Severe neonatal hyporegenerative anemia due to anti-Vw (anti-MNS9) alloantibody

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Sir,

Severe hemolytic disease of the fetus and newborn (HDFN) not related to anti-D (anti-RH1) and requiring in utero transfusion or postnatal exchange transfusion are most commonly caused by anti-Kell (anti-K1) and anti-c (anti-RH4) [6]. However, antibodies directed against low incidence antigen (LIA), particularly those resulting from MNS polymorphism can also lead to life threatening complications [5, 7]. In some fetuses or newborns presenting with severe anemia, and more particularly in the presence of anti-Kell (anti-K1) antibodies, suppression of erythropoiesis may be observed. Anemia is associated with reduced reticulocytosis, less erythropoiesis and less severe hyperbilirubinemia when compared with similar hemoglobin levels but related to anti-D (anti-RH1) alloimmunization [9]. These observations have been supported by in vitro analysis, showing that sera containing anti-Kell as well as monoclonal anti-Kell antibodies suppressed the growth of Kell-positive erythroid progenitors [8]. Here, we report a case of severe hyporegenerative anemia, due to an anti-Vw (anti-MNS9), that was not detected by routine screening of alloantibodies during pregnancy.

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A male newborn from a 32-year-old Caucasian woman, gravida 2 para 1 (1 abortion), without history of transfusion, was delivered by emergency cesarean section at the 34th gestational week because of decreasing fetal movements during the last three days and non-reactive cardiotocogram on admission. The newborn presented with extreme pallor, blueberry muffin spots on the trunk and face, respiratory distress, tachycardia and mild hepatosplenomegaly. Laboratory testing demonstrated severe and isolated hypoproliferative anemia (hemoglobin: 33 g/L; reticulocyte count: 9.6 × 10^9/L, nucleated red blood cell per 100 white blood cells: 2), a metabolic acidosis with a lactate level of 7.6 mmol/L and a moderately increased bilirubin (46 µmol/L). A first transfusion of red blood cell (O Rh negative, non-cross matched) was immediately administered (20 mL/kg). The baby’s condition improved rapidly and the skin lesions disappeared in the next 12 h. Abdominal ultrasound only revealed mild hepatomegaly without ascites. Echocardiography showed good left ventricular function without morphological anomaly. The direct antiglobulin test (DAT) was positive (IgG) on cord blood cells. Both mother and child were O Rh positive. Eluate from the newborn red blood cells was not performed before the first transfusion and DAT became negative thereafter. Indirect antiglobulin test (IAT) of maternal serum was negative with panel cells, but was clearly positive (3+) with the father’s red blood cells, suggesting that a low incidence antigen (LIA) was involved as the cause of immunization. Blood samples of the mother as well as of the father were tested at the reference laboratory of the Blood Transfusion Service (Bern, Switzerland). Anti-Vw (anti-MNS9) was identified in the serum of the mother. The father was MNS:-1,2,3,4,9 (Vw positive). Extensive investigations for infectious diseases (Epstein Barr virus, cytomegalovirus, parvovirus B19, toxoplasmosis, rubella, syphilis, enterovirus) were not contributive. The Kleihauer-Betke test in the mother was negative. A screening for pyruvate kinase and glucose-6-phosphate dehydrogenase deficiency was performed, and yielded normal results. Therefore, the diagnosis of HDFN related to a LIA was confirmed.

Because of ongoing hemolysis with increasing levels of total bilirubin (278 µmol/L on day 4), phototherapy was performed until day 11. A second transfusion (20 mL/kg) performed at day 2 was sufficient to bring the hemoglobin level up to 140 g/L. Four weeks later, the baby was again transfused because of hemoglobin level of 75 g/L.
with a reticulocyte count of $40 \times 10^9/L$. The baby was discharged thereafter. On the last follow-up at 10 weeks, he was healthy and well growing, and showed no evidence of neurological impairment. Hemoglobin was 112 g/L. The parents were informed of the risk in case of future pregnancies.

