OBSTETRICS

Predicting anti-Kell-mediated hemolytic disease of the fetus and newborn: diagnostic accuracy of laboratory management

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BACKGROUND: There is controversy on critical cut-off values of laboratory testing to select pregnancies at increased risk for anti-Kell-mediated hemolytic disease of the fetus and newborn. Without early detection and treatment, anti-Kell-mediated hemolytic disease of the fetus and newborn may result in progressive fetal anemia, fetal hydrops, asphyxia, and perinatal death.

OBJECTIVE: We aimed to determine the value of repeated anti-Kell titer determination and biological activity measurement using the antibody-dependent cellular cytotoxicity test determination in the management of pregnancies at risk for anti-Kell-mediated hemolytic disease of the fetus and newborn.

STUDY DESIGN: This was a retrospective cohort study of pregnancies with anti-Kell and a Kell-positive fetus, identified from January 1999 through April 2015. Laboratory test results and clinical outcome were collected from the Dutch nationwide screening program and the national reference center for fetal therapy in The Netherlands, the Leiden University Medical Center. Diagnostic accuracy was measured (receiver operating characteristic curves, sensitivity, specificity, positive and negative predictive values) for anti-Kell titers and antibody-dependent cellular cytotoxicity test. The relationship between the titer and antibody-dependent cellular cytotoxicity measurements and the 2 foregoing measurements were computed with a Pearson product-moment correlation coefficient.

RESULTS: In a 16-year unselected cohort, representing screening results of 3.2 million pregnancies resulting in live births in The Netherlands, we identified 1026 Kell-immunized pregnancies. In all, 93 pregnant women had anti-Kell and a Kell-positive child, without other red cell alloantibodies. In all, 49 children (53%) needed intrauterine or postnatal transfusion therapy. The first anti-Kell titer showed already a high diagnostic accuracy with an area under the curve of 91%. The optimal cut-off point for the titer was 4 (sensitivity 100%; 95% confidence interval, 91—100), specificity 27% (95% confidence interval, 15—43), and positive predictive value 60% (49—71%). The antibody-dependent cellular cytotoxicity test was not informative to select high-risk pregnancies. Linear regression showed no significant change during pregnancy, when antibody titer and antibody-dependent cellular cytotoxicity test results were compared with every 2 foregoing measurements ($P < .0001$).

CONCLUSION: Early determination of the anti-Kell titer is sufficient to select pregnancies at increased risk for hemolytic disease of the fetus and newborn with need for transfusion therapy. If the Kell status of the fetus is known to be positive, a titer of $\geq$4 can be used to target intensive clinical monitoring.

Key words: alloimmunization, anti-Kell, diagnostic accuracy, hemolytic disease of the fetus and newborn, intrauterine blood transfusion, laboratory tests, red blood cell antibodies, screening program

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is caused by red blood cell (RBC) antibodies developed by the mother and transferred to the fetus.[1,2] Kell (K) alloantibodies are second to RhD alloantibodies in importance as the cause of severe HDFN.[1,2] K alloantibodies cause hemolysis of fetal erythrocytes and inhibit the fetal erythropoiesis.[3–5] Without treatment, HDFN may result in progressive fetal anemia, fetal hydrops, asphyxia, and perinatal death.[6] After birth, neonatal hyperbilirubinemia may lead to "kernicterus," a cause of neurodevelopmental impairment with athetoid cerebral palsy, hearing problems, and psychomotor handicaps.[7–13] Even though the incidence of fetal alloimmune hydrops has declined in the last decades,[14] this condition is still a well-known risk factor for adverse perinatal and long-term outcomes.[15] Severe anti-K-mediated HDFN may develop early in pregnancy, and often presents with hydrops <20 weeks' gestation.[5,15,16] Postnatally, anti-K-mediated HDFN is characterized more frequently by anemia than by hyperbilirubinemia, compared with HDFN caused by anti-D or other types of Rh alloantibodies.[6]

RBC alloimmunization should ideally be detected early in pregnancy upon routine RBC antibody screening. In most centers, to identify pregnancies at risk for severe HDFN, the titer of clinically relevant RBC alloantibodies is determined.[1,8,17,18] If the titer is above a certain threshold, patients are referred to a maternal-fetal medicine center for close surveillance and, if needed, for fetal or neonatal treatment.[17,18] High-risk pregnancies are monitored with ultrasound and Doppler middle cerebral artery (MCA) peak systolic velocity measurements, to predict the presence of fetal anemia.[19–21] Severe fetal anemia can be successfully treated using intrauterine transfusions (IUT). Neonates

EDITORS' CHOICE

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AJOG at a Glance

Why was this study conducted?

