



MEDICINE

Blood-Matching Goes Genetic

Hoping to prevent adverse transfusion reactions and save lives, European researchers are lobbying to replace serology-based blood typing with matching based on DNA tests

Two decades ago, a 20-year-old man entered Duke University hospital for a hip replacement. He had sickle cell disease and because he was anemic, he needed a blood transfusion before the operation. As with most sickle cell patients, he had received transfusions before. But on this occasion, his body rejected the blood.

The doctors tried again and again with different blood, but the man's immune system rebelled against every new transfusion, generating antibodies that killed the new blood cells and some of his own in the process. "He reacted to everything," says Wendell Rosse, a hematologist at Duke at the time. The man's anemia continued to worsen, and 2 weeks later he died. "There wasn't anything we could do," Rosse says.

Following previous transfusions, the man had become alloimmunized, meaning that his body made antibodies to donated blood. He had so many transfused cells mingling with his own and had made so many antibodies that physicians could no longer identify his original blood type nor find suitable blood. Although this rare case of fatal alloimmunization happened more than 20 years ago, Rosse says he's not sure whether the man would have survived today, as there have been few advances in blood-matching tests in hospitals.

Some hematologists believe genetic testing offers a solution. For more than 100 years,

blood matching, also known as typing, has relied on serology, the identification of surface proteins and carbohydrates, called antigens, on red blood cells. Genotyping looks at the genes that determine these antigens, and some scientists say genetic techniques can more closely match blood between donors and recipients, preventing alloimmunization and other immune-related blood reactions.

Now, these claims are being put to the test. France, for one, will begin genotyping a portion of blood donors by the end of April to determine if a switch from serology is scientifically justified and practical. In addition, Canada has begun using genotyping as a screening tool to identify donors



Old-fashioned. Serology tests like this one have been used to match blood samples for more than a century.

with rare blood types that are hard to find in emergency situations.

"The argument that we will have 100% perfect blood for every patient will never be the case," says Neil Avent of the University of the West of England (UWE) in Bristol, who has led a European research consortium exploring blood genotyping. "But when you have all the information available, you can find the best blood."

Matchmaking

Finding the best blood seems simple. Two antigens on red blood cells determine the major blood group—A, B, AB, or O. A person with type A naturally harbors antibodies to B antigens and vice versa. Give type-B blood to a patient with type A, and antibody reactions will cause blood cells to clump together, sometimes with fatal results. AB people don't have antibodies to either antigen, making it easier to find blood for them. But O people have antibodies to both A and B, as their blood cells have neither antigen, so they can only receive O blood.

But there's more to blood than A and B antigens. Physicians also routinely test for D, an antigen in the Rhesus (Rh) blood group that can evoke a strong immune response. They try never to give RhD+ blood to an RhD- person.

The possibilities get even more complicated. Avent, who also directs UWE's Centre for Research in Biomedicine, says there are 29 known blood groups, which are determined by about 200 antigens. A person's full blood type might read: AB, D+, M+, N+, K-, Lea+, and so on.

A simple antibody test can identify a donor's or patient's ABO blood type, and in theory, similar tests could detect the other so-called minor blood groups. But the reagents to identify some of these antigens are costly, not reliable, or simply not available. For patients who receive only one or two transfusions during their lifetimes, mismatches in the minor blood groups pose no obvious problems. Yet people who receive multiple transfusions—including those with sickle cell disease, hemophilia, or leukemia—can develop antibodies to the minor blood group antigens, which is what happened in the Duke University patient. In some cases, the transfused blood can cause acute or delayed hemolytic reactions. The Public Health Agency of Canada, which keeps statistics on such reactions, estimates that as many as 1 in 12,000 transfusions ends in an acute reaction, with as many as 1 in 600,000 ending in death. Delayed reactions occur in as many as 1 in 5000 transfusions but are less often fatal.

Traditional blood typing has other limitations. Some people have a weak or partial version of the D antigen that is difficult to detect. Misdiagnosing D+ donor blood as D- could cause serious problems. A D- person given such mislabeled blood will make antibodies that would react to future transfusions of D+ blood. Furthermore, it's important for women to accurately know their RhD status: If a D- woman had a D+ baby, any subsequent D+ fetus is at risk because the mother will have made antibodies to the D antigen during the first pregnancy. Because serology has trouble deciphering RhD status, says Avent, as many as 40% of pregnant women may receive unnecessary drug treatment, which can have side effects, to limit their antibody production.

Genotyping offers an alternative. By sequencing the 31 genes that determine the surface antigens, hematologists can classify the blood into the 29 groups. For example, a single gene on chromosome 9 controls the ABO antigens, and the versions inherited from each parent determine a person's type. Other blood groups are determined by a single nucleotide polymorphism (SNP), which changes just one base in an antigen-determining gene. Insertions or deletions of DNA within other blood-group genes lead to different antigens. By identifying these different versions of a gene, or alleles, genotyping can predict a person's blood types in fine detail.

