False Neonatal ABO Blood Typing due to Contamination of the Cord Blood

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ABSTRACT

Determining the blood type of a newborn is one of the first laboratory tests to be performed after birth. Precautions should be taken during cord blood collection to avoid contamination of the sample. In the unique case presented, the cord blood sample was contaminated with Wharton’s jelly from the umbilical cord which caused a false agglutination reaction during blood typing. During clinical rotations, medical students have the opportunity to participate in newborn deliveries, and it would be advantageous to understand the proper technique of cord blood collection and the protocols that labs should have in place when typing the cord blood. It is also of the utmost importance to understand the role that agglutination plays in blood typing and the consequences that a false positive result can have on the mother as well as the newborn. This case explores the basics behind blood typing and discusses the genetic variation of ABO blood types and the different phenotypes that can result. In addition, this case highlights the unusual mechanism behind an extremely rare blood phenotype that causes a neonate to have a different blood type than expected.

A 33-year-old with a confirmed O- blood type presented at 28 weeks gestation for prenatal care. The patient refused Rho(D) immunoglobulin administration, insisting that her husband was also O-. Repeated requests for documentation of paternal blood type were unsuccessful at follow-up visits. Twelve weeks later at 40 weeks gestation, labor was induced. Secondary to a sustained fetal bradycardic episode in the second stage of labor, an emergent cesarean section was performed. After delivery, the cord blood was tested and the neonate’s blood type was reported to be AB+. At the request of the delivering physician, the cord blood was retested twice by the laboratory with the blood type again reported as AB+. The mother was immediately given 300 µg of Rho(D) immunoglobulin to decrease her risk of isoimmunization. It was then decided to test the neonate’s venous blood rather than the cord blood sample. This time the reported blood type was O-. The vast discrepancy between the two results was investigated and it was discovered that Wharton’s jelly had contaminated the cord blood sample and caused the false AB+ result.

This case has implications for family physicians providing maternity care, nurse midwives, obstetricians, and medical students. Therefore, care should be taken at delivery to avoid contam
ination of the cord blood sample. To understand the effects that contamination can have on the medical treatment of the mother and newborn after delivery, one must comprehend the role of ABO blood group, Rh(D) antibodies, and timely prophylactic administration of Rho(D) immunoglobulin.

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CASE PRESENTATION

A 33-year-old primigravid married female with controlled hypothyroidism and confirmed O-blood type presented at 28 weeks gestation for prenatal care. She had no vaginal bleeding or spotting complaints prior to her first visit. The patient was documented as O- on numerous antenatal and peripartum labs. At her 28 week appointment, she was counseled on the possibility of isoimmunization due to her Rh(D)- status. She was warned about the potential hemolytic complications for any future pregnancies if she were to not receive Rho(D) immunoglobulin. She declined the administration of Rho(D) immunoglobulin because she insisted her husband was O-. Despite being advised that there was no harm in prophylactic administration, the patient still refused. Documentation of her husband’s blood type was never provided or verified. The remainder of the pregnancy was uneventful. Due to the increased risk of fetal demise due to maternal hypothyroidism, she was induced at 40 weeks gestation. She was given misoprostol vaginally for cervical ripening and the following morning was given intravenous oxytocin for labor augmentation. Twenty hours after the first misoprostol dose, she was fully dilated and effaced. She began to push, but with each contraction, a prolonged fetal bradycardic episode would occur. The labor failed to progress, and the patient underwent an urgent cesarean delivery which resulted in a viable female newborn. During the cesarean delivery, the umbilical cord was incised and clamped. The cord blood was collected using the gravity and glass tube method. The umbilical cord was held directly above the collecting tube, and the blood was allowed to flow down into the tube. The lab reported the neonate’s blood type as AB+. The mother was immediately given the standard 300 μg dose of Rho(D) immunoglobulin. A Klee- hauer-Betke stain was ordered to identify the amount of fetal hemoglobin that was transferred from the fetus to the mother’s bloodstream, which could affect the subsequent amount of Rho(D) immunoglobulin that she should receive.† The lab was called and asked to test the cord blood sample again. The following morning the lab reported the neonate’s blood type to be AB+. According to the Mendelian laws of inheritance, this is rarely possible due to the fact that the maternal blood type was confirmed as type O. Finally, a decision was made to test the neonate’s venous blood, which was later reported to be O-. The parents were informed of the neonate’s true blood type and that there was no risk of isoimmunization for any of the mother’s subsequent pregnancies.

