Case Report

Blood group genotyping in a multitrauma patient: a case report

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Currently DNA-based analysis of blood groups is mainly used to improve transfusion safety by reducing alloantibody formation in multiply transfused patients and by monitoring pregnancies at risk for hemolytic disease of the fetus and newborn. We present a case in which genotyping was performed after massive transfusion with unmatched group O, D– blood in a trauma setting. Our patient was genotyped as *O1A1* and predicted to be D–, and we therefore transfused group A, D– red blood cell concentrates. This case demonstrates how the use of blood group genotyping in an acute setting can lead to a decrease in the unnecessary use of group O, D– blood products. ***Immunohematology* 2012;28:85–7.**

Key Words: genotyping, serology, massive transfusion, unmatched transfusion

Case Report

Our transfusion laboratory received an emergency request for six unmatched group O, D– red blood cell (RBC) concentrates for a patient. These units were to be transfused not inside the hospital but at the scene of a car accident (at a peripheral highway), where the patient was bleeding heavily while trapped in a car wreck. Again, another four unmatched RBC units were ordered together with five group AB, D– fresh-frozen plasma (FFP) units and one randomly selected unit of platelets obtained from five donors. All products were leukocyte reduced, with less than 10^6/L white blood cells per unit. We had not received blood for crossmatching.

When released from the car wreck, the 62-year-old white female patient arrived at the hospital emergency department and was admitted to the operating room immediately. The patient suffered from complex high-impact trauma. Her car had slid underneath a truck, and the patient had been trapped for at least 1.5 hours. She had multiple fractures, in the nose, left wrist, left humerus, bilateral femur, and ramus superior and inferior, and she had a pneumothorax. Also, the patient exhibited various superficial wounds and a head injury that was closed using staples. Besides damage-control surgery, additional acute surgery was necessary to implant a stent because of dissection of the descending aorta. During this procedure the patient was in need of more blood products, and at that time the transfusion laboratory received the first sample for crossmatching. Serologically it was no longer possible to determine a blood group owing to the number of transfused RBC units. Even a mixed-field reaction was hardly visible (Fig. 1). Also, a reverse blood group typing was not possible as a result of the number of FFP units she had received. After the surgical procedure, the patient was stable and was transferred to the intensive care unit with a hemoglobin concentration of 8.8 g/dL (5.5 mmol/L). Three additional FFP units and two five-donor platelet concentrates were transfused because of low levels of fibrinogen (0.87 g/L) and platelets (76/nL), respectively. Altogether, our patient received 18 units of group O, D– leukocyte-reduced packed cells, 12 units of FFP (blood

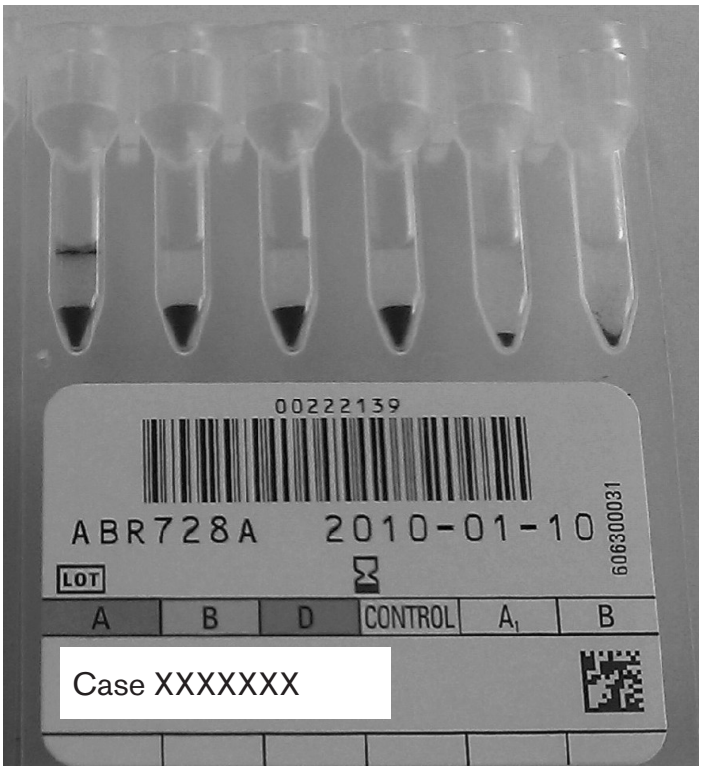

Fig. 1. Blood group typing cards (Ortho Clinical Diagnostics, Inc., Raritan, NJ) from our patient's blood upon arrival at the hospital, showing a mixed-field reaction with anti-A (**left lane**). The mixed-field reaction was only visible after addition of 10x the erythrocyte volume used in the usual procedure. No reaction with the A_1 cells and B cells (Ortho Clinical Diagnostics, Inc.) is visible.

group AB), and 6 units of leukocyte-reduced five-donor platelet concentrates (group O, D–) during her intensive care stay.

Unfortunately, we were unable to obtain information regarding the blood group of our patient (even after contacting the patient's general practitioner and the local blood bank). Therefore, we chose to determine her blood group by means of DNA analysis to avoid further (unnecessary) use of group O, D– RBC products. We collected venous blood samples (anticoagulated in K_3-EDTA) and sent them to the laboratories of the Sanquin Blood Supply Foundation. ABO genotyping was performed using a sequence-specific primer polymerase chain reaction (SSP-PCR).[1] *RHD* genotyping was performed using a multiplex reverse transcriptase (RT) PCR method.[2] Our patient's genotype was determined to be *O1A1,* and she was predicted to be D–.

