Positive Predictive Value and Interpretation of Results of the Harmony® Prenatal Test

Introduction

Professional societies across the world recommend that pregnant women have access to fetal aneuploidy screening and diagnostic testing and that they are appropriately counseled about the benefits and limitations of all testing options.[1–3] As the newest screening method for fetal aneuploidy, cell-free DNA (cfDNA) analysis has received attention in both the scientific literature and lay press due to concerns that health care professionals and patients are not adequately informed of the limitations of cfDNA screening and that, based on reported sensitivities and specificities of >99% for trisomy 21, may misinterpret the results as being diagnostic.[4–8] Authors have encouraged providers to rely less on test sensitivity and specificity and to educate themselves and their patients about the positive predictive value (PPV) of cfDNA screening for trisomy 21 and other conditions. Online 'PPV calculators' have been developed and laboratories performing cfDNA screening have been encouraged to provide information about 'patient- specific PPV.'[9] This focus on PPV challenges providers and laboratories alike since there is no precedent for either clinical discussion or reporting of PPV in previous methods of aneuploidy screening, i.e., serum analyte screening. The purpose of this paper is to address these issues by 1) describing the risk calculation employed by the Harmony test, 2) explaining how results can be interpreted in the context of PPV, and 3) exploring how test results and PPV information can be interpreted within the broader clinical picture for an individual patient.

Aneuploidy screening strategies

Trisomy 21 screening methods have evolved in the last decades but consistently involve risk assessment based on measurable parameters and recommendation of diagnostic testing when risk scores exceed a determined cut-off. Laboratories performing maternal serum screening, including first and second trimester screening, calculate an individual risk by combining serum analyte measurements with a maternal age-related risk (obtained from epidemiological studies of trisomy prevalence) and in some cases ultrasound findings.

The Harmony test uses a comparable approach, but instead of serum analytes and ultrasound findings, results of cfDNA analysis are used in conjunction with the maternal age- related risk to generate an individual probability score.[10] For resulting probabilities of 1/100 (1%) or greater, genetic counseling and consideration of diagnostic testing are recommended. The Harmony test has a higher sensitivity and significantly lower false positive rate than traditional screening for trisomy 21, but the output of the test is similarly a probability score.[11]

The probability score calculated by the Harmony test represents the odds of a sample being trisomic (vs. disomic).[10,12,13]

Harmony test's FORTE algorithm defines the expected chromosome proportions for trisomy and disomy given a sample-specific fetal fraction and computes the odds of that sample belonging to one or the other group.

The output is a fetal fraction-dependent, patient-specific probability score based on the relative quantities of cfDNA sequences in a maternal plasma sample that are derived from the chromosomes of interest. Most scores fall beyond caps set at 0.01% and 99% but scores between 0.01% and 99% may occur.[14]

Harmony test's probability score takes into account:

- Relative quantities of sequences from targeted chromosomes: Assays targeted to clinically relevant chromosomes are used to amplify and quantify the relative proportion of chromosome- specific cfDNA present in the maternal plasma sample.
- Fetal Fraction: The proportion of fetal DNA present in a maternal sample is determined using assays at polymorphic loci (single nucleotide polymorphisms, or SNPs). The algorithm then calculates an odds- ratio based on the premise that an increase in chromosomal dosage resulting from fetal trisomy will be directly related to the fraction of fetal DNA present in a sample. For example, in a sample with 8% of the cfDNA in maternal plasma being from a pregnancy with trisomy 21, a 4% relative increase in chromosome 21 sequences is expected.
- Maternal age and gestational age: The odds ratio is then modified by a prior risk of trisomy (vs. disomy) that is estimated from maternal age and gestational age of the pregnancy.

The Harmony probability score does not take into account:

- Presence or absence of ultrasound findings
- Results of other screening tests
- Pregnancy or family history

In theory, because the FORTE algorithm incorporates a prior risk of trisomy, alternative risk information (other than a risk based solely on maternal age and gestational age) could be incorporated. In practice, obtaining and compiling this information to create a best estimate of a patient's prior risk presents significant challenges for both clinician and laboratory. Comprehensive clinical information has also not been routinely incorporated by laboratories performing serum screening.

