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Usefulness of absorption techniques in doubtful cases of incompatibility Rh maternal fetal: a case report.

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ABSTRACT

INTRODUCTION

This case report traces the diagnostic and therapeutic process of the Immuno-Transfusion Service in a case of fetal anaemia characterized by various inconsistencies between clinical and laboratory data.

The paper describes an alternative technique implemented with the aim of resolving incompatibilities between positive IAT and negative DAT.

In addition, the algorithm necessary to reach a diagnosis in a transfusion medicine laboratory is traced, namely, the performance of first- and second-level immuno-haematological tests, the evaluation of fetal clinical signs, and the preparation of blood components necessary for intrauterine transfusion.

CASE PRESENTATION

The case involves a pregnant woman who comes to the Immuno-Transfusion Service following a positive Indirect Antiglobulin Test and fetal anaemia.

The main questions relate not only the presence of immunization in the woman, which should have been avoided by immunoprophylaxis (being group 0 Rh negative), but also the stability of the antibody titer (or level) throughout the pregnancy, fetal recovery after TIU, and the presence of a negative Direct Antiglobulin Test.

CONCLUSIONS

Questions that were completely resolved at caesarean section with the discovery of a giant chorioangioma that, by sequestering fetal red blood cells, caused anaemia and the confirmation of a rare blood phenotype of the father that caused the woman's immunization.

Keywords: Indirect antiglobulin test (IAT), haemolytic disease of the newborn, Rh alloimmunization, fetal anaemia, giant chorioangioma.

INTRODUCTION

Fetal anaemia is a condition in which the number of circulating red blood cells in the fetus blood decreases. The most common causes can be traced to maternal-fetal isoimmunization (HDNF), parvovirus infections and hemoglobinopathies.

More rarely, fetal anaemia may be the consequence of giant chorioangiomas, which have a prevalence ranging from 1 in 9000 to 1 in 50000. In these cases, the vasculature present in the tumour formation sequesters fetal red blood cells to allow the mass to proliferate and survive.

Haemolytic Disease of the Newborn and Fetus (HDNF) is a condition due to incompatibility between maternal and fetal blood types, specifically maternal antibodies cross the placenta and cause destruction of fetal red blood cells leading to fetal anaemia that, in severe cases, can progress to fetal hydrops, heart failure, and death [1].

HDNF is often classified into three categories based on the specificity of the antibodies that cause it:

- Rhesus HDN is the most common and is related to the presence of isolated Anti-D or associated with anti-C or anti-E; it occurs in Rh negative mothers (immunized in previous pregnancies) with Rh positive fetuses;
- AB0 HDN affects 15 to 25 percent of all pregnancies, however, only 1 percent of people will develop it and it occurs in group 0 mothers with Anti-A, B or from isolated Anti-A or Anti-B antibodies;
- more rarely, HDNF can be caused by the presence of antibodies to antigens of minor systems.

In the mid-20th century, HDNF, mainly the Rhesus type, was a major cause of fetal and neonatal morbidity and mortality.

The introduction of immunoprophylaxis (1968) reduced the mortality rate from 50% to 0.5%, which was further decreased to 0.1% by Rh D antepartum immunoprophylaxis (1970). Despite anti-Rh prophylaxis 1 to 3 women per 1000 Rh negative women develop alloimmunization [2].

Current guidelines recommend immunoprophylaxis with anti-Rh(D) IgG to every Rh(D)-negative non sensitized woman.

Immunoprophylaxis is done as follows: at 28 weeks gestation during each pregnancy, immediately after delivery of any Rh(D)-positive infant, and in the context of any other event that might expose her to Rh(D) antigen (e.g., abortion, abdominal trauma).

The only scenarios in which prenatal administration of anti-D IgG is unnecessary are occurring when the father is also Rh(D)-negative, or if the fetus is successfully typed for Rh(D) status by prenatal free DNA testing on maternal plasma [3].

Laboratory monitoring for diagnosis of maternal-fetal immunization involves the determination of blood groups and detection of any irregular alloantibodies in the mother's plasma by performing an Indirect Coombs Test (TAI) accompanied by the performance of 11- and 22-cell panels.

The woman is then subjected to antibody titer monitoring. A titer above 16 BAU/ml for the antibodies under investigation is empirically determined as a threshold value indicating an increased risk of HDNF and justifies a closer surveillance by a specialist with ultrasound measurements of peak systolic velocity (PSV) in the fetal middle cerebral artery (MCA) [4].

