Advancements in Transfusion Medicine: Utilizing cfDNA for HDFN Risk Stratification.

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Cell Free DNA (cfDNA) analysis is a massive development in recent medical laboratory science, allowing for a wealth of information to be gained from a simple peripheral blood sample. cfDNA has been commercially available since 2011, and one of its most recognized uses is in Non Invasive Prenatal Testing (NIPT).[1] In NIPT, cfDNA has been used to gather abnormal genetic information regarding the fetus, primarily in indications of chromosomal aneuploidies such as Down syndrome, trisomy 18, and chromosome 13 trisomy.[1] In a recent study, prenatal cfDNA analysis has been evaluated for the determination of fetal antigens in alloimmunized pregnancies from an early gestational age. [2]

Cell Free DNA are circulating DNA fragments stemming primarily from apoptotic hematopoietic sites. In pregnancy, the placenta produces cfDNA via apoptotic trophoblasts. The cfDNA released from these cells is reflective of fetal DNA and utilized in assays to reflect fetus status.[3] cfDNA screening is preferred to other fetal testing methods as it can be done from a maternal peripheral smear whereas other tests may require amniocentesis, an extremely invasive and potentially dangerous procedure.[4]

cfDNA analysis begins with the collection of a peripheral blood sample into a specialized tube. This tube contains K_3EDTA and a stabilizing agent that prevents the release of genomic DNA, preserving the purity of the cfDNA.[5] The sample is centrifuged twice, one slow spin and one fast, to separate the plasma from other blood components. The cfDNA is then extracted using silica-based columns or magnetic bead purification systems optimized for short fragment recovery.[5]

The fragments then undergo preparation for Massively Parallel Sequencing, a library sequencing methodology. The 5’ end of the fragments are phosphorylated and ligated by sequence adapters.[6] Unique Molecule Identifiers (UMIs), tag each original cfDNA molecule.

UMIs allow for low-frequency mutations to be differentiated from sequencing errors.[6] If similarly abnormal sequences share a UMI, it is likely to be a real mutation rather than a sequencing mistake. Following UMI and adapter ligation, PCR amplification with high fidelity polymerases proceeds. The genetic information obtained in the sequences give insight into genetic phenotypes, and the UMIs and Massively Parallel Sequencing methodologies ensure a high-confidence in the data.

A recent study by Rego et. al., "Fetal Red Blood Cell Antigen Genotype in Individuals With Alloimmunized Pregnancies" highlights how cfDNA may be implemented to assess individual risk of hemolytic disease of the fetus and newborn. Alloimmunization in pregnancy is the development of maternal antibodies against fetal antigens inherited through the father.[2] Sensitization of fetal antigens by the mother can result in a condition called Hemolytic Disease of the Fetus and Newborn (HDFN) that can be fatal for the fetus if untreated.[2] Traditional risk stratification for HDFN requires paternal antigen testing or invasive amniocentesis testing, which can be dangerous for the fetus and mother.[4]

This study evaluates the use of cfDNA analysis strategies against postpartum fetal buccal swabs to determine the accuracy of detecting fetal antigens. The clinical studies were conducted on English or Spanish-speaking pregnant women who received cfDNA analysis in the United States as part of prenatal testing. The participants had confirmed sensitizations to any of the following antigens: antigens K1, Fy^a, C, c, E, and D.[2] One hundred and fifty six pregnant women, four of whom were pregnant with twins, were included in the study. The median gestational age of the participants was 16.4 weeks.[2] The women were alloimmunized to a total of 191 antigens, the most common of which being E (34.0%). Thirty four of the women had sensitization to more than one antigen. Of the tests, 91 fetal antigen results were positive for

antigen presenting phenotypes and 100 were negative. Of the 190 fetal cfDNA tests done for antigens to which the mothers were alloimmunized, 190 tests were in concordance. Additional tests on patients who were not alloimmunized to any of the listed antigens, but at risk of sensitization, passed with 100% concordance out of 456 tests.[2]

The clinical implications for transfusion medicine are substantial. Early and accurate identification of fetal antigen status allows physicians to distinguish between pregnancies requiring intensive monitoring or intervention, and those at minimal risk. The ability to determine fetal genotype safely and early could shift immunohematological and obstetric practices from reactive to proactive care.

While promising, limitations of cfDNA analysis should be acknowledged. Adequate fetal DNA fractions within maternal circulation are required for reliable detection. Very early gestational ages or multiple gestations may reduce analytical sensitivity.[1] Additionally, widespread implementation would require access to specialized molecular laboratories and may raise the cost of prenatal care. Nonetheless, the demonstrated accuracy and non-invasive nature of cfDNA antigen testing makes it a powerful addition to modern transfusion medicine practice.

In conclusion, cfDNA analysis has evolved beyond aneuploidy screening to become a valuable tool in the management and identification of alloimmunized pregnancies. By applying massively parallel sequencing and quantitative analysis to fetal RBC antigen genotyping, clinicians can precisely identify pregnancies at risk for HDFN and treat them proactively. This advancement showcases room for the integration of molecular diagnostics into transfusion and immunohematology medicine.

References

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