Advocates of genotyping predict that with better matched blood, they can prevent between 80% and 90% of alloimmunization and also eliminate partial and weak versions of the D antigen from the blood supply. Rosse says such tests might have helped his patient. "If we had his genotype, we could have matched it with a donor's genotype," Rosse says. "We might have found a match."

Europe takes the lead

Researchers have been using polymerase chain reaction (PCR)-based techniques to genotype blood samples in the lab for a decade. But if blood genotyping is extended into widespread clinical use, cheaper and faster methods will be needed.

In 2002, the European Union gave €2.35 million to BloodGen, a consortium of universities and blood centers across Europe that planned to standardize blood genotyping techniques and prove that they beat serology. The consortium, led by Avent, has since developed the BLOODchip, a gene chip that tests a person's DNA using blood samples. PCR alone would require 60 or more tests to determine the blood type as comprehensively as the chip can with just one test. The current

chip, produced by the company Progenika, looks at nine blood groups, including the genes that code for the A, B, and D antigens.

In initial tests, the BloodGen team genotyped 1000 blood samples and found 42 cases that conflicted with serology, some of which were in the Rhesus blood group. Further analysis revealed that two of these errors were the fault of genotyping and 40 were the fault of serology. Progenika has full clinical approval for seven of the blood groups on its chip and is currently seeking approval for RhD. The next round of tests will look at 3000 samples.

BioArray Solutions in Warren, New Jersey, has developed another genotyping product called BeadChip that tests for 11 blood groups but not A, B, and D. Although the omission is in part practical, some researchers have said it reflects a larger debate in the scientific community about the potential of genotyping to replace, as opposed to supplement, serology.

A bloody debate

Avent is adamant that genotyping will replace serology for most blood groups within the decade and in some cases sooner. He predicts European hospitals will demand blood genotyping of patients expecting to receive a transfusion. "For multitransfused patients, I expect a change in policy this year," Avent says. "Then I would expect other vulnerable groups [such as pregnant women to be] tested. Then I would like to see blood centers genotyping cohorts of donors."

Researchers in the United States are taking a more cautious approach. They believe genotyping has a way to go for the A, B, and D antigens. Researchers have identified more than 100 alleles for the ABO blood type and more than 200 for the Rhesus system, and new mutations are discovered frequently, which makes some people nervous about relying on current gene chips. "I don't think we have found all the alleles," says Marion Reid, an immunohematologist at the New York Blood Center.

Avent predicts that within the next few years, researchers will have compiled the majority of those alleles, and he adds that genotyping is already superior for identifying

the D antigen. Willy Flegel, a transfusion medicine specialist at University Hospital Ulm in Germany, agrees. He has genotyped more than 46,000 blood donations identified as D- based on serology and found that 47 are actually D+. "The smart serologist will apply molecular techniques now for the benefit of the patient," Flegel says. "The scientific arguments are clearly in favor of genotyping."

Connie Westhoff, scientific director at the American Red Cross, concurs that genotyping is the future, but she doesn't expect that the United States will fully adopt it for another 15 years at least, and even then, she does not expect the country to completely abandon serology. "We would never throw out our old toolbox," she says.


Moving forward

A few countries are already forging ahead with blood genotyping on a large scale. Canada is among the leaders. In December, for example, Quebec announced plans to genotype 22,000 blood donors. Currently, when a patient with a rare blood group needs a transfusion, practitioners have to blindly order blood from banks based on ABO and D grouping alone and then test it with more detailed serology on site. This trial-and-error approach wastes time and money. The screening should make the process faster by narrowing initial blood selection, but serological testing will still serve as a follow-up.

Jean-Pierre Cartron, scientific director at France's national institute of blood transfusion, says his blood center will be genotyping using both the BLOODchip and the BeadChip by the beginning of April. "We want to see them together on the same population of donors and patients," Cartron says.

Flegel says the ABO group will take time to work out, but for all other groups, genotyping should begin now. Only by using the available genotyping tools can hematologists locate unknown alleles and make future gene chips more accurate. "We need to learn what we are missing," he says. He agrees that hematologists need to proceed with caution. But, he says, "we don't need to wait."

—ELIZABETH QUILL



Blood Type	NUMBER OF ANTIGENS	
	BLOODchip	BeadChip
RhCE and RhD	18	
RhCE		4
KEL	7	2
JK	3	2
FY	3	2
MNS	6	4
DI	2	2
DO	2	4
CO	2	2
SC		2
LU		2
LW		2
ABO	4	

Chipping away. Two new gene chips (BLOODchip above) can test for many blood groups beyond ABO.