



# Final Commentary on Anti-M Alloimmunization Following Term Stillbirth: A Case Report and Review of the Literature

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In a unique editorial exercise, perhaps for the first time in the history of medical publishing, it was decided to forward the reply to a critique of a manuscript<sup>1</sup> to the authors of the critique, to possibly close the circle, or, finally leave it open for further inputs from the realm of future research and understanding.

The following reviews were received. These have been edited to exclude overlapping sections and confidential feedback.

These make for very relevant feedback to the very process of scientific logic and are intended to facilitate open thinking. These should not be viewed in the perspective of rejection of the conclusions of the original manuscript. They need to be viewed in the open space of differences of opinion in emerging multispecialty medical fields.

In a letter to the editor, Abels and Jacobs commented that a label of hemolytic disease of the fetus and newborn (HDFN) mediated by antibodies against non-Rhesus blood group antigen systems was not justified by the methods used.

In their opinion, the authors did not provide compelling evidence that the antibody causing the fetal anemia was anti-M other than a positive indirect antiglobulin test (IAT) for anti-M in maternal plasma and a coincidentally positive direct antiglobulin test (DAT) on neonatal cells. They felt the need for information regarding plasma studies in the newborn, elution studies, and low-incidence antibody testing, and stated that a positive newborn DAT is nondiagnostic and requires further evaluation, as even a negative maternal IAT does not preclude the possibility of HDFN to a low-incidence maternal alloantibody. They went on to state that contemporary and historical literature of larger case series

found that in HDFN caused by anti-M the neonatal DAT is more frequently negative than positive.<sup>2–4</sup> Therefore, the positive DAT cited by Beck et al could theoretically lower one's suspicion for the cause of HDFN being solely due to anti-M. Additionally, when discussing any case of HDFN, but especially with anti-M where the antibody's isotype and reacting temperature are questionable, the testing methods including platform technology, temperature, and enhancement media are vital to the discussion. They went on to state that although this case could be an important addition to the growing body of evidence supporting anti-M as a cause of HDFN, further investigation and reporting are required to definitively establish this conclusion.

Beck et al furnished replies<sup>5</sup> clarifying newborn M antigen typing, the technique of breast milk collection, the methodology for red cell antibody screen, indirect Coomb's test (ICT), IAT, and the methodology for column agglutination technique (CT) for titers for anti-M red cell if found positive.

As mentioned earlier, to bring closure to the discussion, we considered a novel approach of sending the clarification for review to the critics themselves.

The comments of Abels were as follows and are being reproduced verbatim to open a possibly different perspective on the matter by our readership.

“There are many other antibodies including private antibody/antigens that have been known to cause HDFN. Anti-M is an extremely common antibody in pregnancy and the M antigen is a very common antigen. Therefore, HDFN caused by anti-M should be a diagnosis of exclusion. Rare antibodies should be ruled out (more

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than an 11-cell panel would be needed for this), and at minimum, an eluate should be done for the positive DAT to confirm the antibody. This reviewer believes this case presents vital information for the HDFN literature, however, the conclusions drawn by the authors are not possible given the testing performed. This mom is clearly an antibody former given her history of anti-Jkb, anti-Fyb, anti-Lea, and anti-M. She has had multiple pregnancies from the same father and thus private antigen or antibody of low incidence is a very real concern here. This reviewer believes the appropriate conclusion should be: anti-M is a possible cause, however, the case highlights the importance of ruling out the low incidence and private antigens in cases of fetal demise/HDFN.”

Upon review of the original manuscript, there was no direct evidence that anti-M was responsible for the fetal anemia. Indeed, from the data provided, it was not even definitively secondary to maternal alloimmunization. The authors did not exclude other causes of hydrops and fetal anemia, including inherited red blood cell (RBC) disorders and hemoglobinopathies, which can present with essentially identical findings.

However, if we assume this was secondary to alloimmunization, the authors have not sufficiently addressed the original letter writers' comments definitively proving anti-M as the cause. For example, the authors report the mother had anti-Fy(b) and anti-Jk(b), in addition to the anti-M (and anti-Leb) antibodies. This individual is clearly a “hyper-responder,” and care should be taken to exclude these antibodies, as well as additional antibodies to low-prevalence antigens. Thus, at minimum, neonatal plasma should have been tested to identify the antibody(ies) present.

Second, the only supporting evidence the authors provide for anti-M-mediated HDFN is the presence of low-titer anti-M in maternal plasma, a positive neonatal direct Coombs, and in the reply, positive M antigen typing on neonatal RBCs. While we appreciate the information that the neonate expresses the M antigen, this by itself does not confirm anti-M HDFN and, at most, is circumstantial.

To more conclusively support the authors' diagnosis, the methodology and reactivity strength of the neonatal's direct Coombs test should be reported, as it is not unusual for direct Coombs testing on cord blood to be falsely positive, particularly using column agglutination technology (usually < 2+ reactivity). More importantly, demonstration of anti-M in neonatal plasma as well as an eluate prepared from neonatal RBCs containing anti-M (and not other antibodies, such as Fyb or Jkb) is paramount to confirm this diagnosis.

Jacobs pointed out that he is:

“...appreciative of the author's contribution to the literature for unusual causes of HDFN; however, more evidence

is needed to substantiate these claims. Thus, if the authors have this information available, it should be added to the original report. Alternatively, if this information is unavailable, the authors' conclusions should be less definitive, and they should suggest ‘monitoring for HDFN in the setting of anti-M alloimmunization’ as their case represents a potential case of HDFN secondary to anti-M, but lacks data to represent a definitive case. In addition to the above-mentioned points, the authors' statement that 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, should be removed or altered. The fact that the mother and neonate have the same ABO and RhD type only excludes ABO antibodies (and most cases of anti-D antibodies) as causes of neonatal anemia. There are numerous causes of neonatal anemia and jaundice—these causes are not limited to antibodies against minor RBC antigens but include a variety of inherited RBC and hemoglobin disorders. Thus, [I] recommend removing this statement entirely. The authors did include one new piece of data in their reply—that being, the neonate was antigen typed as M-positive. However, the only other evidence they have to support their diagnosis is a low titer anti-M in maternal plasma and reportedly positive neonatal DAT. They did not provide any new evidence to substantiate their presumed diagnosis—particularly demonstrating anti-M in neonatal plasma and especially anti-M in an eluate prepared from neonatal RBCs. I encourage the authors to include this[sic] data if available, as it would strengthen their report and likely become a highly cited case of low-titer anti-M mediated HDFN—quite a rare occurrence, and a hot topic in the HDFN and blood bank communities.”

This matter awaits the test of time and interpretation of new technologies.

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