Groups urge phase-in of *RHD* genotyping

Anne Paxton

October 2015—It may not be quite like boxing frogs or herding cats. But gaining broad consensus on a laboratory medicine practice can be difficult, especially where multiple organizations must agree. A new joint statement on *RHD* genotyping by the CAP and the AABB, plus four other organizations, shows that such consensus is possible, however, even where it involves a laboratory medicine practice in place for more than 50 years—especially when advances in molecular testing are offering a solution to a problem.

But gaining broad consensus on a laboratory medicine practice can be difficult, especially where multiple organizations must agree. A new joint statement on RHD genotyping by the CAP and the AABB, plus four other organizations, shows that such consensus is possible, however, even where it involves a laboratory medicine practice in place for more than 50 years—especially when advances in molecular testing are offering a solution to a problem.

The problem, in this case, is a lack of standard practice in the U.S. for laboratory testing and interpreting of the Rh blood type of patients, including pregnant women, with a weak D phenotype. Blood typing of persons with a weak D phenotype is "really unlike any other test we do in the laboratory," says Anne Eder, MD, PhD, vice president of national medical affairs for the American Red Cross.

As a 2014 CAP Survey revealed, some laboratories test blood samples from patients, including pregnant women, by methods intended to avoid detecting a weak D phenotype and thereby to interpret the result as Rh-negative. This practice, recommended by AABB standards since 1958, has been intended to protect Rh-negative persons, particularly Rh-negative women of childbearing potential, from inadvertent exposure and alloimmunization to Rh-positive red blood cells.

But *RHD* genotyping methods are now available that would make the practice obsolete. The CAP, the AABB, America's Blood Centers, the American Red Cross, the American College of Obstetricians and Gynecologists, and the Armed Services Blood Program agree on the need to bring more precise decision-making to obstetrical practice and transfusion medicine, and a workgroup consisting of representatives from these six organizations was organized by the CAP and the AABB to explore how *RHD* genotyping might help.

This year the workgroup announced that high-quality evidence from observational studies backed its main recommendation: *RHD* genotyping should be performed whenever a weak D phenotype is detected by routine Rh blood typing of pregnant women and other women of childbearing potential. Such genotyping should be phased in "as a feasible and appropriate first step to delivering specific benefits of molecular science to a well-defined and relatively limited number of patients," says a joint statement by the six groups. The data and arguments backing up the statement were published in the March 2015 issue of *Transfusion* (Sandler SG, et al. 2015;55:680–689).

The interdisciplinary effort leading to the joint statement represented a unique level of cooperation, says workgroup chair S. Gerald Sandler, MD, director of transfusion medicine and professor of medicine and pathology at MedStar Georgetown University Hospital. "I have no recollection of six national organizations endorsing a transfusion practice before."

Approximately 0.2–1.0 percent of Caucasians inherit a weak D phenotype, and under conventional laboratory practices of long standing, patients have been Rh-typed using methods that interpret weak D phenotypes as Rh-negative. But, as Dr. Eder explains, current practice is inconsistent. In most clinical laboratories, pregnant women are not tested for weak D.

Dr. Eder

“This is a conservative approach to avoid inadvertent exposure to D positive cells and the potential of alloimmunization and complications in pregnancy,” she says. “For blood donors, on the other hand, we use a test to detect weak D and call them ‘D positive.’ So the method selection is based on the circumstances, not on getting a real answer for the patient.” In fact, the CAP’s Survey showed that some labs conduct the test for weak D serological testing for women when they’re patients, but then, no matter what the result is, report it as Rh-negative, Dr. Eder notes.

“Unlike any other test in the laboratory, we’re saying we’re not even going to test you for weak D if you’re a patient; we’re going to manage you conservatively and just assume you’re Rh-negative when the woman is in the hospital, and we’re going to give you Rh immune globulin [RhIG] when you might not need it. It goes completely against the grain of a clinical mindset,” Dr. Eder says. “The upshot is a woman can be told she’s D positive when she’s a blood donor, and when she’s pregnant be told she’s D negative and needs Rh immune globulin.”

Even though only a small percentage of the population has a weak D phenotype, about 2.5 million women are screened for RhD every year. “So it’s not a small number of women.” And many of them will be given RhIG when they don’t need to receive it because they’re not at risk. “They’re weak D type 1, 2, or 3 so they can be managed safely as Rh-positive.”