To assess the performance of anti-Kell (K) titer and antibody-dependent cellular measurements in K-alloimmunized pregnancies with a K-positive fetus, and to predict severe hemolytic disease of the fetus and newborn (HDFN) requiring transfusion therapy.

Key findings

The first titer with a cut-off value of 4 has the best diagnostic accuracy to select pregnancies at risk for severe HDFN. The antibody-dependent cellular test has no additional value to the titer.

What does this add to what is known?

This 16-year unselected cohort actualizes that severe K-mediated HDFN can occur with low titers, the importance of fetal K typing in selection of high-risk cases, and shows that the K titer is not changing significantly during pregnancy.

may require phototherapy or neonatal (exchange) transfusions.[22]

In The Netherlands, fetal K genotyping is performed with cell-free fetal DNA isolated from maternal plasma.[23] K-alloimmunized pregnancies with a K-positive fetus are monitored by serial antibody titer measurements and by the antibody-dependent cellular cytotoxicity (ADCC) bioassay, a monocyte-based assessment of the destructive capacity of the antibodies.[24–26] However, there is still controversy on which critical titers and ADCC cut-off levels indicate a high risk for anti-K-mediated HDFN.[17,18,27–31]

The aim of this study was to assess the performance of anti-K titer and ADCC measurements in K-alloimmunized pregnancies with a K-positive fetus, to predict severe HDFN requiring transfusion therapy.

Materials and Methods

Setting and prevention program in The Netherlands

In The Netherlands, all pregnant women are typed for ABO, RhD, and Rhc blood group antigens and screened for RBC antibodies at the first-trimester booking visit. All screen-positive samples are sent to 1 of 2 national reference laboratories for confirmation and determination of the antibody specificity. These laboratories are Sanquin Diagnostics, Amsterdam (90% of the pregnant population) and the Special Institute for Blood Group Investigations, Groningen (10% of the pregnant population). When clinically relevant RBC antibodies are detected, ie, antibodies with the potency to destroy fetal RBCs, the father of the fetus is typed for the cognate antigen(s). In case the father is antigen-positive, or his type is not known, noninvasive fetal typing with cell-free fetal DNA isolated from maternal plasma has been offered (for RhD, RhC, Rhc, RhE, and K), since 2004.[23] If the fetus is antigen-positive, serial testing (starting with every 4 weeks, from 24 weeks every 3 weeks, from 36 weeks every 2 weeks) of maternal antibody titers and the ADCC test is performed. Following current Dutch guidelines, a K antibody titer $\geq$2 and/or an ADCC test result $\geq$30% indicate a substantial risk for K-mediated HDFN, and the fetus will be monitored weekly or every 2 weeks with MCA Doppler measurements.[17] Laboratory follow-up is stopped if these thresholds are reached. Severe fetal anemia is treated with IUTs at the Leiden University Medical Center (LUMC), which is the national Dutch reference center for fetal therapy. The threshold for suspected severe fetal anemia requiring IUT was: (1) a MCA peak systolic velocity of 1.5 multiples of the median for gestational age, detected by Doppler measurement; and/or (2) the presence of other signs of anemia at ultrasound examination (cardiomegaly, ascites, hydrops); or (3) amniotic fluid delta optical density measurements reaching the upper part of Liley zone II or zone III (only in the early years of this study).[19,32]

Laboratory testing

Both reference laboratories assess antibody titers, in phosphate-buffered saline by doubling dilutions, with the indirect antiglobulin test, using an anti-IgG reagent and heterozygous K-positive RBCs.[33]

The ADCC test, as described by Engelfriet and Ouwehand,[24] is only performed at Sanquin Diagnostics in Amsterdam. Fetal K typing is also only performed at Sanquin Diagnostics.[23]

Study design

We performed a retrospective cohort study, including all pregnancies diagnosed with anti-K in The Netherlands, from January 1, 1999, through April 1, 2015. All K immunization cases were identified at the 2 national reference laboratories. Women with K alloimmunization and antibody titers $\geq$2 and/or ADCC test results >30% were usually referred to the LUMC for monitoring or treatment. All these cases could therefore also be identified in the LUMC database. We only included pregnancies with a K-positive fetus.

Outcomes

The primary outcome was the diagnostic accuracy (sensitivity, specificity, and predictive values) of antibody titers and ADCC tests to predict severe K-mediated HDFN, which was defined as the need for IUT or postnatal transfusion.

Data collection

We collected the results of laboratory monitoring during pregnancy from Sanquin Diagnostics and data concerning clinical monitoring and IUT treatment during pregnancy from the LUMC databases. Neonatal outcome data on treatment with blood transfusion(s) or phototherapy during the first 3 months of life were extracted from their medical files, by contacting the obstetric care provider, the pediatrician, or the local hospital laboratories.