The probability score calculated by the Harmony test does not represent the actual odds of the fetus being trisomic.

Biological factors with the potential to cause discordance between cfDNA results and fetal genetic status include confined placental mosaicism, fetal mosaicism, maternal chromosome changes, and the presence of an unrecognized, nonviable (or viable) twin. There are published case reports of 'false positive' cfDNA results with evidence for these factors as underlying biological causes but limited data addressing frequency and potential impact on cfDNA test performance.[15–21] Without sufficient data to incorporate the likelihood of these and other potential confounding factors into the test algorithm, the probability score can only represent the odds of a sample (meaning the specific patient's plasma cfDNA sample) being trisomic and not the odds of the fetus being trisomic. This is analogous to serum screening in which biological factors such as placental health and underlying maternal disease can impact serum analytes.[22,23]

Positive Predictive Value

Positive predictive value (PPV) is a clinically relevant statistical measure that indicates how likely individuals that screen positive are to be affected by the condition assessed. It can be considered in pre-test decision making to set expectations of test utility. For a given test in a given population, the PPV is the proportion of all positive test results that represent true positives. For example, in a population of 15,841 women presenting for routine prenatal aneuploidy screening (the Harmony - NEXT study), 38 of 47 high risk Harmony results were confirmed by diagnostic testing.[11] This corresponds to an observed PPV of 80.9% for trisomy 21 in this population.

PPV depends not only on test performance but also on the prevalence of the condition in the population studied. In a lower risk subset of the NEXT study population, the observed PPV was lower.[11] Lower disease prevalence, by definition, results in lower PPV as the proportion of true positives decreases relative to the false positives. As a general rule, when comparing tests with similar sensitivities in comparable populations, the test with the lowest false positive rate (highest specificity) will have the highest PPV.

PPV is a population-based statistic. To apply observed PPV in a clinical setting, the population or individual being screened must be directly comparable to the original population tested. If not, PPV as observed in a defined study population may not necessarily be applicable to a specific patient with her own unique clinical factors. Online PPV calculators, such as the "NIPT/Cell Free DNA Screening Predictive Value Calculator" (at http://www.perinatalquality.org/Vendors/NSGC/NIPT/), attempt to account for this by calculating a theoretical PPV, allowing the user to input an estimated patient-specific prior risk as well as expected test sensitivity and specificity. Although it has been suggested that this calculation is more appropriately referred to as an 'estimation of post- test risk,'[24] this document will use the term 'PPV,' albeit not wholly accurate, because of its frequent use in this context.

Theoretical PPV calculations make significant assumptions (see box below) and their output depends on the accuracy of pre-test risk estimation. Clinicians should exercise caution when using these calculations. They are intended for educational purposes and not for direct clinical application.

Table 1 presents the output of theoretical PPV calculations as a function of estimated pre-test risk. The sensitivity and specificity inputs for trisomy 21, trisomy 18, and trisomy 13 mirror performance data for the Harmony test across multiple clinical studies.[25] Because test sensitivity and specificity information is based on a binary classification of results ('high' or 'low' based on a 1% cut-off), theoretical PPV calculations must likewise treat all high risk probability results similarly and low risk probability results similarly, regardless of the specific score provided to the patient.

Limitations to consider when applying theoretical PPV calculations to the Harmony test

- Sensitivity and specificity information is obtained from pooled data across a range of risk categories. The calculations assume that sensitivity and specificity remain constant for all patients.
- PPV calculations are based on a binary (high probability vs. low probability) result and are not able to take into account the individual numeric score calculated by the Harmony test.
- This table is based on an estimated pre-test risk; however, prior risk based on maternal age and gestational age of the pregnancy has already been factored into the probability score by the FORTE algorithm (and so is essentially 'double-counted').
- Although our understanding of cfDNA biology is evolving, there are still many unknowns. For example, the factors used to estimate pre-test risk (e.g., serum analytes or ultrasound findings) and cfDNA screening outcomes may not be completely independent of one another.