Dr. Sandler, left, with Dr. Flegel, center, and Dr. Queenan. Dr. Sandler was chair of the AABB/CAP interorganizational workgroup. Dr. Flegel was among the original investigators who established the molecular basis for the RHD gene. Dr. Queenan

is an obstetrician-gynecologist who has focused on
Rh hemolytic disease of the fetus/newborn.

“A lot of people say there really is no harm in giving women Rh immune globulin because reactions are few,” Dr. Eder adds. “But the workgroup estimated if the recommendations were followed, then more than 13,000 women could be managed safely as D positive. That would avoid 24,700 doses of RhIG annually.”

Testing and interpretation are all over the board when it comes to RhD typing, agrees workgroup member Louis M. Katz, MD, chief medical officer of America’s Blood Centers. And as a result of erring on the side of safety, supplies of Rh-negative red blood cells can be hard hit. “I think it’s true that, when in doubt, clinicians will do more rather than less, but Rh-negative red blood cells are in very tight supply right now because of things like massive transfusion protocols in the emergency room. We’re at the point where we’re asking for more Rh-negative cells than are available.” Although the number of women affected by the genotyping recommendations appears relatively small, “our Rh-negative donor base is being stretched really thin, so every little bit helps.”

Dr. Sandler points to two important aspects of the Rh-negative supply problem. “One, there’s a seasonal shortage, in that a lot of elective surgery occurs over vacation periods, but fewer donors are donating at that time. The other thing is a lot of people are getting Rh-negative blood who don’t need it. The logistics of getting a blood sample collected, transported, identified as urgent, and confirmed as to type, and then getting an answer back to the trauma center are such that, in a lot of cases, it’s easier to just get a unit of O negative and move on.”

Performance characteristics of different laboratories’ testing methods are also an issue, says workgroup member Susan T. Johnson, MSTM, MT(ASCP)SBB, director of clinical education at BloodCenter of Wisconsin and a board member of the AABB. “Serologic testing is becoming automated, but there are still many labs that are doing manual testing, with its inherent subjectivity. We have many different anti-D reagents available, and each manufacturer meets the FDA requirements. But the requirements are based on serologic observations. The tests cannot differentiate variants identified by genotyping, so it’s not enough in this day and age, with everything we know.”

This interorganizational project on *RHD* genotyping arose in large part from CAP proficiency testing, explains Jerome L. Gottschall, MD, senior medical director of the BloodCenter of Wisconsin and chair of the CAP Transfusion Medicine Resource Committee. “The Survey asked how much Rh immune globulin would you give based on what was detected. Adequate Rh immune globulin is a separate issue from weak D phenotype policy, but the Survey generated a lot of interest, and as red cell genotyping has become available, it has allowed us to look at ways of determining whether a mother who has a weak D blood type truly needs Rh immune globulin,” Dr. Gottschall says.

Progress in technology for genotyping is certainly part of what made the interorganizational effort possible, he adds. “We’ve been detecting weak D in mothers for a long time, and with the advent of red cell genotyping and particularly of the *RHD*, we are able to determine variants of weak D that do not elicit an immune response and therefore the mother doesn’t need RhIG and can be treated as Rh-positive.”

“Giving a person RhIG when they don’t need it may seem like a somewhat minor issue, but there’s no person who would want any type of injection if they didn’t have to have it,” Dr. Gottschall says.

In a 2014 paper, the committee compared answers of 2012 Survey participants to answers that 1999 Survey participants gave to the same questions, which related to weak D phenotype testing policies and procedures. The comparison showed a decrease in the percentage of transfusion services performing a serological weak D test on patients as a strategy to manage those with a weak D as Rh-negative—from 58.2 percent to 19.8 percent. That led the committee to conclude that “selective integration” of *RHD* genotyping policies and practices might be advisable (Sandler SG, et al. *Arch Pathol Lab Med.* 2014; 138:620–625).

“That was the first step—a recognition by the committee that there wasn’t a standard way of technically typing for Rh at the bench and for interpreting the result for the serological weak D,” Dr. Sandler says. “The CAP’s

observation, together with the AABB's support for the group and joint appointment of the representatives, resulted in the AABB/CAP-sponsored interorganizational workgroup."

Dr. Johnson

It was time to start looking at a different way to address these RhD typing discrepancies, Johnson says. "Over the last 15 years or so, the science has developed greatly from typing for RhD by serology. We've all struggled with how to address these discrepant typings and what Rh type to call the patient. It's been easier to err on the side of caution, but then we're giving Rh-negative blood to patients who don't need it, and we're always stressed for inventory of Rh-negative blood."

