of educating laboratory and medical personnel and improving patient care.

In this study, we examined accuracy of the calculated dose of RhIG for postpartum women with excessive FMH after delivering an Rh(D)-positive infant. Depending on the calculation method used, 2% to 9% of laboratories recommended a dose of RhIG that was too low for the volume of fetal RBCs they had measured in a proficiency testing specimen. We also found that part of this shortfall was due to inaccurate calculations. In 3 of 4 exercises in which the calculation method was clearly delineated for the participants to follow, about 20% to 30% of laboratories still recommended a dose of RhIG that was too low.

Our study also found that 11.5% of laboratories using the AABB Technical Manual method would have recommended too many vials of RhIG for the FMH level they reported. Overuse of RhIG would subject patients to extra biologic product exposures and expenses, and would unnecessarily consume a product which is especially complicated to make.

Failure to prevent Rh(D) alloimmunization despite post-

### Table 1. Laboratory Accuracy of Rh Immune Globulin (RhIG) Dose Calculation for Excess Fetomaternal Hemorrhage in a Proficiency Test Specimen*

<table>
<thead>
<tr>
<th>% Fetal RBCs Measured</th>
<th>Doses of RhIG from AABB Technical Manual Method</th>
<th>Laboratories Recommending, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correct Dose</td>
<td>Overdose</td>
</tr>
<tr>
<td>0.0–0.2</td>
<td>1</td>
<td>15 (88.2)</td>
</tr>
<tr>
<td>0.3–0.8</td>
<td>2</td>
<td>70 (86.4)</td>
</tr>
<tr>
<td>0.9–1.4</td>
<td>3</td>
<td>318 (78.3)</td>
</tr>
<tr>
<td>1.5–2.0</td>
<td>4</td>
<td>162 (80.6)</td>
</tr>
<tr>
<td>2.1–2.6</td>
<td>5</td>
<td>35 (76.1)</td>
</tr>
<tr>
<td>2.7–3.2</td>
<td>6</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>3.3–3.8</td>
<td>7</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>3.9–4.4</td>
<td>8</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>4.5–5.0</td>
<td>9</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>≥5.1</td>
<td>≥10</td>
<td>6 (75.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>621 (79.3)</td>
</tr>
</tbody>
</table>

* Laboratories participating in College of American Pathologists proficiency test 2006 HBF-B for fetal red blood cell (RBC) detection, which reported using the AABB Technical Manual method for calculating RhIG dose. The target value for this specimen was 1.4% for Kleihauer-Betke methods and 1.0% for flow cytometry. The number of doses from the Technical Manual for each laboratory’s reported percentage of fetal RBCs was compared with the laboratories’ recommendations for the number of doses to give.

**COMMENT**

College of American Pathologists proficiency tests are widely used in the United States and other countries to allow laboratories to determine the comparability of their results. Supplemental questions appended to these laboratory exercises have been a valuable source of information on laboratory and medical practices for many issues of interest in a broad cross section of health care facilities.

asked 4 supplemental questions to test the accuracy of the calculations of the participants. Two questions supplied the Technical Manual method to be applied, and two questions provided a detailed calculation method based on 30 mL fetal blood per dose in a 5000-mL maternal blood volume (Table 2). From 1442 to 1446 laboratories answered these questions. For 1 question, only 0.5% of participants submitted a low dose. However, in the other 3 questions, 21% to 30% of laboratories calculated too few doses of RhIG for the percentages of fetal RBCs given. Most of these responses were 1 dose too low. Including all responses, 62% to 77% of laboratories submitted the intended doses for the 4 questions.

