Assessment of the fetal kel and rhce genotype in alloimmunized pregnant women

Durdova V, Bohmova J, Kratochvilova T, Vodicka R, Lubusky M
Obstetrics and Gynecology Clinic of the Faculty of Medicine UP and the University Hospital Olomouc, Olomouc, Czechia

Objective
All pregnant women in the Czech Republic undergo the screening of irregular anti-erythrocyte antibodies in their first trimester. The result is positive in about 5% of all cases (in the Czech Republic 5000 women annually), but only in about 1.5% of the cases (1500 women) there is a clinically significant anti-erythrocyte alloantibody. The fetus and the newborn is at risk by the development of a significant form of a hemolytic disease only when the complementary antigen is present on their erythrocytes. Using the non-invasive assessment of the genotype of the fetus from the free fetal DNA circulating in the peripheral blood of pregnant women, it is possible to exclude the fetuses that should lack the antigen and thus they should not be at risk by the development of the hemolytic disease. From the clinically most significant alloantibodies, the antibody anti-E is the most frequently diagnosed antibody in the Czech Republic while the alloantibodies anti-D, c, K belong between the clinically most significant alloantibodies. The Anti-K (Kell, KEL1) is diagnosed in about 0.1% of the cases (in the Czech Republic annually 100 women). Assessing the KEL genotype of the fetus, we can exclude up to 95% of the fetuses (95 fetuses annually), which lack the KEL1 allele that correspond to the presence of the erythrocyte “K” antigen. The Anti-c is diagnosed in about 0.1% of the cases (in the Czech Republic annually 100 women). Assessing the RHCE genotype of the fetus, we can exclude up to 44% of the fetuses (44 fetuses annually). These fetuses lack the variant of the RHCE gene that correspond to the presence of the “c” antigen. The Anti-C is diagnosed in about 0.1% of the cases (in the Czech Republic annually 100 women). Assessing the RHCE genotype of the fetus, we can exclude up to 56% of the fetuses (56 fetuses annually). These fetuses lack the variant of the RHCE gene that correspond to the presence of the “C” antigen. The Anti-E is diagnosed in about 0.6% of the cases (in the Czech Republic annually 600 women). Assessing the RHCE genotype of the fetus, we can exclude up to 74% of the fetuses (504 fetuses annually). These fetuses lack the variant of the RHCE gene that correspond to the presence of the “E” antigen.

Methods
The non-invasive assessment of the KEL genotype of the fetus from the free fetal DNA in the plasma of pregnant women was carried out by the minisequencing method using the capillary electrophoresis (called SNaPshot). This method is based on extending primers of different lengths by one base (fluorescently labeled dideoxynucleotides). Using the capillary electrophoresis, the length of the extended primer and the type of the fluorescent length is assessed. The method was consequently used to assess the RHCE genotype of the fetus.

Results
In the total of 295 pregnant women (between the 8th and the 23rd gestational week), the genotype of the fetus was assessed from the peripheral blood using the minisequencing method. Consequently, the genotype of the fetus was verified by testing the cells of the newborn from the buccal smear. The minisequencing method has proven to be reliable. The sensitivity and specificity of the method have reached 100%.

Conclusion
The non-invasive assessment of the RHCE and KEL genotype of the fetus enables to exclude the fetuses which lack the complementary antigen and thus are not at risk by the development of the hemolytic disease of the fetus and the newborn. On the contrary, it determines which fetuses might have the antigen present and thus, they should be monitored in specialized centers focusing on the hemolytic disease of the fetus and the newborn.