Molecular is the future, Johnson says. "And it's the 'now' as well. We're seeing more and more blood centers genotyping their donors, and at some blood centers we have the ability to genotype for almost every blood group antigen that's known. With serology, it takes many hours to do typing, whereas today we can do it in a short amount of time for donors. The free-for-all is at the 3,000-plus hospitals in the U.S. that are doing ABO/Rh typing routinely for transfusion practice and prenatal testing. That's really why the workgroup came together, specifically to address the women of childbearing potential."

The main problem for such women is that when they become pregnant and have Rh-negative blood types, they are at risk of Rh alloimmunization, says workgroup member John T. Queenan, MD, professor of obstetrics and gynecology at the Georgetown University School of Medicine, and co-editor of a book on protocols for high-risk pregnancies.

"This occurs mostly by transplacental passage of red blood cells or antigens when the fetus has inherited the Rh-positive factor from the father. Then the fetus develops hemolytic anemia and there's a very high morbidity and mortality risk." In the late 1960s, obstetricians started Rh immune globulin in these cases at 28 weeks of gestation and about 10 years later added postpartum RhIG. "And between the antepartum and postpartum RhIG, we became 98 percent effective in preventing alloimmunization. So basically the disease has almost disappeared."

What the discussion is about now, Dr. Queenan says, is the patient who early in pregnancy is detected as a serologic weak D phenotype. "Many of these patients are technically Rh-positive, and they do not need the RhIG." About 13,000 patients a year fall into this category. By using the antiglobulin weak D test, labs can detect this type of patient. "But almost half of the time, where a potential pregnancy is involved, some labs are doing one type of test while most labs doing blood transfusions are doing a more complete test and picking up the weak D. The College and the AABB said now we really have to find a more unified way of explaining to labs and clinicians how to manage these patients in the future," Dr. Queenan explains.

Genotyping is able to identify weak D types 1, 2, or 3, he points out, and that's why the six national organizations are urging the phase-in of genotyping. "It's not only available; the prices are coming down. They now have gotten a request out for some of the genetic companies to participate in doing some future testing, and I think this is something that will become more widespread."

He also thinks the recommendation will be accepted fairly quickly. "I think you'll find a number of places that start offering *RHD* genotyping. It will be much more available in a year or two. The genotype only has to be done one time, and if the mother has two or three babies, they don't repeat it, so that saves a huge amount of cost from the standpoint that she doesn't need the RhIG and from the standpoint of the availability of blood if she needs

transfusion."

The *RHD* genotyping effort is one prime example of how modern genetics can help screen patients so they get personalized care that's appropriate, Dr. Queenan adds. "As we do this, genotyping will cost less over time, and the need for more RhIG will decrease because we're finding who really needs it."

Dr. Gottschall

If the joint statement is followed, it will improve the uniformity of practice in the U.S. in regard to provision of RhIG to women with a weak D blood type, Dr. Gottschall says. Whether there is a change in practice, he believes, will depend on how obstetricians decide to organize around the issue. That was one reason why it was considered important for the workgroup to have a representative from ACOG and from the Armed Services Blood Program, Dr. Sandler says. "The Armed Services program was included because domestically, it has a very significant number of pregnant women, and the population we're addressing is predominantly pregnant women and women of childbearing age."

Molecular testing in blood services is definitely on the rise, says Dr. Eder. The American Red Cross' national molecular laboratory has seen a 31 percent increase in patient samples submitted this year for *RHD* genotyping, and the number continues to grow. The National Institutes of Health and BloodCenter of Wisconsin also have growing programs, she notes.

However, moving to *RHD* genotyping will be a dramatic change from following the same policy for 50 years, says Willy A. Flegel, MD, a scientific consultant to the workgroup and chief of the laboratory services section of the NIH Clinical Center Department of Transfusion Medicine. "Now we are suddenly saying a subgroup needs to be tested at the molecular level. That is a drastic change of approach. We still have to make it happen in the lab, and we're not quite there."

The concept is not new, he says, and the U.S. should be moving ahead on it now. The scientific basis for what should now become U.S. policy was laid in the 1990s. "This is not something we dreamed up yesterday," he says. A 2000 paper from Dr. Flegel's laboratory (Wagner FF, et al. *Blood.* 2000;95:3662–3668) proposed the improved *RHD* typing and transfusion strategy in weak D patients, which is now being recommended. Fifteen years ago clinical red cell genotyping technology was very limited in Europe and in the U.S. "Now there are a multitude of platforms that are much improved and easier to use, including 10 or 12 different products that have been CE-marked in Europe for a decade."

