Can cffDNA technology be used to determine the underlying cause of pregnancy loss to better inform future pregnancy planning?

Compared with direct sequencing of the pregnancy tissue, **cell-free fetal DNA via maternal serum has a reasonable sensitivity of 85% (95% CI, 79%–90%) and specificity of 93% (95% CI, 88%–96%) for the application of ploidy determination following a pregnancy loss**, according to a prospective cohort study of 1,000 women with spontaneous pregnancy loss before 149 days of gestation.

Hartwig TJ, Ambye L, Gruhn JR, et al. Cell-free fetal DNA forgenetic evaluation in Copenhagen Pregnancy Loss Study (COPL): a prospective cohort study. Lancet. 2023;401:762-771. https://doi.org/10.1016/S0140-6736(22)02610-1.

EXPERT COMMENTARY
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A devastating outcome for women, pregnancy loss is directly proportional to maternal age, estimated to occur in approximately 15% of clinically recognized pregnancies and 30% of preclinical pregnancies.[1] Approximately 80% of pregnancy losses occur in the first trimester.[2] The frequency of clinically recognized early pregnancy loss for women aged 20–30 years is 9% to 17%, and these rates increase sharply, from 20% at age 35 years to 40% at age 40 years, and 80% at age 45 years. Recurrent pregnancy loss (RPL), defined as the spontaneous loss of 2 or more clinically recognized pregnancies pregnancies, affects less than 5% of women.[3] Genetic testing using chromosomal microarrayanalysis(CMA)hasidentifiedaneuploidy in about 55% of cases of miscarriage.[4]

Following ASRM guidelines for the evaluation of RPL, which consists of analyzing parental chromosomal abnormalities, congenital and acquired uterine anomalies, endocrine imbalances, and autoimmune factors (including antiphospholipid syndrome), no explainable cause is determined in 50% of cases.[3] Recently, it has been shown that more than 90% of patients with RPL will have a probable or definitive cause identified when CMA testing on miscarriage tissue with the ASRM evaluation guidelines.[5]

Details of the study

In this prospective cohort study from Denmark, the authors analyzed maternal serum for cell-free fetal DNA (cffDNA) to determine the ploidy status of the pregnancy loss. One thousand women older than age 18 were included (those who demonstrated an ultrasound-confirmed intrauterine pregnancy loss prior to 22 weeks' gestation). Maternal blood was obtained while pregnancy tissue was in situ or within 24 hours of passage of products of conception (POC), then analyzed by genome-wide sequencing of cffDNA.

For the first 333 recruited women

FAST TRACK

The cause of repeat pregnancy loss can be identified in more than 90% of cases with chromosomal microarray analysis

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WHAT THIS EVIDENCE MEANS FOR PRACTICE

The best management course for unexplained RPL is uncertain. Despite its use for a euploid miscarriage or parental chromosomal structural rearrangement, in vitro fertilization with preimplantation genetic testing remains an unproven modality.[6,7] Given that approximately 70% of human conceptions never achieve viability, and 50% fail spontaneously before being detected,[8] the authors' findings demonstrate peripheral maternal blood can provide a reasonably high sensitivity and specificity for fetal ploidy status when compared with direct sequencing of pregnancy tissue. As fetal aneuploidy offers a higher percentage of subsequent successful pregnancy outcomes, cffDNA may offer reassurance, or direct further testing, following a pregnancy loss. As an application of their results, evaluation may be deferred for an aneuploid miscarriage.

—MARK P. TROLICE, MD, MBA

FAST TRACK

When products of conception were unavailable for chromosomal analysis using single-nucleotide polymorphism testing, cffDNA obtained from maternal serum yielded conclusive results nearly 90% of the time in this study

(validation phase), direct sequencing of the POC was performed for sensitivity and specificity. Following the elimination of inconclusive samples, 302 of the 333 cases demonstrated a sensitivity of 85% and specificity of 93%. In the subsequent evaluation of 667 women, researchers analyzed maternal serum from the gestational age of fetuses ranging from 35 days to 149 days.

Results. In total, nearly 90% of cases yielded conclusive results, with 50% euploid, 46% aneuploid, and 4% multiple aneuploidies. Earlier gestational ages (less than 7 weeks) had a no-call rate (ie, inconclusive) of approximately 50% (only based on 16 patients), with results typically obtained in maternal serum following passage of POC; in pregnancies at gestational ages past 7 weeks, the no-call rate was about 10%. In general, the longer the time after the pregnancy tissue passed, the higher likelihood of a no-call result.

Applying the technology of single-nucleotide polymorphism (SNP)-based CMA can improve identification of fetal and/or maternal sources as causes of pregnancy loss with accuracy, but it does require collection of POC. Of note, samples were deficient in this study, the authors cite, in one-third of the cases. Given this limitation of collection, the authors argue for use of the noninvasive method of cffDNA, obtained from maternal serum.

Study strengths and weaknesses

Several weaknesses of this study are highlighted. Of the validation cohort, one-third of pregnancy tissue could not be analyzed due to insufficient collection. Only 73% of cases allowed for DNA isolation from fetal tissue or chorionic villi; in 27% of cases samples were labeled "unknown tissue." In those cases classified as unknown, 70% were further determined to be maternal. When all female and monosomy cases were excluded in an effort to assuredly reduce the risk of contamination with maternal DNA, sensitivity of the cffDNA testing process declined to 78%. Another limitation was the required short window for maternal blood sampling (within 24 hours) and its impact on the no-call rate.

The authors note an association with later-life morbidity in patients with a history of pregnancy loss and RPL (including cardiovascular disease, type 2 diabetes, and mental health disorders), thereby arguing for cffDNA-based testing versus no causal testing; however, no treatment has been proven to be effective at reducing pregnancy loss. ●

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