In Response: Hemolytic Disease of the Fetus and Newborn: Understanding the Testing Needed to Confirm the Identity of the Causative Antibody

Manisha M. Beck[1] V. Hamsavardhini[2] Preethi Navaneethan[1] Manish Kumar[3]

[1] Fetal Medicine Unit, Christian Medical College and Hospital, Vellore, Tamil Nadu, India
[2] Departement of ObGyn, Christian Medical College and Hospital, Vellore, Tamil Nadu, India
[3] Department of Neonatology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India

Address for correspondence Manisha M. Beck, MD, Professor and Head, Fetal Medicine Unit, Department of ObGyn, Christian Medical College and Hospital, Vellore, 632004, Tamil Nadu, India (e-mail: beckmanisha@yahoo.com; fmu@cmcvellore.ac.in).

J Fetal Med 2023;10:137–138.

First, we would like to thank the authors for the thought-provoking questions, which are relevant to the case report. The authors have asked the question about how the diagnosis of anti-M alloimmunization was made in the neonate.

Mother's plasma and breast milk had shown anti-M antibodies (the process of detection and quantification has been described in detail below). There was fetal anemia and signs of early hydrops on fetal scans prior to delivery. At birth, the neonate had presented with hemolytic hyperbilirubinemia. M antigen typing of the newborn was done and showed a positive reaction. Both mother and newborn had the same blood group, A positive, which eliminates other causes of neonatal anemia and jaundice such as ABO or Rhesus incompatibility.

The authors have further raised queries about the methodology involved in the detection and quantification of anti-M antibodies in maternal plasma and breast milk. We did not mention these in the original case report, considering the readership would be mostly obstetricians and fetal medicine specialists, and the key message we want to convey through this case report is that anti-M alloimmunization is a reality and that there is a phenomenon of ethnic susceptibility, hence it is much more common in Asians than Caucasians.[1] The methodology of anti-M detection and quantification are described below.

Breast Milk Collection

Breast milk samples were collected after sufficient feeding was established. There was no protocol available for the collection of breast milk. We, therefore, decided to collect samples within 2 to 5 days of establishment of feed to maintain uniformity in the study. Samples were collected before giving the next feed. At least 1 mL of breast milk (~ 16 drops) was collected from every mother in a sterile container without anticoagulant. Fresh uncentrifuged breast milk was used for testing.[2]

Antibody Screen and Identification

Red cell antibody screen and indirect Coombs test (ICT)/indirect antiglobulin test (IAT) were performed using column agglutination technique (LISS/Coombs IDCard, DiaMed Gmbh, Bio-Rad Laboratories, Switzerland). Fifty microliters of screen cells and 25 µL of mother's plasma/breast milk were incubated at 37°C for 15 minutes and then centrifuged for 10 minutes in the DiaMed card centrifuge. ICT was performed using 3 to 5% suspension of saline-washed pooled phenotyped "O" group cells and patient plasma along with negative and positive controls. Positive control for IAT was 1 in 10 dilutions of immunoglobulin G anti-D, while negative control was AB group plasma. Identification of antibody, if present, was performed using 11-cell identification panel (ID-DiaPanel, DiaMed Gmbh, Bio-Rad Laboratories, Switzerland) in the Coombs phase.

Titers of Anti-M Red Cell Alloantibodies

Titers were performed for anti-M red cell alloantibodies if identified using the column agglutination technique. Titers were performed in the saline (NaCl, Enzyme test and Cold agglutinins DiaMed Gmbh, Bio-Rad Laboratories, Switzerland)

article published online
December 12, 2023

DOI https://doi.org/10.1055/s-0043-1777434.
ISSN 2348-1153.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

and Coombs (LISS/Coombs IDCard, DiaMed Gmbh, Bio-Rad Laboratories, Switzerland) phase. Breast milk was titrated in the same way as the plasma. For the Coombs phase titer, the plasma and milk were treated with dithiothreitol (Sigma Aldrich Co., St. Louis, MO, USA) for inactivation of immunoglobulin M antibodies. The titer was determined by testing the serial twofold (2-1024) dilution of the plasma/breast milk against selected red cells. One hundred microliter of plasma/breast milk was added to a tube containing 100 µL saline labeled as 1:2. Further, 100 µL was transferred from the tube 1:2 to 1:4 and continued through the remaining tubes that contained 100 µL saline each. Twenty-five microliters from each of these dilutions were added to the correspondingly labeled columns in the card. Fifty microliters of 0.8% red cells (M antigen positive O red cells) were added to the columns and titers were reported as the column with 1+ agglutination.

Conflict of Interest
None declared.

Acknowledgements
The authors would like to thank Gayathiri Kaduvetti Chellaiya, Assistant Physician, Department of Transfusion Medicine and Immunohematology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India.

References

1 Yu M, Graham K, Pasalic L, Alahakoon TI. Recurrent fetal hydrops with maternal M alloimmunisation: not a benign condition. BMJ Case Rep 2019;12(07):e230552
2 Kumar S, Rasalam JE, David VT, et al. Breast milk contains red cell isohaemagglutinins: an observational study of 176 mothers. Vox Sang 2022;117(06):847–852