He points to the CAP Survey as opening the way for the field to move in a more scientific direction on handling weak D patients. "The Survey asked: If you get a weak D, what do you do? Do you transfuse Rh-positive or Rh-negative? And the answers were 50/50. Half said Rh-positive and half said Rh-negative. So therapy depended on which lab the blood sample was sent to. That's not exactly science. That's chance," Dr. Flegel says. From there, people have started feeling the need to move to a scientific approach to weak D patients rather than a "throw of the dice" that depends on the bias of the lab.

For some populations, he points out, the risk is higher. "Many patients actually can be labeled properly as Rh-positive and should not receive RhIG. But if you happen to be an African-American pregnant female, the chance that Rh-positive is the right label is lower. If you do not give some of them RhIG, then they could develop an anti-D and the baby may be stillborn. So you have to make sure that those patients, African-Americans in particular, get

the RhIG, and that won't happen consistently if it just depends on where your sample is sent."

While decisions about cut points can get technical and debatable, he adds, "In the end, somewhere between 85 percent and 95 percent of Caucasians, even in the U.S., do not need RhIG, and for African-Americans that number is lower, but it's still more than 50 percent."

He expects greater attention to blood group issues now, and he considers that a positive thing. "Unfortunately, testing weak D effectively is not possible by serology, and that is generally not understood." Dr. Flegel says he frequently gives presentations on weak D issues to physicians who want to do the right thing for their patients. "At the end, they say, 'This is fine; tell us how can we do that through serology.' And the answer is you can't, you have to do it molecularly."

The run-of-the-mill serologic lab, however, was not set up to do any molecular biology until recently, and often still isn't, Dr. Flegel says. "To bring *RHD* genotyping into the lab is a momentous task, and that's actually why the joint statement is saying you should not do it." Of the molecular systems for blood groups that are available now, he notes, only one platform is FDA-approved, but the approved test kit doesn't have weak D on it. The workgroup recommends that laboratories send the test to a reference lab or to another pathology lab set up to do the testing.

With the release of the *RHD* genotyping joint statement, Dr. Flegel thinks there will be a market for such testing. And the data have shown it is cost-efficient. A 2015 study coauthored by Dr. Sandler found that compared with common practices, *RHD* genotyping and management according to presence or absence of weak D types 1, 2, or 3, over 10 or 20 years, would bring a two- to four-cent cost savings per pregnancy (Kacker S, et al. *Transfusion.* 2015;55:2095–2103). Dr. Flegel points out that this does not even include the beneficial effects on the supply of RhIG shots and Rh-negative blood that would otherwise be given to women who do not need it. "RhIG is a safe product, but there could be agents in it that we may not even know of, and these young females have another 60 or more years to develop consequences."

The recommendations in the joint statement, based on the fact that *RHD* genotyping would need to be performed only once per patient and many doses of RhIG could be avoided, would also be cost-effective, Dr. Eder says. "When a study aligned the costs of this approach, and if you consider all the doses that would not have to be given, *RHD* genotyping turns out to be clinically beneficial without increasing overall costs."

For transfusion medicine, *RHD* genotyping is one of the first actionable applications of molecular medicine outside the use of PCR for detection of transmissible pathogens, the ABC's Dr. Katz points out. "This is just a little tiny microcosm of the molecular revolution that's allowing us to be smarter. It's kind of our precision medicine, in a sense. As we adopt these molecular methods, even if they look more expensive at the start, it creates a market, people will develop the assays we need, and that will drive the price down."

Dr. Katz

He refers to this as a kind of "Field of Dreams" medicine—"If you build it, he will come." "Right now genotyping is more expensive than doing two agglutination tests, or a card test, or putting a sample on an automated immunohematology analyzer. But if we don't create movement toward the use of precision medicine, the price won't get driven down. You have to jump in with both feet to create the market, so the people who build our devices and tests will build cheap assays."

The fact that the American Medical Association recently approved addition of a new analyte for CPT code 81403 for

RHD genotyping (tier 2 molecular pathology procedure, level 4), and some payers are setting reimbursement rates for the tier 2 code, will help push the process along, Dr. Katz believes. The bulk of *RHD* genotyping is done in clinical laboratories and large national laboratories, he notes, not in ABC members' immunohematology reference labs. "But blood centers are very well situated to centralize this technology. We're already using red cell genotyping for the other 30 to 35 clinically significant red cell antigens that are important, so we already have the expertise to add on this test."

The joint statement recommends a phase-in of *RHD* genotyping because of the complexity of making the shift, says Johnson. "So far, it's not a test that's done routinely in the lab. It's more done by reference labs. AABB and CAP are trying to educate laboratories on what to do, but there's also a big need for education of obstetricians."