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DISCUSSION

To comprehend how contamination affects the agglutination reaction when determining blood type, the basic ABO antigen-antibody reactions must be understood. To determine blood type, a blood sample is mixed with anti-A antibodies and anti-B antibodies. The sample is then checked to see whether or not agglutination has occurred. Type AB red blood cells have both A and B antigens on their surface, therefore there are no antibodies in the plasma. Type O red blood cells have no antigens on their surface, therefore there are both anti-A and anti-B antibodies in the plasma. Type A red blood cells have A antigens on their surface and have anti-
B antibodies in the plasma. Type B red blood cells have B antigens on their surface and anti-A antibodies in the plasma. When testing a blood sample, if agglutination occurs with anti-A serum, the blood sample is type A. This is because the anti-A antibodies in the serum will cause agglutination in the presence of A antigens on the surface of red blood cells. If the blood sample agglutinates with both anti-A and anti-B serum, the blood is type AB. If the blood sample does not agglutinate with either, the blood is type O.

There is a commonly used second step in blood typing called back typing, also known as reverse typing. In this step, only the plasma from the blood sample is mixed with blood that is known to be type A and type B. If the plasma agglutinates when type A blood is added, the blood sample is type B due to the presence of anti-A antibodies in the plasma of type B blood. If agglutination occurs with both A and B blood, the blood sample is type O due to the presence of anti-A and anti-B antibodies in the plasma of type O blood. Back typing is not performed in newborns. This is due to the fact that newborns have not synthesized antibodies to A or B antigens yet, so there is no built-in quality assurance check when typing their blood.2

Once the process of blood typing is understood, it becomes clear that certain substances could catalyze the false agglutination reaction, thus distorting the blood type result. In fact, there are substances within the umbilical cord that can cause this reaction. Wharton’s jelly is a gelatinous component within the umbilical cord that supports and protects the umbilical blood vessels. This viscous material is composed of cells that originate from extraembryonic mesoderm and is made up of hyaluronic acid and chondroitin sulfate. Wharton’s jelly aids in the physiological clamping of the umbilical cord shortly after birth and can coat the neonate’s red blood cells and make them polyagglutinable, causing a false agglutination reaction of cold agglutinins.3,4 Reverse blood typing would resolve this inconsistency, but it is never performed on neonates.

Contamination of the cord blood sample is a rare occurrence, but nevertheless it is tremendously important to consider. There are various techniques for the collection of cord blood that can help lower the risk of contamination with maternal blood as well as bacterial contamination. Protocols for cord blood collection are in place to avoid interfering with the delivery of the baby while preserving sterility. Most importantly, cord blood collection should never compromise the well-being of the mother or the neonate. Regardless of the technique that is used, the earlier the blood is collected the less likely that clotting will occur and hinder the collection.

There are two main techniques for cord blood collection: the syringe method and the gravity and glass tube method. The syringe method involves clamping the cord prior to delivery of the placenta. A four to eight inch area is cleaned with antiseptic and a 16 gauge needle is inserted into the umbilical vein and the cord blood is allowed to drain into the collection bag by gravity. To avoid clotting of the cord blood during the collection process, the collection bag contains an anticoagulant solution. By using the syringe method, the exposure to air is minimized and sterility is maintained.5

The second cord blood collection technique, the gravity and glass tube method, allows blood to drain from the incised end of the umbilical cord into a glass tube. When the cord blood is collected via the gravity and glass tube method it is important to wash the red blood cells 3-4 times in saline before determining the blood type of the neonate. It is also important not to squeeze the umbilical cord while collecting the cord blood because the increased pressure on the cord could expel Wharton’s jelly into the collection.
tube. Furthermore, if the blood sample is contaminated by Wharton’s jelly and cannot be removed by washing the red blood cells in normal saline, hyaluronidase must be added to negate the agglutination effect of Wharton’s jelly. Many labs have protocols in place to reject contaminated cord blood. The Duke University Clinical Lab “rejects any cord blood sample that has been contaminated with Wharton’s jelly, which may result in a false positive reaction, and therefore, is not accepted for testing.” Additionally, the American Association of Blood Banks requests that cord blood be collected using a needle and syringe which avoids contamination and the need for additional washing of the cells.

