Case Report

A mild case of hemolytic disease of the fetus and newborn due to anti-Sc2

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We report the case of a newborn girl with jaundice due to increased indirect bilirubin with a positive direct antiglobulin test (DAT) and compensated hemolysis. The result of the newborn's DAT was discrepant with the negative result of the mother's indirect antiglobulin test. The multiparous mother had a previous history of fetal hydrops miscarriage, with no known cause, and no record of the cause was found at the hospital where she was treated. After referring samples from the mother and newborn to a reference laboratory, the rare alloanti-Sc2 was identified in the mother's plasma and in the newborn's eluate. HEA BeadChip genotyping of the newborn's DNA sample predicted the SC:1,2 phenotype. ***Immunohematology* 2021;37:122–125. DOI: 10.21307/immunohematology-2021-018.**

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Hemolytic disease of the fetus and newborn (HDFN) occurs when the pregnant mother is alloimmunized with immunoglobulin (Ig)G-type antibodies specific for antigens present on the red blood cells (RBCs) of the fetus. These antibodies cross the placenta, and hemolysis occurs when the maternal antibody binds to the fetal RBC antigens, generating a binding to the Fc receptor of macrophages in the spleen of the fetus. After delivery, the continuous destruction of RBCs can cause progressive anemia and hyperbilirubinemia, or only hemolysis if the newborn is able to compensate for the anemia.[1–3]

During gestation, the destruction of fetal RBCs releases hemoglobin, which is transformed into bilirubin. This bilirubin passes through the maternal circulation and is metabolized by the maternal liver. After birth, however, bilirubin remains in the neonatal circulation. The immature liver of the newborn cannot conjugate bilirubin efficiently, causing excess unconjugated bilirubin to cross the immature blood–brain barrier of the newborn and, in significant amounts, can result in permanent brain damage, known as kernicterus.[1–3]

If the antibodies involved are specific against common RBC antigens, it is possible to perform the investigation in most blood banks. However, if the specificity of the antibody is directed against an antigen for which there is no commercial antiserum and if, in addition, the antigen is not represented on conventional reagent RBC panels, the investigation is complex and can only be performed in an immunohematology reference laboratory (IRL).

The Scianna blood group system consists of eight antigens: one pair of antithetical high- and low-prevalence antigens (Sc1 and Sc2), one antigen of low prevalence (Sc4), and five antigens of high prevalence. The eighth high-prevalence antigen in this blood group system was recently characterized by Srivastava et al.[4] and was named "SCAR"; there are still no reports of hemolytic transfusion reaction (HTR) or HDFN due to antibodies against this antigen. All antigens are localized in the glycoprotein of the immunoglobulin superfamily, ERMAP. The *SC* gene is linked to the *RH* genes on chromosome 1p.[4,5]

There are few reports of antibodies directed against antigens in the Scianna blood group system. These antibodies react more efficiently in the antiglobulin phase and do not bind complement. Two cases of HDFN due to anti-Sc2 have been previously reported, and the first case of a severe acute HTR caused by anti-Sc2 was reported in 2018.[6–8]

We herein report the first case in Chile of compensated hemolysis and jaundice caused by blood group incompatibility between mother and fetus due to anti-Sc2.

Case Report

A sample from a 31-year-old pregnant Chilean woman typed as group A, D+ with a negative indirect antiglobulin test (IAT). The woman had a history of a previous fetal hydrops miscarriage at 29 weeks of gestation and of receiving transfusions previously. At 34 weeks, in a premature delivery in the city of Temuco, IX region of Chile, she gave birth to a baby weighing 2080 g, 43 cm in length, whose blood sample typed as group A, D+ with a positive direct antiglobulin test (DAT) and negative IAT.

The newborn presented with jaundice at 48 hours after delivery, with a progressive increase in bilirubin: a 9-day peak of 16.3 mg/dL of total bilirubin (normal <12.6 mg/dL) and