Dr. Sandler describes the process as "getting our toe in the water here for broader integration of genotyping into the industry of blood banking." Having Dr. Queenan, whose background includes work on the original RhIG studies, as a champion for genotyping serologic weak D in obstetrics will give the effort a major boost, Dr. Sandler believes. He hopes that manufacturers will recognize the potential market for genotyping to make a workable turnaround time for a test that could even become available on a point-of-care instrument. "That is not on the table right now, but it will be as we go into genotyping version 3.0, 4.0, 5.0, and so on."

At MedStar Georgetown University Hospital, "we converted to *RHD* genotyping several years ago. Our hospital is one of the hospitals that types all donors and recipients using two stages. If the first stage is negative, that's an anti-D test and we do a weak D, which is the second stage. We're going beyond what is required by taking transfusion recipients and typing them like donors. And we're finding that about 90 percent of serologic weak D are Rh-positive."

Some of his colleagues, Dr. Sandler reports, expect that serological typing will be history and everything will be done through genotyping and electronic matching. "I don't see that in the foreseeable future, but I do think that genotyping for the three percent of the population that is multiply transfused and form antibodies is a realistic goal, and can be done even within the recognition that Congress wants us to reduce the rate of increase of health care expenses." The joint statement advocated "selective integration," he notes. "This means it's not our intent at this time to go beyond very limited, selective integration of RHD genotyping."

The workgroup did focus on consensus, so not every proposed recommendation made the final cut. Originally, Dr. Sandler says, "The six members of our group recommended genotyping for three groups with serological weak D: women who are pregnant, women of childbearing potential, and transfusion recipients. We took that recommendation to the six pertinent national organizations, and at that level, as you could expect, the viewpoint was more conservative than that of the individual investigators, so the joint statement was limited to just the first two groups."

He thinks two things will be needed before it would be possible to extend the recommendation to include transfusion recipients: faster genotype testing, which now requires more than a day for a hospital to get a result, and a permanent electronic record for every patient. Once the industry is able to bring genotyping into the real time of transfusion recipient requirements—and he thinks that will happen—"we're going to save a lot of the Rh-negative blood that right now is going to people who really don't need it."

"They did make decisions along the way to limit their recommendation to pregnant women and women who might become pregnant," Dr. Eder says. "They stopped short of making recommendations that would be more controversial, and the Red Cross was involved and fully endorsed the final product." Johnson believes the workgroup decided not to make a strong recommendation on genotyping of pretransfusion patients in part because of cost. "Many are not convinced. It's easy to just pull Rh-negative units off a shelf, whereas ordering genotyping is adding extra cost."

Says Dr. Katz: "I think we just wanted to start with a little bite, and just deal with the issue in prenatal testing of pregnant women. Once we have that piece, we'll have experience using these technologies, and we will decide whether we can move it further along."

Joint statements are unusual, the workgroup members agree. Dr. Eder notes that the American Red Cross has had joint statements with the ABC and the AABB on matters related to blood donor selection and transfusion policy for 10 years or so—for example, in advocating that donor selection criteria be scientifically based. “But I really can’t think of any joint statements that have been so cross-disciplinary.”

Dr. Gottschall gives credit to the AABB, the CAP, and to Dr. Sandler for organizing the process and to the workgroup for bringing it to a successful conclusion. “As technology evolves, we are able to apply it to medical situations that will benefit patients, and this is one example where that has occurred. And it points out that if organizations come together around an issue, they can drive a change in practice for the good.”

The workgroup’s accomplishment is significant, Dr. Katz agrees. “We had a group of scientific advisors whose understanding of the sequences involved in the RHD gene is encyclopedic, and they guided us through this. So it was collegial, it was evidence-based, and it was reality-based in terms of how far we can move the community in the short run.”

The main target of the joint statement, Dr. Katz says, is really clinicians and laboratories. “As experts in the area of blood collection and blood processing, as clinical pathologists who run transfusion services, we’re saying to clinicians and laboratories in the real world, at the bedside, that we think you should do this and adopt it. And I think after a period of time, it will start showing up in the AABB standards and the CAP checklists. We’re not there yet, but we hope it will become the standard of care to do it this way. So we’ll need a little bit of time to make sure the resources are in line. If it can be made affordable, which I think it absolutely can, then I think it should be the standard of care.”

“The bottom line is that clinical laboratory people have to adopt this and say this is the thing to do. Then we’ll move forward.”
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