First-trimester Ultrasound and Cell-Free DNA Screening: Improved Patient Care

Introduction

Professional societies around the world recommend that pregnant women have access to screening and diagnostic testing for fetal aneuploidy.[1,2,3] Options for screening now include first and second trimester serum biomarkers, often including a nuchal translucency (NT) assessment, and cell-free DNA (cfDNA) analysis. The high performance and efficiency of cfDNA for the detection of common aneuploidies in a single step, versus the multistep process of NT plus biomarkers, has led some to question the role of NT and first-trimester ultrasound in the context of cfDNA screening.[4] If the only objective is screening for trisomy, cfDNA analysis alone may be sufficient;[5] however, complementing it with detailed/comprehensive ultrasound evaluation in the first trimester, provides critical information for pregnancy management, aids in cfDNA test interpretation and detects fetal anomalies beyond aneuploidy. The purpose of this paper is to review how ultrasound and cfDNA analysis, used together, provide optimal patient care.

Ultrasound PRIOR to Cell-free DNA testing

Accurate Dating

The most accurate method for establishing or confirming the estimated date of delivery (EDD) is an ultrasound of the embryo or fetus performed in the first trimester of pregnancy.[8] Traditionally, the EDD is determined by adding 280 days to the first day of the last menstrual period;[6] however, menstrual cycle irregularities, variable timing of ovulation, and maternal recall can all affect the precision of this method. Unlike maternal serum screening, cfDNA testing does not have a narrow window in which it can be performed; however, establishing an accurate gestational age ensures that cfDNA is performed after 10 weeks gestation, when most pregnancies have sufficient fetal cfDNA for testing.

Multiple Gestations

Early ultrasound is key for determining the number of gestations that are present. According to 2018 data, almost 1 in 30 babies born are from a twin pregnancy. Multiple gestation pregnancies have increased complexity of obstetrical care. The performance of cfDNA testing for trisomy 21 in twin pregnancy is similar to that reported in singleton pregnancy;[7] however, it is critical that the pregnancy be identified as a twin during testing to allow accurate test interpretation. Performance data for screening in higher-order multiple pregnancies is lacking.[8] The performance of the Harmony test in twin gestations is supported by numerous publications and is detailed in a whitepaper titled “Cell-Free DNA Screening in Twin Pregnancies using the Harmony® Prenatal Test”.

Chorionicity versus Zygosity

A description of the number of placental membranes that separate the fetuses of a multiple gestation is termed chorionamnionicity (often shortened to chorionicity). Multiple sources of perinatal morbidity and mortality in multiple gestations correlate with chorionicity, including intrauterine growth restriction, twin anemia polycythemia sequence (TAPS) and twin-twin transfusion syndrome (TTTS), with monochorionic pregnancies being at increased risk. Therefore, early determination of chorionicity is essential to the management of twin and higher order gestations. Using standardly defined ultrasound criteria, optimally assessed in the first trimester, the accuracy of prenatal prediction of chorionicity is greater than 95%.[9] This accuracy cannot be replicated by an assessment of zygosity with cfDNA analysis, no matter how accurate, as 25% of monozygotic twins are dichorionic.[10]

Vanishing Twin

The loss of a fetus in a multiple pregnancy is a complicating factor for aneuploidy screening.[11] cfDNA from the non-viable conception is released into the maternal bloodstream and creates the possibility of a discordant (“false positive” or “false negative”) result. The amount and duration of this biological process is not well understood,[12,13,14,15,16] leading professional societies to advise against the use of cfDNA testing when ultrasound reveals a vanishing twin.[2,3] Early identification of such pregnancies with ultrasound prior to cfDNA testing allows the consideration of alternative testing options.

Identification of structural birth defects

Three to six percent of pregnancies are found to have fetal malformations.[17] While some patterns of birth defects are associated with specific genetic conditions, many malformations have etiologies other than aneuploidy and will not be detected by cfDNA screening. A detailed first trimester ultrasound may identify many issues, including but not limited to cystic hygroma, enlarged nuchal translucency, acrania, heart defects, abdominal wall defects, spina bifida and limb defects. When fetal malformations are identified, diagnostic testing with microarray is indicated because significant genetic copy number variations are found in up to 6% of cases.[18,19] "Genome wide" cfDNA tests are commercially available but are not a substitute for diagnostic testing because of their low sensitivity for microdeletions smaller than 7 Mb and high risk of false negative results.[20,21]

Ultrasound FOLLOWING cfDNA testing

Management of high probability results

Confirmatory diagnostic testing is recommended after a cfDNA test indicates a high probability of fetal trisomy. Patients faced with these results may ask their provider how likely it is that the result is an accurate reflection of their fetus. This question is best answered by looking at the positive predictive value (PPV) of the test. In an average risk population, the PPV for trisomy 21 is higher than the PPV for trisomies 18 and 13 due to the relative prevalence of the condition. Ultrasound can be used to provide additional information to assist in counseling. For example, if anomalies are identified on a detailed ultrasound, the probability of an affected fetus increases. Conversely, an unremarkable ultrasound evaluation decreases the probability of fetal trisomy. Ultimately, a diagnostic procedure would be required for definitive information; however, ultrasound may inform the decision whether to proceed with CVS, which has the advantage of earlier diagnosis but the potential disadvantage of being complicated by placental mosaicism, or wait for amniocentesis.[22]

Management of redraw requests

Ultrasound may assist management in some cases if a cfDNA test does not yield a result on the first blood draw. Although redraw is a viable option and most patients who are redrawn receive results with a second sample,[23] the association between "no-results" on cfDNA testing and increased aneuploidy risk has led to recommendations that women consider invasive testing. Specifically, trisomy 18 and trisomy 13, rather than trisomy 21, have been associated with lower fetal fraction.[24] When examined by a fetal medicine specialist, over 90% of fetuses affected by trisomy 18 or trisomy 13 will demonstrate a malformation.[25] Detailed ultrasound evaluation of the fetus might provide information to aid in counseling about testing options after no result on cfDNA testing.

Summary

- Prenatal ultrasound can be used prior to cfDNA or following a cfDNA result. A paradigm that uses both the cfDNA and ultrasound can allow for accurate and early first trimester assessment of clinically relevant conditions in pregnancy. Neither assessment independently can provide the same level of detection as both assessments combined.
- An understanding of the association of structural abnormalities with specific aneuploidies can help clinicians determine when diagnostic testing is more appropriate than screening.
- For twin gestations, chorionicity and amnionicity are critical information that can only be ascertained by ultrasound assessment. Zygosity assessment, by any means, cannot replace the need for this ultrasound assessment.
- A detailed ultrasound following a high-risk result in the first trimester may help clinicians and patients choose between an immediate CVS or waiting until an amniocentesis.
- A detailed ultrasound study following a redraw request may help clinicians choose between a repeat sample submission or immediate diagnostic testing.

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