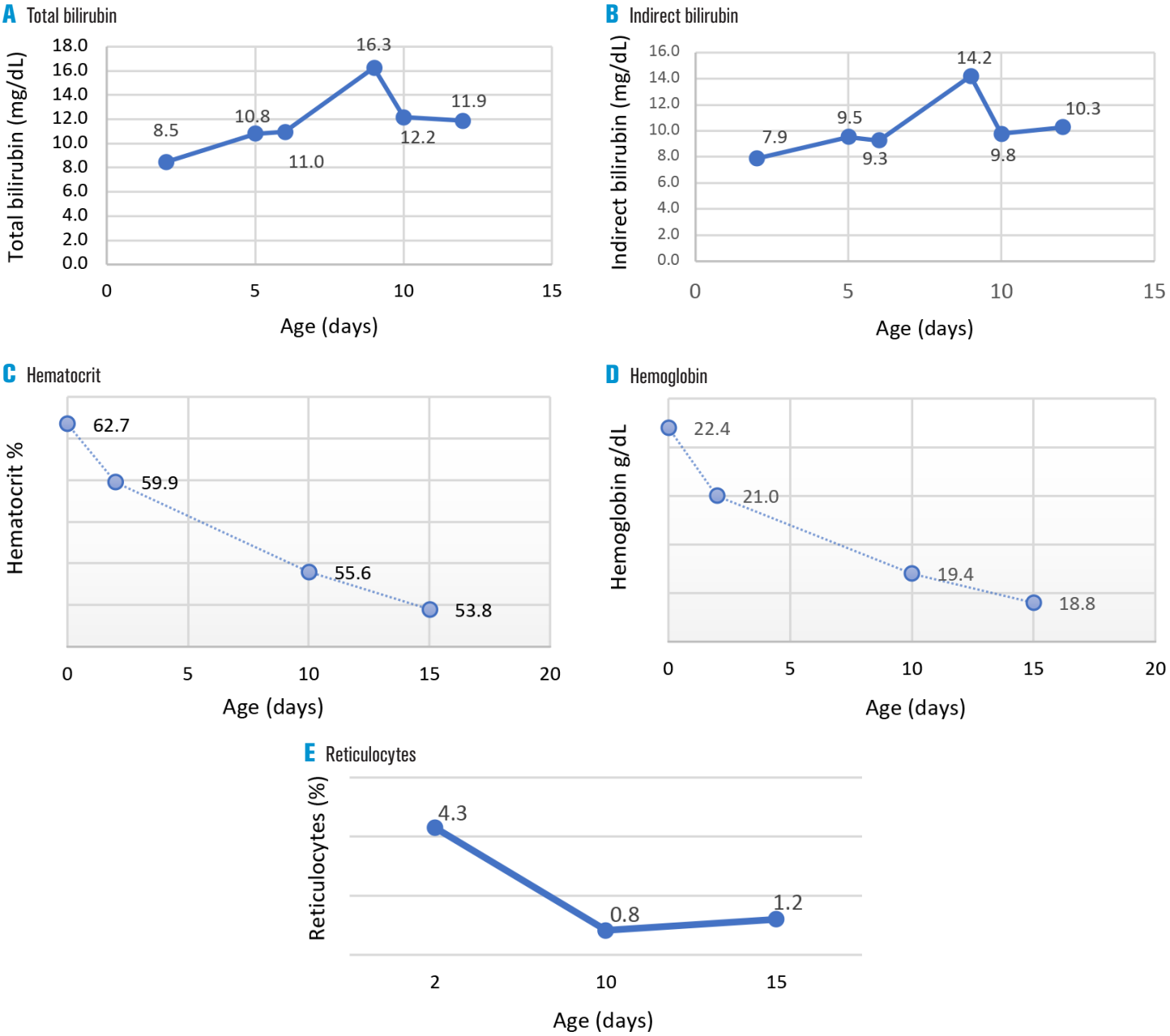

Fig. 1 (A) Total bilirubin concentration of the newborn on days 2, 5, 6, 9, 10, and 12 after birth. (B) Indirect bilirubin concentration of the baby on days 2, 5, 6, 9, 10, and 12 after birth. (C) Newborn hematocrit values at birth and on days 2, 10, and 15 after birth. (D) Hemoglobin values of newborn at birth and on days 2, 10, and 15 after birth. (E) Reticulocyte values measured on days 2, 10, and 15 after birth.

14.2 mg/dL of indirect bilirubin (normal range not provided) (Fig. 1A and B). In the hemograms performed on days 0, 2, 10, and 15 after birth, decreases in hematocrit and hemoglobin were observed (Fig. 1C and D). The reticulocyte count is shown in Figure 1E.

The discrepancy between the negative IAT result in the mother's plasma and the positive DAT result on the baby's RBCs suggested an antibody against a low-prevalence antigen. Compatibility tests between the mother's plasma and the father's RBCs, which typed as group O, D+, and between the baby's eluate and the father's RBCs were performed. The results are shown in Table 1.