Although the gravity and glass tube method is technically easier and used more commonly in hospitals, it comes with the great disadvantage of higher cord blood contamination rates. The gravity and glass tube method has a contamination rate of 14%, as compared to a 4% contamination rate for the syringe method. In these studies, contamination was from bacteria as well as maternal blood. The syringe method has principally been used by designated umbilical cord blood collection centers with specially trained staff, but this method is worth the additional steps due to lower contamination rates.

Contamination is a major cause of false ABO blood type results. However, when contamination is not a causal factor, false paternity must be investigated as the inciting factor that would cause a different ABO result based upon the mother’s known blood type. In this case, there was no concern of false paternity because the mother was confirmed to be O. No matter what the father’s blood type is, an AB neonate very rarely occurs. The A and B alleles are dominant as compared to the recessive O alleles. A and B can also be co-dominant with each other. Since the mother has the genotype of (OO), even if the father is (AB), the possible genotypes of their offspring could be: (OA) or (OB). This could

\begin{table}
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\begin{tabular}{|c|c|c|c|c|}
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& \textbf{A} & \textbf{B} & \textbf{AB} & \textbf{O} \\
\hline
\textbf{A} & & & & \\
\text{Genotypes AO or AA} & AA \footnote{type A} & AO \footnote{type A} & AA \footnote{type A} & AO \footnote{type A} \\
& AO \footnote{type A} & BO \footnote{type B} & AO \footnote{type A} & AO \footnote{type A} \\
& OO \footnote{type O} & AB \footnote{type AB} & BO \footnote{type B} & AB \footnote{type AB} \\
\hline
\textbf{B} & & & & \\
\text{Genotypes BO or BB} & AO \footnote{type A} & BO \footnote{type B} & AO \footnote{type A} & BO \footnote{type B} \\
& BO \footnote{type B} & BB \footnote{type B} & BO \footnote{type B} & BB \footnote{type B} \\
& AB \footnote{type AB} & OO \footnote{type O} & AB \footnote{type AB} & OO \footnote{type O} \\
\hline
\textbf{AB} & & & & \\
\text{Genotype AB} & AO \footnote{type A} & BO \footnote{type B} & AO \footnote{type A} & BO \footnote{type B} \\
& AO \footnote{type A} & BB \footnote{type B} & AO \footnote{type A} & BB \footnote{type B} \\
& BO \footnote{type B} & AB \footnote{type AB} & BO \footnote{type B} & AB \footnote{type AB} \\
\hline
\textbf{O} & & & & \\
\text{Genotype OO} & AO \footnote{type A} & BO \footnote{type B} & AO \footnote{type A} & BO \footnote{type B} \\
& AO \footnote{type A} & OO \footnote{type O} & AO \footnote{type A} & OO \footnote{type O} \\
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\end{tabular}
\caption{Possible Neonate Blood Types resulting from various Maternal and Paternal Blood Genotypes}
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only result in blood type A or B, but rarely AB. An AB neonate usually only results from the union of an A, B, or AB mother and an A, B, or AB father; neither parent can have an O blood type. The neonate has to inherit the A allele and B allele from each parent (see Table 1). The A and B alleles cannot be inherited together from the same parent. There is one exception to the prior statement, known as the cis-AB phenotype. In this situation, both the A and B alleles are inherited from one parent. The cis-AB phenotype is an ABO allele that encodes a glycosyl transferase that is known to synthesize both A and B alleles. A structural mutation in the type A or B glycosyl transferase produces a single enzyme with bifunctional activity. The cis-AB phenotype has only been researched in certain populations. Based on a study done on blood samples from the Japanese population, the gene frequency of cis-AB was $1.1 \times 10^{-5}$.