From these evaluations, the need for intrauterine transfusion (IUT) may be requested, allowing a distinction to be made, depending on gestational age, between areas of medium and high risk

for anemia. In experienced hands, IUT is now considered a relatively safe procedure and the most effective transfusion practice in rapidly correcting severe fetal anaemia [5].

In this clinical case, it was also important to study some rare phenotypes of the D antigen because a partial D phenotype was analysed, which is characterized by blood cells that lack one or more epitopes of the antigen in question.

Most of the D-positive hematomas show clear macroscopic agglutination with an anti-D serum after centrifugation; for some D+ red blood cell samples, the determination of the presence of the D antigen requires more caution; in fact, some categories of partial D cannot be determined with monoclonal anti-D reagents. Therefore, adding small aliquots of IgG-class anti-D antibodies to IgM-class monoclonal sera produces a mixture of antibodies that will also react with partial D antigens, allowing their identification [6].

The alternative technique performed in case resolution was based on this principle. The purpose of this report is to review the diagnostic procedure undertaken for the management of a particular clinical case in transfusion practice and to provide useful suggestions for the future management of similar cases.

CASE PRESENTATION

The case involves a pregnant woman at 19 weeks gestation, 31 years old and of Arab ethnicity who came for observation at the Immuno-Transfusion Service for a positive Indirect Coombs Test finding.

The woman's medical history showed a single immunization event represented by a full-term pregnancy in 2017 accompanied by a positive TAI (Indirect Coombs Test) since then.

As per the algorithm various tests were performed which included blood group determination and phenotype which was found to be 0 Rh negative ccdee kk and positive TAI with uneven scores (2+,3+,0) leading to hypothesize the presence of an antibody mixture, confirmed by performing 11 and 22-cell panels, like the presence of a mixture of anti-D and anti-C. Antibodies .. were then defined as alloantibodies since self-testing was negative as well as the Direct Coombs Test (TAD). All tests were performed on "BIOVUE" cards from Ortho-Clinical Diagnostics® (Ortho-Clinical Diagnostics, Ins. Pencoed, UK). As indicated above, antibody titration is performed to assess the degree of pregnancy risk. The anti-C antibody titer was found to be clinically insignificant because <1:2, unlike the anti-D titer which was found to be 1:256, thus critical.

The woman underwent antibody titer monitoring every 4 weeks.

At 28WG (week of gestation) on flowmeter examination there was evidence of altered peak of the middle cerebral artery flow velocity (MCA - PSV) and fetal cardiomegaly, both signs of fetal anaemia. These alterations lead to transfusion support.

Therefore, the preparation of E.C. units (concentrated erythrocytes/RBC) useful for IUT, fresh (no more than 5 days after collection), CMV safe and with extended erythrocyte set-up as close as possible to maternal.

Compatibility testing was performed between the pregnant woman's plasma and the chosen unit; this was then washed with saline and further concentrated.

A CBC was performed to assess the hematocrit, which was found to be 80% (expected values 70-80%). The bag was irradiated and then sent to the ward.

The newborn was transfused with a concentrated blood volume of 22ml, appropriately diluted to have a hematocrit of 50%.

During the intrauterine transfusion, a funicular blood sample was taken on which were performed: blood group determination and phenotype found to be A Rh negative Ccdee kk and TAD found to be negative. A HDNF would have been characterized by a positive TAD. In this particular case, the TAD was negative, so other hypotheses were considered to justify the fetal anaemia.

Given the woman's immunization toward anti-D and the TAD result, it was necessary to formulate new hypotheses, described more fully in the "Conclusions" section, which needed the development of an alternative serologic technique to explain the inconsistency between the positive TAI and the negative TAD and also included the father of the child because of his particular phenotype (A Rh NEGATIVE CCdee kk). It was then decided to react the maternal plasma with the father's blood cells, as the mother produced the anti-D antibodies specific for the paternal D antigen as a result of the antigenic stress she had with her first pregnancy and thus after coming in contact with half of her husband's genetic makeup.

This method, however, presented problems in as the mother's plasma being group 0, presented, in addition to the presumed anti-D natural, anti-A antibodies that would bind to the A antigens present on the father's blood cells (group A) thus leading to a false-positive reaction that would not allow the presence of the D antigen to be discriminated.