Crossmatching results suggested the presence of an antibody reactive by IAT against a low-prevalence antigen inherited by the newborn, expressed on the father's RBCs, and absent on the reagent RBCs used for screening. Samples

Table 1. Crossmatching between mother, father, and newborn

Sample	Results at AHG phase of IAT
Mother's plasma and father's RBCs	2+
Newborn's eluate and father's RBCs	2+

AHG = antihuman globulin; IAT = indirect antiglobulin test; RBCs = red blood cells.

from the mother and the newborn were sent to an IRL for the identification of the antibody. The results obtained in the reference laboratory are shown in Table 2.

Table 2. Immunohematologic results obtained on samples from the mother and newborn at the reference laboratory

Test performed	Method used	Results on maternal sample	Results on newborn's sample
ABO and D typing	Microplates, NEO*	Group A, D+	Group A, D+
IAT	Capture, NEO*	1+	Negative
Antibody identification	Capture, NEO*	Anti-Sc2	
DAT (anti-IgG)	Capture, NEO*		3+
DAT (anti-C3b, -C3d)	Tube method, monoclonal murine antiserum*		Negative

*NEO; Immucor, Norcross, GA.
IAT = indirect antiglobulin test; DAT = direct antiglobulin test.

An eluate using an acid elution test (ELU-KIT II; Immucor, Norcross, GA) performed on the newborn's RBCs demonstrated the presence of anti-Sc2. Anti-C^w, -Co^b, -Js^a, -Go^a, -Lu^a, -Kp^a, -Di^a, and -Mt^a specificities were excluded. The results are shown in Table 3.

Table 3. Detection and identification of antibody results obtained in the newborn's eluate at the reference laboratory

Test performed	Method used	Results of newborn's eluate
IAT	Capture, NEO*	1+
Antibody identification	Capture, NEO*	Anti-Sc2

*NEO; Immucor, Norcross, GA.
IAT = indirect antiglobulin test.

To confirm the presence of Sc2 on the newborn's RBCs, DNA was extracted from the newborn's peripheral blood using a DNA mini-kit (QIAamp; Qiagen, Hilden, Germany), and genotyping was performed using a DNA array platform (HEA BeadChip; BioArray Solutions, Immucor), which includes the detection of gene polymorphisms encoding for the low-prevalence antigens: V, VS, Js^a, Co^b, Di^a, Lu^a, Kp^a, and Sc2. Genotyping results by HEA BeadChip only showed the presence of the *SC2* gene polymorphism responsible for the expression of the low-prevalence antigen, Sc2. In addition, the presence of Yt^b was also excluded with an *in vitro* diagnostic testing kit (BAG Health Care GmbH, BAG Diagnostics GmbH, Lich, Germany).

Discussion

This report presents the first case of HDFN due to anti-Sc2 in Chile as well as the first case of the presence of Sc2 in Chileans. We do not know if the presence of this antigen is associated with any Amerindian ethnicity. In this case, the father indicated that his paternal great-grandmother had a Mapuche surname (the Mapuche compose the major ethnic group in Chile). Nevertheless, to determine whether there is an association between the presence of Sc2 and Mapuche ancestry, a larger number of samples is required.

There are only two cases previously reported of HDFN due to anti-Sc2—one of them very mild and the other requiring neonatal RBC transfusion.[6,7] The case we presented herein was mild. The newborn presented with jaundice, increased total and indirect bilirubin, and decreased hematocrit and hemoglobin, although these values remained within the normal range, without observed anemia. Newborn laboratory tests (hemogram and reticulocyte count) were reviewed by a pediatric hematologist, who diagnosed this case as a compensated hemolysis. The degree of hemolysis and the difference in the severity of the disease may be due to the immunoglobulin (Ig)G subclass present, the antibody titer, and the number of RBC antigen sites. Antibodies of the IgG1 and IgG3 subclasses are more efficient in producing hemolysis than those of IgG2 or IgG4, although it was not possible to determine the IgG subclass in this case.[1]

To determine the clinical significance of an antibody, the monocyte monolayer assay (MMA) can be performed. The MMA is an *in vitro* functional cellular assay, used to differentiate between clinically significant and insignificant RBC antibodies. In this workup, the use of MMA would have allowed a more accurate assessment of the clinical significance of the anti-Sc2; this testing was not done, however, because this method is not available in Chile.[9]

In this case, the first diagnosis of HDFN was based on jaundice and on the result of a positive DAT in the newborn, which was not possible to confirm in the patient's hospital of origin. This situation emphasizes the importance of IRLs to evaluate complex clinical cases, integrating serologic and molecular methods.

Knowledge of the cause of maternal–fetal incompatibility is important because it allows the diagnosis to be made and counseling to be provided to the parents about the risks of future pregnancies so that they can make informed decisions. This report is also important to make the medical community aware of the existence of HDFN due to antibodies against rare blood group antigens.

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