Cell-Free DNA Screening in Twin Pregnancies using the Harmony® Prenatal Test

Introduction

Pregnancies with more than one fetus are not uncommon. According to 2018 data, almost 1 in 30 babies born are of a twin pregnancy.[1] Infants born from a pregnancy with multiples are at a higher risk for adverse outcomes including birth defects, low-birth weight and preterm deliveries, and therefore, professional societies around the world recommend increased surveillance and screening for these pregnancies.[2–7]

Cell-free DNA (cfDNA) analysis for aneuploidy risk assessment has been used in twin pregnancies since 2013; however, published data regarding the performance of different test methodologies in twin pregnancies is less abundant as compared to singleton pregnancies.[8] cfDNA analysis of twin pregnancies is confounded by unique technical and biological factors. Proper interpretation and clinical implementation of cfDNA analysis in twin pregnancies requires evidence supporting this specific use of the technology.[9–11]

The purpose of this paper is to review the published data for the Harmony® prenatal test and highlight the technical and clinical considerations involved in cfDNA testing of twin pregnancies.

The Harmony test and twin pregnancies
9 Publications supporting Harmony use in twin pregnancies
36 of 40 Trisomies detected in published data sets of twin pregnancies[12,13]
Accurate method for fetal fraction assessment, evaluated in over 250,000 published pregnancy samples (twins & singletons)
Available for donor egg pregnancies, including gestational surrogates
Fetal sex analysis available
Available > 10 weeks gestation

The Harmony test and twin pregnancies: considerations
Two fetuses may contribute different amounts of cfDNA to maternal plasma
Redraw requests may be more frequent, as compared to singleton pregnancies, possible due to lower fetal fractions
Sex Chromosome Aneuploidy Panel not available
cfDNA analysis should not be used in pregnancies with a vanished or demised fetus

Robust Validation Studies Support the Harmony Test Performance in Twin Pregnancies

The Harmony prenatal test was used in two publications where 25 of 27 twin pregnancies with trisomy 21 were given a high-risk result.[12,13] Six false positive results were reported among 1,149 euploid fetuses in twin pregnancies (Table 1). In contrast, traditional first trimester combined screening for trisomy 21 in twin pregnancies has detection rates of 75-90% with false positive rates of 5-9%.[14-17] Experiences with the Harmony test in twin pregnancies have been reported in nine publications with >3500 analyses, as of November 2019 (Table 2).

Table 1. The Harmony test results in twin validation studies

Study	Trisomy 21	Trisomy 18	Trisomy 13	Euploid	Fetal Sex
Gil et al (2014)[12]	9 of 10		1 of 1	181 of 181	
Gil et al (2019)[13]	16 of 17	9 of 10	1 of 2	962 of 968	
Jones et al[18]					39 of 39
Totals	25 of 27	9 of 10	2 of 3	1,143 of 1,149	39 of 39

Table 2. Peer-reviewed publications with twin pregnancy samples and the Harmony prenatal test

Publication Year	Study	Type of study	# Twin Samples	Dichorionic	Monochorionic	Mean gestational age	In vitro fertilization
2014	Gil et al[12]	Cohort, retrospective Cohort, prospective	207 68	174	101	12.0	
2014	Struble et al[9]	Cohort, retrospective	70	35	35		
2015	Stokowski et al[19]	Cohort, blinded	40				
2015	Bevilacqua et al[20]	Cohort, prospective	515*	301	67	13.0	272
2016	Sarno et al[21]	Cohort, prospective	438*	373	65	11.5	246
2017	Jones et al[18]	Cohort	51			16.7	
2019	Galeva et al[22]	Cohort, prospective	224*				
2019	Galeva et al[23]	Cohort, prospective	928*	806	122	11.9	517
2019	Gil et al[13]	Cohort, prospective	997	854	143	12.1	231

*included in Gil 2019

Clinical scenarios included in published Harmony test twin studies
Monochorionic Twins
Dichorionic Twins
Twins conceived with in vitro fertilization
Twins conceived naturally
Pregnancies where both twins have aneuploidy
Pregnancies where only one twin has aneuploidy
First trimester testing
Second trimester testing

Technical Complexities in Twin Pregnancies

Accurate Fetal Fraction Assessment

The major difference between cfDNA analysis of singleton and twin pregnancies is the assessment of fetal fraction. The obstetrics community has come to recognize that accurate measurement of fetal fraction is critical for the performance of all NIPT methodologies.[24] The Harmony prenatal test uses single-nucleotide polymorphisms (SNPs) to assess the contribution of cfDNA from the pregnancy. This methodology has demonstrated accuracy[25] and has been utilized in the validation studies for singletons, twins, sex chromosome aneuploidies and 22q11.2 deletion, as well as in the commercial laboratory.[26-28]