### Table 2. Laboratory Accuracy of Rh Immune Globulin Dose Calculations Using 2 Specified Methods*

<table>
<thead>
<tr>
<th>Doses Recommended</th>
<th>Dose Calculated According to Method A†</th>
<th>Laboratories Recommending, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.2% Fetal RBCs</td>
<td>2.6% Fetal RBCs</td>
</tr>
<tr>
<td>1</td>
<td>7 (0.5)$</td>
<td>1 (0.1)$</td>
</tr>
<tr>
<td>2</td>
<td>1043 (72.1)$</td>
<td>5 (0.4)$</td>
</tr>
<tr>
<td>3</td>
<td>381 (26.4)$</td>
<td>10 (0.7)$</td>
</tr>
<tr>
<td>4</td>
<td>12 (0.8)</td>
<td>411 (28.5)$</td>
</tr>
<tr>
<td>5</td>
<td>3 (0.2)</td>
<td>893 (61.9)$</td>
</tr>
<tr>
<td>6</td>
<td>0 (0)</td>
<td>107 (7.4)</td>
</tr>
<tr>
<td>7</td>
<td>0 (0)</td>
<td>5 (0.4)</td>
</tr>
<tr>
<td>≥8</td>
<td>0 (0)</td>
<td>10 (0.7)</td>
</tr>
<tr>
<td>Total</td>
<td>1446 (100)</td>
<td>1442 (100)</td>
</tr>
</tbody>
</table>

* RBCs indicates red blood cells.
† Method A: Assume a maternal blood volume of 5000 mL and calculate the volume of fetal blood present. Give 1 dose per 30 mL fetal blood.
‡ Method B: Assume a maternal blood volume of 5000 mL and calculate the volume of fetal blood present. Divide by 30 mL per dose. If the fractional remainder is less than 0.5, round down and then add 1 vial. If the fractional remainder is 0.5 or more, round up to the next dose and add 1 dose.
§ Inadequate doses.
|| Intended responses.
partum prophylactic administration of RhG may be due to several causes. Certainly among these is biologic variability in immune response, but there are several systematic issues to be considered as well. These include failure to administer scheduled antenatal RhG at 28 weeks, treat FMH at the time of antenatal events that may have caused excess hemorrhage, test for excess FMH when these events occurred after 20 weeks of gestation (when the fetal blood volume begins to exceed 30 mL), seek excess postpartum FMH, and treat excess postpartum FMH when it is identified. In retrospective studies, some of these omissions may have been in documentation rather than performance, but documentation is also important in case of later alloimmunization. Our study adds to these problems the potential failure of the laboratory to recommend enough RhG when more than 1 dose is needed.

Although the amount of RhG needed to protect against a given volume of Rh(D)-positive RBCs is fairly precise and generally accepted in the United States (300 µg for 15 mL), there is no single universally recommended method for calculating RhG dose from the proportion of fetal RBCs detected in maternal blood. The AABB Technical Manual, the most widely used source in our survey, considers the degree of imprecision inherent in manually counting fetal RBCs in the Kleihauer-Betke-type method, and rounds up an additional dose when values are obtained with a remainder of 0.5 to near 1.0. Although flow cytometry is more precise than manual staining, and therefore perhaps rounding up is less necessary, most flow cytometry laboratories also used the Technical Manual calculation method in our survey.

The next most common calculation source in our survey was the RhG manufacturers’ instructions. Table 3 shows pertinent excerpts from the prescribing information of the 4 RhG manufacturers approved in the United States. The degree of detail in their directions is variable. One manufacturer refers to a mixed field positive D* (weak D) test result as an indication to assess the patient for a large FMH. Although this statement is true, a positive weak D test is an insensitive marker for excess FMH and should not be the sole screening criterion.

None of these methods for RhG dose calculation takes into account the mother’s actual blood volume (as derived from her size) within which the fetal RBCs are contained. For a mother more than 70 kg whose blood volume (70 mL/kg) is well above 5000 mL, the volume of fetal RBCs that the woman has is quite large. Technique to calculate the woman’s blood volume can be refined by entering the woman’s height and weight, as shown here.
present (and the level of RhIG achieved) could be substantially underestimated in these formulas.\textsuperscript{21} Corrections for maternal blood volume would make the calculations more complex and more prone to error. However, if a computerized algorithm were available to enter the desired variables and calculate the number of doses in a standardized way, this could reduce mathematical and treatment errors. The CAP Transfusion Medicine Resource Committee has developed an online algorithm in a spreadsheet that can be downloaded to assist in the calculation of RhIG for excess FMH (www.cap.org; Figure). Its formula is based on the AABB Technical Manual method, with maternal weight included if desired. Laboratories using this or other formulas should validate the calculations with a range of sample FMH levels before use to verify that results are consistent with their written procedures.

In conclusion, our study indicates that dosage recommendations for RhIG after excess FMH may be inaccurate in many cases. Laboratories performing quantification of FMH should review their procedures and training for calculating RhIG dose in order to avoid potentially preventable D alloimmunization in women at risk.

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References