Role for serial prenatal anti-Vel quantitative serologic monitoring with 2-ME serum treatment during pregnancy: case report

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Anti-Vel is an uncommon antibody to a high-prevalence antigen. Its clinical significance and management in the prenatal setting are not well characterized. We present a case that demonstrates the utility of serial prenatal anti-Vel quantitative serologic monitoring with 2-ME serum treatment during pregnancy. The patient is a 23-year-old Hispanic woman with history of prior pregnancy and prior transfusion who was discovered to have an antibody to the high-prevalence Vel antigen in the first trimester (week 7) of her second pregnancy. Interval measurements of the serologic antibody titers were performed during the next 26 weeks. The untreated serum (IgM and IgG) titer increased from a baseline of 4 to 16 during that interval, while the 2-ME (presumed IgG component) titer remained stable at 4. Responding to ultrasound findings suspicious for fetal anemia, the child was delivered without complications at 34 weeks' gestation. At birth, the DAT was negative and there was no evidence of HDN. Placed in the context of other similar reports, this case demonstrates the importance of separately reporting the IgG fraction (after either DTT treatment or 2-ME treatment) from the untreated (IgM and IgG) fraction and the importance of correlating the treated serum titer with potential clinical significance.
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Vel is a high-prevalence antigen first described by Sussman et al. in 1952 in association with a transfusion reaction.[1] Although several additional cases quickly confirmed these authors' findings,[2] our knowledge of the Vel antigen (collection) remains incomplete. The presence of the Vel-negative phenotype in the general population is estimated to be 1 in 4000 individuals.[3] Vel alloimmunization can be mediated through IgG or IgM. Anti-Vel is thought to be associated rarely with hemolytic disease of the fetus and newborn (HDFN) because many examples of anti-Vel are mostly IgM and Vel is weakly expressed on the surface of fetal RBCs. Although the risk may be low, it is reasonable to monitor prenatal serologic titers serially during pregnancy.[4] The findings published in a recent case report by van Gammeren et al.,[5] of severe HDFN from anti-Vel, support this approach. The van Gammeren case report describes discovery in a prenatal patient of an anti-Vel, which at initial presentation was determined to be composed exclusively of IgM. At the end of the pregnancy, an anti-Vel titer of 64 was demonstrated in untreated serum, whereas the DTT-treated serum (presumed IgG content) titer rose to 16. In their case, the infant exhibited severe jaundice and reticulocytosis. As a corollary to the van Gammeren case report, we present a case of an anti-Vel for which the IgG titer as determined by 2-ME treatment was stable through pregnancy despite a significant rise in the titer of the anti-Vel in the untreated serum. At birth, the child showed no evidence of HDFN. This case report supports the importance of serial determination of the prenatal IgG titer to help assess risk of HDFN.

Case Report

A 23-year-old Hispanic woman, with a history of prior blood transfusion and one successful pregnancy, presented initially early in the first trimester of the index pregnancy. Her RBCs were typed as group O, D+. Routine serologic testing demonstrated variable reactivity using gel technology. The autocontrol was negative. Further testing using tube technology with PEG as enhancement also demonstrated variable weak reactivity, again with no specificity. No reactivity was demonstrated when using tube technology with LISS. A repeat serologic evaluation was performed 4 weeks later to help clarify the nature of this reactivity. The subsequent serologic evaluation demonstrated reactivity with all RBCs tested whether using gel or test tube technology with LISS or PEG methods. The autocontrol was consistently negative. As an antibody to a high-prevalence antigen was suspected, the sample was referred to a large nationally recognized immunohematology reference laboratory. With use of rare RBCs lacking various high-prevalence antigens, reactivity was determined to be that of anti-Vel. The patient's RBCs were shown to lack the Vel antigen. The initial quantitative serologic titer was 4 when tested against Vel+ RBCs using both 2-ME-treated and untreated serum. The Marsh score ranged from 14 with untreated serum to 4 with 2-ME-treated serum. The developing baby's RBCs were presumed to be Vel+ as the father's RBCs were demonstrated to express the Vel antigen. Subsequent quantitative serologic monitoring was performed in approximately 4- to 6-week intervals for the next 20 weeks of pregnancy (Table 1).

At 33 2/7 weeks' gestation, Doppler ultrasonography showed an increase in middle cerebral artery velocity (>1.5 multiples of the median [MoM] for gestational age) suspicious for fetal anemia. This finding was a significant change from previous Doppler ultrasound studies and corresponded with the change in quantitative serologic titer of the

untreated serum from 4 at presentation to 16 by week 26 (Table 1). A multidisciplinary risk-benefit analysis, including consideration of potential maternal and fetal need for transfusion with an extremely limited blood supply (two available units), the unpredictable timing issues encountered with induction, and the limited time-availability for transfusion after thawing the available units, prompted the decision to move toward delivery in a manner that optimized the availability of blood for the newborn, if it was indeed anemic. The patient underwent cesarean delivery at 34 3/7 weeks' gestational age, 2 days after the initial administration of Celestone to promote fetal pulmonary maturity. A 3088-g male newborn with Apgar scores of 7 at 1 minute and 9 at 5 minutes was born without complication. The child's RBCs typed as group A, D+, and the antibody screen was negative. Initial laboratory data were also remarkable for a Hb level of 16.6 g/dL (normal range), reticulocyte count of 3.5% (normal range), and negative DAT. Approximately 1 week after delivery, a mild rise in bilirubin was observed, reaching a peak of 10 mg/dL. Given that the DAT and the initial screen were negative, the mild hyperbilirubinemia was thought not likely to have resulted from transplacental anti-Vel or a possible anti-A from a group O mother. Moreover, although laboratory data are a bit limited, there was no evidence of anemia or hemolysis. Importantly, the mild hyperbilirubinemia quickly resolved, and the newborn was released to home and is doing well approximately 10 months after birth.