Twin fetuses may contribute differing amounts of cfDNA into maternal circulation.[9] Insufficient fetal fraction from one twin may lead to discordant cfDNA analysis results. The FORTE algorithm takes into account the twin pregnancy to estimate the fetal fraction contribution from each twin and to use the contribution of one twin as the basis to determine the probability score.[9, 12,13,19-22]

Fetal Sex and Sex Chromosome Aneuploidy

The Harmony prenatal test **can** evaluate for fetal sex in a twin pregnancy. A "female" result indicates the absence of Y chromosome and a "male" result indicates presence of Y chromosome. For twin pregnancies, a male result indicates one or two male fetuses. The Harmony prenatal test has published validation data for fetal sex analysis in twin pregnancies.[18] Fetal sex assessment in 39 twin pregnancies correctly reported 18 of 18 twins with two female fetuses and 21 of 21 twins with at least one male fetus.

cfDNA analysis of the X and Y chromosomes requires a rigorous and advanced algorithm. Harmony evaluates the probability for five different sex chromosome aneuploidy conditions (monosomy X, XXX, XYY, XXY and XXYY) in singleton pregnancies.[18,29] The presence of more than one fetus exponentially increases the complexity of the analysis. Therefore, the Harmony prenatal test is not validated to assess for sex chromosome aneuploidy in twin pregnancies.

Clinical Considerations in Twin Pregnancies

Zygosity Does Not Determine Chorionicity

cfDNA analysis cannot provide information about chorionicity and amnionicity of the twin pregnancy, as these are structural rather than genetic features. Optimal management of a twin pregnancy relies on understanding

the placenta and membrane structures.[4,5,30] There is no substitute for ultrasound, which in the first trimester is an effective and reliable tool for determining these clinical characteristics and has been shown to improve outcomes for twin pregnancies.[5,31]

Twin fetuses who share a common placenta are at an increased risk for vascular abnormalities of the placenta such as twin-twin transfusion syndrome (TTTS).[31,32] Fewer than 15% of monochorionic twins may develop TTTS, which can be diagnosed with ultrasound alone. Optimal twin pregnancy outcomes rely on understanding chorionicity; however, knowledge of twin zygosity may not contribute to clinical care.

There is scant published data supporting the reliability of cfDNA analysis for twin zygosity assessment.[33-36] Less than 150 samples from dizygotic twin pregnancies have been published, suggesting zygosity screening may be possible through cfDNA analysis; however, performance remains unknown. Furthermore, rare instances of non-identical monozygotic twins have been reported and may be more common in pregnancies achieved with assisted reproduction.[37-39] Prenatal cfDNA analysis is a screening test and potential zygosity testing through noninvasive methods would not be equivalent to diagnostic tests.

Redraw Request Rate May be Increased for Twin Pregnancies

Twin pregnancy samples may be more likely to not receive a probability score result on the first blood draw, depending on the gestational age of the sample draw, maternal weight and other unknown factors.[21] Twin pregnancies have lower fetal fraction levels as compared to singleton pregnancies.[12,20,21] Some studies suggest that in vitro fertilization (IVF) is associated with lower fetal fraction although current data regarding this theory is very limited.[20,40] Test performance is based on sufficient fetal fraction from each fetus in the sample, and is critical for quality assurance.

Vanishing Twin

The loss of a fetus in a multiple pregnancy is recognized to be a complicating factor when using maternal plasma for aneuploidy screening.[41] cfDNA from a non-viable embryo or fetus is released into the maternal bloodstream; however, the amount and duration of this biological process is not well understood.[11,42-45] Presence of this additional cfDNA may lead to an increased chance of a discordant NIPT result (both “false positives“ and “false negatives“). For this reason, the Harmony prenatal test is not validated for use when a demised twin has been identified. Our validation data for twin pregnancies is based on the presence of two live fetuses in the uterus. Patients who have a demised fetus should consider other methods of evaluating the pregnancy for aneuploidy.

Summary

- The Harmony prenatal test provides accurate aneuploidy risk assessment in twin pregnancies, with robust validation studies.[12,13,18] Traditional maternal serum screening has false positive rates of 5-9% in twin pregnancies.[14-17]
- Reliable estimation of cfDNA contribution from each fetus is especially important when there are two fetuses contributing fetal cfDNA. Redraw requests may be more frequent in twin pregnancies, as compared to singletons, and reflect the quality control measures utilized in the Ariosa Diagnostics CLIA-certified laboratory.
- Chorionicity and amnionicity of twin pregnancies is critical information and can only be determined by ultrasound assessment. Zygosity assessment, by any means, cannot replace the need for this ultrasound assessment.
- Fetal sex may be reported from a twin pregnancy and reflects either one or both fetuses having the reported sex. The sex chromosome aneuploidy panel is not offered for twin pregnancies, due to the complexity of the analysis.
- The presence of a demised fetus within a pregnancy may increase the risk for a false negative or false positive cfDNA result. The Harmony prenatal test should not be ordered for a patient who is known to have a demised or vanished twin.

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