Vw (Verweyst) belongs to the MNS blood group system and is secondary to a gene conversion between genes encoding for glycophorin A and glycophorin B (hybrid A-B-A). The insert encoding the Vw gene results in an amino acid polymorphism at position 28 (Threonine → Methionine) leading to the loss of N-glycosylation of the adjoining asparagine at position 26. It is a LIA but its prevalence has been reported to be as high as 1.43% in southeastern Switzerland [7]. This antibody has been associated with severe HDFN [5, 7]. As expected, in all reported cases, prenatal IAT were negative and, since pregnancies were clinically uncomplicated, diagnosis was only made at birth. Newborns were treated with exchange transfusion and/or transfusion and intensive phototherapy. Retrospectively, some older asymptomatic siblings were also tested Vw positive. In our case, HDFN occurred already in the second pregnancy after a probable immunization during the first pregnancy (termination).

This case report highlights two different aspects that are severe anemia of the newborn despite negative screening tests and apparent suppression of hematopoiesis due to alloantibodies. Alloantibodies against LIA are of little concern in pretransfusion testing, because the risk for a patient to receive incompatible red cell units remains very low. In practice, the screening red blood cells used in antenatal testing are identical to those used in pretransfusion testing. According to the recommendations of the Swiss Transfusion Service, the panel cells that are used must include antigens with a frequency of more than 8% of the Caucasian population. Thus, it is important to remember that routine IAT testing ignores most antibodies that are specific for LIA and missing such antibodies may be of great concern in some cases, taking into account that the probability of fetal inheritance of the incompatible antigen can be as high as 100%, if the father carries the antigen. Therefore, if the antibody screen is negative but alloimmune hemolytic disease is still suspected, testing of the father’s red blood cells with the maternal serum is of great interest because reagents for rare antigen are not available in all laboratories. In presence of ABO incompatibility between the mother and the father, the absorption of anti-A and anti-B should be performed.

The present case is also relevant because hemolysis was associated with severe reticulocytopenia and absence of erythroblastosis at birth, and was quite prolonged, therefore a transfusion was still necessary at day 27 of life. A similar observation was reported in the literature (absence of significant reticulocytosis and late transfusion requirement) [5]. These findings suggest that a suppression of erythropoiesis at the progenitor-cell level by anti-Vw, exacerbating anemia, may be involved in such cases. Inhibition of erythroid progenitors has already been demonstrated in vitro for anti-Kel sensitization [8, 9], and more recently for anti-Gerbich 3, an antigen of glycophorin C and D [1, 3]. Glycophorin A appears to present early in maturation stage of red cells (before RhD but after Kell antigens), as demonstrated with assays on erythroid cultures [2]. Nevertheless, pathogenicity of anti-Vw remains unknown and this hypothesis requires further in vitro investigations. Glycophorin contributes to most of the carbohydrates on the red blood cell membrane, which have abundant sialic acid, and confers negative charge, but nothing is known about a putative role in erythroid differentiation or growth.

In this case, the diagnosis of HDFN was only suspected after birth because the DAT was positive. DAT is known to have a limited usefulness to screen HDFN. A retrospective study of 1724 DAT analyzed on unselected umbilical cord blood samples showed a positive predictive value of 23% and a sensitivity of 86% for the subsequent development of hyperbilirubinemia [4]. However, the significance of a positive DAT should not be neglected in the presence of non-regenerative anemia of the newborn, even if IAT of maternal serum is negative.

This case confirms that anti-Vw may be responsible for severe HDFN, which is certainly always missed during routine screening, and may be associated with suppression of erythropoiesis. Unfortunately, no cost-effective routine prenatal testing can be offered to prevent such a complication. However, we should keep in mind, that if fetal anemia is suspected, crossmatch of the paternal red blood cell with the maternal serum can help to rule out or confirm alloimmunization.

In case of subsequent pregnancies (or of a similar situation in another patient), the following recommendations can be proposed. We strongly favor regular scanning of the middle cerebral artery peak velocity to detect fetal anemia early in pregnancy and to propose intrauterine fetal transfusions, if necessary. Prenatal phenotyping or genotyping [7] would be of great interest, since homozygous individuals are exceptional. However, the risk of complications of an invasive procedure should be carefully taken into consideration. Finally, PCR analysis of free fetal DNA in maternal blood samples should be discussed, taking into account the fact that this test has not been validated for “routine” MNS polymorphism evaluation. Titration of anti-Verweyst antibodies with paternal red cells is also possible but is not recommended because of its potentially poor predictive value, as in anti-K titration.

In conclusion, this case confirms the clinical and biological heterogeneity of HDFN, and highlights the limits of routine prenatal IAT. The question is whether the increasing use of Doppler ultrasound to detect fetal anemia will diminish the number of late diagnoses.
References


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