Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Development of a noninvasive prenatal test for the determination of fetal blood cell antigens

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Morbus haemolyticus neonatorum is caused by intolerance of blood group antigens, e.g., the rhesus D protein or KEL1 protein between mother and child. A rhesus D or KEL1 negative mother can produce antibodies against the foreign antigens on the child's erythrocytes' surface inherited from the father. In the case of rhesus D, this antibody formation can be prevented by administering immunoglobulins to all pregnant rhesus D negative women. But a targeted treatment of women who are expecting a rhesus positive child would relieve the health insurance companies and reduce the possibly unnecessary treatment of women who might suffer from side effects like anaphylactic reactions. No protective immunoglobulin treatment is available for the KEL blood group, and only continuous ultrasound examinations and blood transfusions can safely protect the child from severe damage to health. Early knowledge about the child's antigen status could help to initiate these important examinations early and reduce the likelihood of disabilities or stillbirths.
However, with blood platelets, incompatibilities can occur between mother and child, resulting in fetal/neonatal alloimmune thrombocytopenia. So far, the probability for fetal alloimmune thrombocytopenia has been determined using the antibody titer but only at later pregnancy stages. In case of a diagnosed fetal/neonatal alloimmune thrombocytopenia, a continuous ultrasound examination has to be carried out in combination with platelet transfusions or immunoglobulin administration to prevent an intracranial hemorrhage during pregnancy. As for the blood group antigens, early knowledge of the fetal status could avoid severe damage to the child's health.
In all described cases, a noninvasive method could determine the child's antigen status from the mother's blood and thus protect the child from health damage. The term noninvasive refers to the fact that the examination is noninvasive for the fetus. In several countries like Denmark or Netherlands, the noninvasive determination of the fetal rhesus D status is already in routine use. But these tests are conducted during the second trimester in pregnancy using qPCR. Especially for the KEL blood group and platelet complications, no standard tests are in the routine jet.
Therefore, this work aims to develop a noninvasive prenatal blood test to identify blood cell and platelet antigens from the fetus only using a blood sample from the mother. The test should have a high sensitivity so that it can already be used at the stage of early pregnancy. To achieve this, a further development of quantitative polymerase chain reaction, namely digital polymerase chain reaction, was used. To implement this relatively new technique in a protocol for routine use and analyze its potential, this work has been divided into three main parts. The first part describes the protocol's development from cell-free deoxyribonucleic acid isolation to completing the digital polymerase chain reaction.
In the second part, the protocol was subjected to a technical validation for sensitivity and specificity. For this, attempts were made to simulate pregnancies in various stages using plasma mixtures consisting of a homogeneous carrier (mother) and a small amount of heterogeneous plasma (child). Mixes of 5,00 % down to 0.10 %, referring to the fact that the target allele is included with 2.50 to 0.05 %, respectively, were prepared. The 0.1 % mixture represents a very early pregnancy, and 5.00 % mixture means pregnancy in the third trimester. During this validation, it was shown that it was possible to reliably identify a proportion of as low as 0.05 % of the rhesus D exon 5 and exon 7. For the KEL blood group, a detection limit of 0.25 % was evaluated. In addition, the test was also validated for the human platelet antigens -1, -2, -3, -5, and -15. Here the test for human platelet antigens -1a and -15b showed a detection limit of 0.05 %, and the threshold value for the human platelet antigens -1b, -2b, and -3a was determined at 0.25 %. Only for the human platelet antigens -1a and -15b the test indicated a threshold of 0.50%. As a fetal marker, amelogenin was used, and for this, the test indicated a detection limit of 0.50 %. However, this marker only identifies male fetuses because, during analysis, the female fetus's

signal cannot be distinguished from the mother's signal. Therefore, nine autosomal markers from the single nucleotide polymorphism for identification panel were tested as fetal markers with 1.00 % mixtures indicating 0.50 % target proportion. In all tested assays, the target could be identified.
In the third part of this thesis, real pregnant women were investigated in a small clinical study, and the results were compared with the technical validation. With approval by a local ethics committee, blood samples from 50 pregnant women were analyzed with the newly developed tests. It was possible to confirm the results of the technical validation. An statistical analysis indicated that from all 17 analyzed assays, 13 revealed significant results ($p > 0.05$). To further confirm the results, the accuracy of the collected genomic desoxyribonucleic acid of children after birth could be tested and compared to the prenatal results in 126 cases. There was a discrepancy in 6 cases, which could be corrected through small test protocol optimizations. Overall, the study shows how reliable the developed protocol can be used in early stages of pregnancy, starting from week seven after conception. Therefore as a next step, it is recommended to carry out a more extensive confirmation study to possibly offer this very sensitive method to women with a high-risk pregnancy for fetal/neonatal alloimmune thrombocytopenia or Morbus haemolyticus neonatorum.