This case highlights the risks of cord blood contamination and emphasizes the importance of Rho(D) immunoglobulin administration and the consequences that can occur in its absence. Had the neonate truly been AB+, the mother could have already made IgG antibodies to the neonate's Rh(D)+ red blood cells that entered the mother's circulation due to silent fetal-maternal hemorrhages during pregnancy or due to blood mixing during the cesarean delivery. Any subsequent pregnancy can be affected since the patient did not receive Rho(D) immunoglobulin at 26-28 weeks gestation. Subsequent pregnancies are affected due to IgG antibodies against Rh (D), which are formed during the first pregnancy, and later cross the placenta and opsonize the fetal red blood cells to be destroyed by the fetal spleen. Simple ABO blood type incompatibility between the neonate and the mother does not cause this severe type of hemolytic disease due to the fact that antibodies against ABO blood group antigens are IgM and do not cross the placenta; thus, they cannot cause any hemolytic disease. Timely administration of Rho(D) immunoglobulin is extremely important to avoid severe hemolytic disease of the newborn. The current standard of care in the United States is to administer a single dose of 300 μg of Rho(D) immunoglobulin early in the third trimester. The 28 weeks recommendation comes from evidence that 92% of women who develop anti-D antibodies do so at or after 28 weeks gestation. It is also recommended to give an additional 300 μg dose of Rho(D) immunoglobulin within 72 hours of delivery of an Rh(D)+ neonate to protect against maternal sensitization from as much as 30 mL of fetal Rh(D)+ whole blood entering the maternal circulation. The incidence of fetal-maternal hemorrhage greater than 30 mL at delivery is about 1 in 200-300 deliveries. In the patient case that was presented, Rho(D) immunoglobulin was not administered at 28 weeks. She was immediately given 300 μg when the neonate was suspected to be Rh(D)+ because the earlier that Rho(D) is given, the lower the risk of isoimmunization. If Rho(D) immunoglobulin is not given at 28 weeks but is administered within 72 hours of the birth of a Rh (D)+ neonate, there is a 2% chance of isoimmunization. If Rho(D) immunoglobulin is never administered the risk of isoimmunization is 16%. There was an additional potentially fatal complication that was avoided by retesting the newborn’s blood and proving the newborn was truly O-. AB+ blood types can potentially accept O, A, B, or AB blood type transfusions since AB+ blood types do not have any antibodies to ABO blood group antigens in their plasma. If the presumed AB+ neonate had needed a blood transfusion, she potentially could have received a transfusion of any blood type. Commonly O- blood is given in an emergency and since the newborn was truly O- there would have been no harmful outcome. If there had been a shortage of O- and any other blood type was given, a life-threatening hemolytic reaction could have resulted.
In summary, care should be taken to collect cord blood samples with the lowest risk of Wharton’s jelly contamination and the laboratory should have policies in place to adequately prepare cord blood samples prior to blood typing. If contamination of the cord blood sample is suspected based on the blood type of the neonate with respect to the mother’s blood type, the neonate’s venous blood should be drawn and typed. Additionally, parents should be properly counseled on the risks of not receiving Rho(D) immunoglobulin at 28 weeks gestation.

LEARNING POINTS

1. In the process of blood typing, blood samples are mixed with known anti-A and anti-B antibody serum, and are then monitored for agglutination. If agglutination occurs, then the particular ABO antigen(s) on the red blood cell surface have reacted with the antibodies present in the serum. Wharton’s jelly can contaminate blood samples by coating the red blood cells and making the cells polyagglutinable, leading to a false positive reaction. When possible, cord blood should be collected using the syringe method rather than the gravity and glass tube method due to the former’s lower rate of contamination with Wharton’s jelly.

2. 300 μg of Rho(D) immunoglobulin should be administered at 28 weeks gestation to all Rh (D)- women. If a Rh(D)- mother delivers a Rh (D)+ newborn, an additional 300 μg of Rho(D) should be administered within 72 hours.

3. The rare cis-AB phenotype causes both the A and B alleles to be inherited from the same parent. This parent’s unique ABO blood group alleles encode a single enzyme with bifunctional activity.

REFERENCES