Table 1. Quantitative serologic monitoring

Approximate gestational age (wk)	Serologic findings	Untreated serum titer	Untreated serum score	2-ME-treated serum titer	2-ME-treated serum score
7	Weak, variable reactivity	N/A	N/A	N/A	N/A
14	Anti-Vel	4	24	4	24
18	Anti-Vel	4	27	4	14
22	Anti-Vel	8	N/T	N/T	N/T
26	Anti-Vel	16	25	4	18
34	Anti-Vel	16	30	4	14

N/A = not applicable; N/T = not tested.

Methods

ABO and D testing and DAT were performed using commercially available reagents according to manufacturers' protocols using tube method (Immucor Gamma Inc., Norcross, GA). Antibody detection and identification were performed using either gel technology according to the manufacturer's protocol using reagent cells (RBCs 0.8% Surgiscreen and reagent RBCs 0.8% Resolve Antigram, ID-MTS Gel Test; Ortho-Clinical Diagnostics, Raritan, NJ) or tube method with commercially available cells and reagents (PEG and LISS, Immucor Gamma Inc.). Titration studies performed at the immunohematology reference laboratory used tube method. The patient's serum was diluted in 6% albumin. A 30-minute 37°C incubation was performed, followed by a saline IAT (Anti-IgG, ImmucorGamma Inc.).[6] Reaction scoring was performed using the system originally described by Marsh.[7] The patient's serum was treated with 2-ME (Fisher Scientific Co., Hanover Park, IL) and dialyzed overnight using dialysis membranes (Spectra/Por2, 12,000 to 14,000 dalton molecular weight cut-off [MWCO], Spectrum Laboratories, Inc., Rancho Domingtuez, CA) in PBS, pH 7.3. Dialyzed serum was tested using the methods previously described.

Discussion

This case adds to the limited published information regarding the natural history of anti-Vel in the prenatal setting, and it supports the value of sorting immunoglobulin classes as an aid to proper prenatal management. It is worth noting that at initial presentation, weak, variable serologic reactivity was demonstrated. Despite use of several standard methods, specificity could not be assigned to the serologic reactivity. In the initial serologic evaluation, reactivity was present using gel technology and tube technology with PEG as enhancement. Tube testing with LISS showed no reactivity. Although a LISS tube method is considered an appropriate antibody detection method in the prenatal setting,[8] this method would not have detected the antibody. A repeat study approximately 7 weeks after the initial study did demonstrate the presence of strong serologic reactivity using tube testing with LISS and PEG as well as with gel. The titer of the untreated serum and 2-ME–treated serum was 4. Presumably, late in the first trimester of pregnancy the mother was exposed to fetal RBCs expressing the Vel antigen because by week 14 she demonstrated a specific serologic immune response. Interestingly, for the remainder of the pregnancy, the titer of the anti-Vel in the 2-ME (presumed IgG component)–treated serum remained stable although the titer in the untreated serum (presumed IgM and IgG) increased by two dilutions. This observation may be useful to laboratories without the capacity to treat patient serum with 2-ME or DTT. Namely, in this case anti-Vel titer in the untreated serum appeared to rise before the anti-Vel IgG titer in the 2-ME–treated serum.

The rise in antibody titer in the untreated serum combined with the abnormal Doppler velocimetry prompted the preterm delivery. At birth, the DAT and antibody screen were negative, and the hemoglobin and reticulocyte count were in the normal range. As the anti-Vel present in the maternal serum contained an IgG component, the negative DAT suggests that Vel antigen on the fetal RBCs may either be weakly or partially expressed[9] or the antibody avidity to the antigen was poor. Regardless, in this case, an anti-Vel titer of 4 does not appear to be independently sufficient for HDFN, whereas a titer of 16 in the case reported by van Gammeren et al.[5] was associated with HDFN. The cause of the rise in

the bilirubin level after birth remains uncertain, although liver immaturity is the most likely explanation. Finally, the abnormal Doppler velocimetry in the absence of true fetal anemia reflects the inherent limitations of a screening test that is known to have a 12 percent rate of false-positive results.[10]

The Vel antigen (collection) and anti-Vel remain enigmatic and are in need of further study. To date, it is known that the Vel-negative phenotype is universally uncommon. The incidence of the Vel-negative phenotype in the Hispanic population is not known with certainty. Anti-Vel is famously associated with in vitro hemolysis, severe transfusion reactions, and shortened RBC survival,[11] although there is at least one case report of Vel+ blood being successfully transfused to a recipient possessing anti-Vel.[12] An auto-anti-Vel[13] has been described, although it is uncertain to what degree the autoantibody contributed to the patient's RBC destruction. Anti-Vel can be difficult to detect, and it can simulate the serologic profile of a cold-reactive autoantibody, with disastrous consequences if improper analytic methods are used.[14] The Vel antigen appears to have a variable antigenic expression and appears to be best expressed on adult cells.

When viewed in concert with the recent case report by van Gammeren et al.,[5] our case report further supports the need not only to properly identify anti-Vel in the prenatal setting but also to perform serial quantitative serologic monitoring using methods to clearly separate the IgG fraction (DTT treatment or 2-ME treatment) from the untreated (IgM and IgG) fraction. Although both the untreated and treated serum titer and score results may be reported, changes in the treated serum fraction, representing the IgG component, may be a better trigger to initiate additional clinical investigation. Additional cases may help identify the most appropriate critical 2-ME or DTT titer for anti-Vel.

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