Fortunately, the patient appeared to no longer need blood products. She stayed in the intensive care unit for another 20 days, during which time only one unit of RBCs was transfused (blood group A, D–). At that time the patient's blood sample was serologically typed as group A, D– (without mixed-field reactions), and the screening for irregular antibodies was negative (using a three-cell screening panel, Ortho Clinical Diagnostics, Inc.). She was then moved to the surgery department for necrotomy of wounds and was transferred to a nursing home 2 months after the car accident.

Results

The chosen SSP-PCR is based on the presence of O1, O2, B, or A2 genes (the presence of glucosyltransferases on chromosome 9q34-2) in DNA isolated from peripheral blood samples.[1] A PCR product is produced only when the specific chosen primer binds to and amplifies the sequence of interest. Eight mixtures of four allele-specific primer sets are used. The PCR products are transferred to an ethidium-bromide gel, and patterns are read and interpreted manually. Extrapolating results of eight different incubations (O1, O2, B, A2, non-O1, non-O2, non-B, and non-A2) led to the genotype. Therefore, this assay cannot be used for ABO variant analysis. In our case, blood group A1 was predicted as determined by exclusion of the other genotypes present. This phenotype has a frequency of 33 percent in the white population. The presence of an A_2 blood group was excluded, as was the presence of a B or Bombay (O_h) phenotype. The frequency of other type A (or B) blood groups is very rare in the white population, and would not have led to a mixed-field reaction of the patient's RBCs with the anti-A serum (Fig. 1).

We were not able to confirm the blood group by reverse blood typing because of the amount of FFP received. However, after several days, serum anti-B was found but no anti-A; this is consistent with the ABO genotype we found.

The multiplex PCR for genotyping the Rh blood group system is based on single-nucleotide polymorphisms (SNPs) using six different primer sets that cover exons 3, 4, 5, 6, 7, and 9 of *RHD* and is able to identify most *RHD* variants.[2] Based on the genotyping results, our patient was predicted to be D– , and for our white patient, the frequency of *RHD*ψ or a variant D is very low. We did not confirm genotyping with buccal cells because the clinical consequences of a false-negative result are far less severe than those of a false-positive result. Serologically, on increasing volumes of RBCs to observe mixed-field reaction in our column technique, the D phenotype remained negative (Fig. 1). When blood group serology was repeated before transfusion 2 weeks after the accident, no mixed-field agglutination was observed, although transfused RBCs were likely to have been present. This was consistent with a D– phenotype and showed concordance between genotype and phenotype results. Again, using a three-cell screening panel, we found no irregular erythrocyte antibodies.

Of course, interpretation of genotyping results should be performed carefully because ethnic differences (e.g., presence of *RHDψ,* or the DAR variant) and silencing alleles (e.g., GATA box mutations) exist that could lead to discrepancies between genotype and phenotype.[3,4] When the patient is of a certain ethnicity with a higher risk of ABO variants, sequencing may be an option, although costly and time-consuming.

Discussion

Recently, excellent reviews[5,6] have been published regarding genotyping of the blood donor population and its possible drawbacks. However, in transfusion practice, genotyping is not commonly performed on patient samples. The Dutch national guideline currently states that a blood group can only be confirmed when it has been established by testing two different samples, irrespective of the method used.[7] One method may be genotyping.

From 2001, leukocytes are depleted from all blood products within the Netherlands by means of filtration, resulting in the presence of less than 1×10^6 white blood cells per unit. In case of massive transfusion of RBCs and platelet or plasma units, the load of donor white blood cells in the patient remains low (undetectable when compared with the patient's white blood cell count, on average 5×10^9/L), hence genotyping on DNA from the patient's white blood cells is achievable. Our patient

had a white blood cell count of 4.5 leukocytes/nL at the time of DNA analysis. Of these, a maximum of 4 percent (0.16/nL: 16 blood products times 10^6 white blood cells) could potentially originate from the received RBC units. To circumvent these limitations or when white blood cell counts in peripheral blood are too low to perform genotyping, other material can be used, such as nails or buccal epithelial cells (with potential errors in bone marrow transplant patients).[3,4,8] Therefore, leukocyte counting for the patient is essential before performing these analyses, and care should be taken in case of neutropenic or lymphopenic patients.

Ideally, all transfusions should be closely monitored, especially transfusions of unmatched RBC units, to detect acute transfusion reactions in case of transfusion of incompatible blood group(s). Unfortunately the clinical practice shows that unmatched units are transfused in emergency situations, in which close monitoring of transfusion can be difficult. Because transfused RBCs remain in circulation for several weeks and can cause mixed-field agglutination when antigen typing is performed, genotyping can determine the blood group of trauma patients who have received repeated unmatched RBC units.

Conclusions

As has previously been suggested, blood group genotyping can be helpful in acute situations, when serologic (hemagglutination with antisera) blood group typing is not possible or yields inconclusive results (i.e., mixed-field agglutination, discrepant results).[6,8] The success of genotyping in cases of emergency depends on the speed of processing and the turnaround time. Improvements in these will eventually lead to fewer unmatched transfusions. The blood transfusion laboratory should, however, always be aware of the limitations of genotyping (vs. serologic determination of blood groups). In a trauma center, genotyping a patient's sample to determine ABO and D might be a suitable alternative to transfusing group O, D– RBCs, thus reducing the unnecessary use of such blood products.

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