Therefore, the mother's plasma was placed in contact with Rh negative A blood cells for 2h at 37°C (Absorption Technique) so as to cleanse the mother's plasma of anti-A.

Once the absorbed plasma and the father's blood cells were brought into contact, a strong positive reaction (agglutination with 2+ score) was obtained, thus allowing the detection of a hitherto undetectable antigen. In addition, to evaluate the detection power of the anti-D monoclonal sera (or serums) supplied to us by the company OrthoClinical Diagnostic®, mixes containing in varying ratios monoclonal serum and mother's plasma were created as per below:

- Mix 1: 50 ul of the child's mother's plasma + 50 ul of anti-D monoclonal serum supplied by OrthoClinical Diagnostic.
- Mix 2: 70 ul of mother's plasma + 30 ul of the company's serum.
- Mix 3: 30 ul of mother's plasma + 70 ul of company serum.

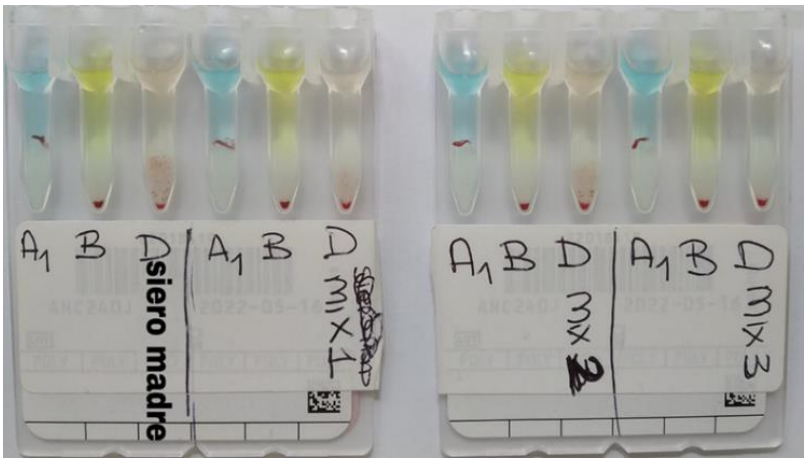

Figure 1: cross test between the father's blood cells with the prepared polyclonal sera and the mother's serum

The results obtained showed that agglutination is much more evident in wells where the ratio is in favour of maternal plasma. In fact, the reaction scores can be defined as follows:

- Mix 1: a very weak reaction positivity is shown (score + 0.5);
- Mix 2: positive reaction with score 1+;
- Mix 3: negative reaction.
- Maternal plasma: score 2+.

These results confirmed the presence of a serologically undetectable D variant phenotype of the father by normal means (Figure 1).

After transfusion support, there was an increase in fetal haemoglobin and a reduction in fetal cardiomegaly was clearly seen.

The pregnant woman underwent antibody titer monitoring every week until the date of planned caesarean delivery at 38 weeks, with consistent results.

During surgery, a cord blood sample was collected on which group A Rh negative was confirmed and TAD was performed, which was positive with specificity for IgG (Figure 2).

Acid glycine elution was performed to identify antibodies adhered to the blood cells, and the eluate showed only the presence of anti-A antibodies with a titer of 1:2, which was not clinically significant, further ruling out the hypothesis of ABO HDN.

Thus, the presence of anti-D, which was thought to be responsible for the presumed fetal haemolytic anaemia, was not detected.

At the cesarean section, the presence of a giant placental chorioangioma, which sequestered haemoglobin from the fetus and rendered it anaemic, was noted.

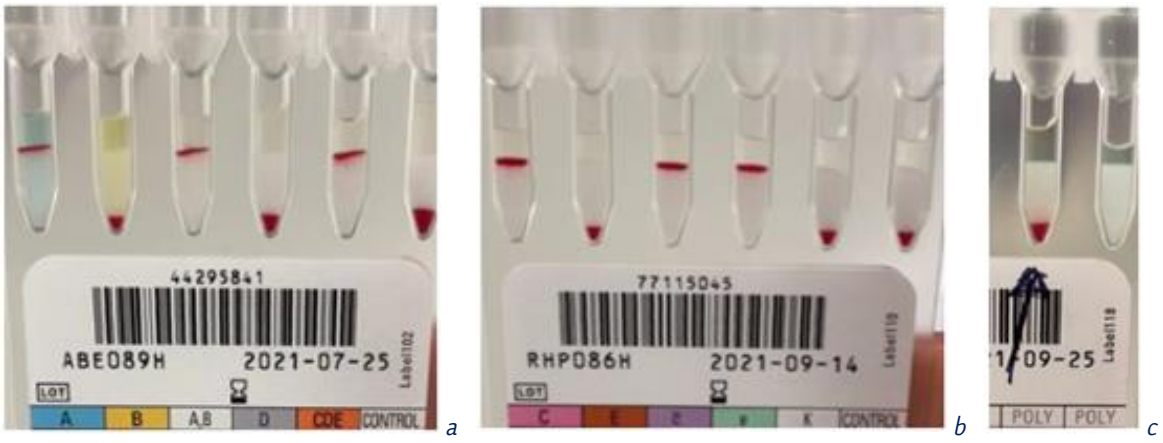

Figure 2: tests performed on cord blood: a) direct group determination: A Rh negative; b) phenotype determination: ccdee kk; c) TAD: positive

CONCLUSIONS

This clinical case raised many questions during its resolution starting right from the pregnant woman's arrival at our Immuno-Transfusion Service.

The presence of anti-D in the woman's plasma, highlighted by antibody identification, cast doubt on the actual immunoprophylaxis recommended for all Rh-negative women provided by the ISS (Istituto Superiore della Sanità – Italian National Institution of Health) guidelines. Her immunization led to speculation of either failure of previous immunoprophylaxis or failure to administer it.

Extensive data collection confirmed the failure to administer anti-D immunoglobulin by virtue of the child's father's blood type, which was found to be A Rh NEGATIVE CCdee kk [3].

At this point, the woman's immunization could be attributed to two other hypotheses: 1) a suspicion of paternity, ruled out after private interview with the pregnant woman; 2) possible D variant of the father, a hypothesis corroborated by the paternal phenotype: ccdee kk which turns out to be extremely rare in individuals lacking the D antigen.

In view of this suspicion, samples from the father of the unborn child were subjected to molecular analysis of the Rh genotype and phenotype.

At the same time, it was decided to undertake the alternative serologic method mentioned above to identify the possible D antigen present on the father's blood cells and confirm its presence.

When, at 28WG the newborn was transfused, on the cord blood, in addition to blood group determination (A Rh negative ccdee kk), TAD was performed which was negative, thus leading to the hypothesis that fetal anaemia could be found in other causes.

Also, in favour of this hypothesis was the improvement in fetal condition after IUT and the constant maternal antibody titer throughout pregnancy. This is because in HDNF, the antibody titer tends either to increase gradually by virtue of antigenic stress, or to decrease abruptly suggesting that IgG has crossed the placenta and bound to fetal antigens.

Although doubts had not yet been resolved, at 38WG the woman underwent caesarean section and the following were performed: blood group determination confirmed as A Rh negative and TAD which was positive.

An acid glycine elution was then performed to identify the antibody adhered to the newborn's blood cells, and it was found to be an anti-A antibody with a titer of 1:2, which was so low as to rule out even the hypothesis of an AB0 HDN.

Thus, the presence of the anti-D that was thought to be responsible for the presumed fetal haemolytic anaemia was not detected.

The cause of the anaemia had not yet been found, until the presence of a giant chorioangioma was detected at caesarean section, which sequestered haemoglobin from the fetus and made it anaemic.

There are several cases in the literature that report the presence of chorioangioma, evidenced by ultrasound checks, as a cause of fetal anaemia [5,6].

In this specific case, the signs of fetal anaemia (haemoglobin drop, altered MCA - PSV, and cardiomegaly) coincided with the known data and guidelines, but the inability to define with certainty the tumour formation as the cause of fetal anaemia did not allow the achievement of an early diagnosis.

On the contrary, that case, raised further doubts as the emerging results of immuno-haematological analysis and clinical signs did not collide.

Therefore, it remains a case in which the collaboration of various professional profiles (laboratory technician, transfusion physician, gynaecologist, obstetricians) was necessary both for the protection of the health of the pregnant woman and the fetus and for the reconstruction of the entire clinical picture since there were no guidelines indicating how to act in the literature, given the particularity of the events.

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