Table 1. Theoretical PPV as a function of estimated pre-test risk based on sensitivity and specificity data

Estimated pre-test risk	Trisomy 21 (Sensitivity 0.993 Specificity 0.9996)[25]	Trisomy 18 (Sensitivity 0.974 Specificity 0.9998)[25]	Trisomy 13 (Sensitivity 0.938 Specificity 0.9998)[25]
1:10	99.64%	99.82%	99.81%
1:50	98.06%	99.00%	98.97%
1:100	96.17%	98.01%	97.93%
1:250	90.88%	95.14%	94.96%
1:400	86.15%	92.43%	92.16%
1:800	75.65%	85.91%	85.44%
1:2,000	55.39%	70.90%	70.12%
1:6,000		44.81%	43.88%
1:10,000		32.75%	31.93%
1:15,000		24.51%	23.82%
1:20,000			19.00%

This table is provided for educational purposes only and not for clinical use. The values from this table are not intended to be applied to individual Harmony test results.

Negative Predictive Value

Negative predictive value (NPV) is a clinically relevant statistical measure that indicates how likely individuals that screen 'negative' are to be unaffected by the condition assessed and thus provides some reassurance regarding the question of how likely a fetus is not trisomic. Theoretical NPV calculations based Harmony test performance input for trisomy 21, trisomy 18 and trisomy 13 determine NPV to be at least 99.3% and generally greater than 99.9% for all of the populations listed in Table 1.

Clinical Correlation

Proponents of PPV calculators have suggested that theoretical PPV and NPV be applied to specific patient results and reported by laboratories.[9] Because PPV is intended to be applied to testing of a broad population, not an individual result, PPV and NPV cannot be applied individually in this manner. Each patient presents with her own unique clinical situation. Neither the probability score calculated by the Harmony test, nor test PPV information can be used in isolation without correlation with other clinical factors. The following (fictitious) case examples are intended to illustrate how Harmony test results and correlation within the broader clinical context can aid in counseling and patient decision making.

Case Example 1:

Megan is a 31-year-old woman presenting for ultrasound at 11 weeks gestation. Based on her age-related risk and enlarged nuchal translucency (NT) measurement, her provider estimates the risk for trisomy 21 to be 1:50 in this pregnancy. Megan is counseled about the diagnostic options of chorionic villus sampling (CVS) and amniocentesis as well the option of trisomy 21 assessment, using the Harmony prenatal test. She considers CVS but is uncertain about accepting the associated risk of fetal loss. Her provider suggests that if a Harmony test were to result in a high probability score for trisomy 21, diagnostic testing is likely to confirm the result. She also clarifies that although the NPV for trisomy 21 is expected to be greater than 99.9%, there may be other underlying reasons for the enlarged NT. Megan feels that trisomy 21 is her primary concern and opts to have her blood drawn for the Harmony test.

Case Example 2:

Susan is a 24-year-old woman who has opted for cfDNA screening in her pregnancy. She has no significant history and had an unremarkable 10- week ultrasound. Her Harmony test results in a greater than 99% probability score for trisomy 13. Her provider counsels her about her increased risk for trisomy 13 and the availability of prenatal diagnosis by CVS or amniocentesis. She understands that there is significant concern for trisomy 13 based on the Harmony test result but also that the PPV for trisomy 13 is lower than for the other trisomies due the relative rarity of the condition. Susan decides to schedule an early second trimester ultrasound for more information before proceeding with a diagnostic procedure. At 16 weeks, ultrasound reveals mild growth restriction and an oral cleft. The Harmony test result in the context of these ultrasound findings prompts Susan to proceed with amniocentesis.

Summary

Education and the appropriate clinical interpretation of results are identified challenges for the widespread implementation of fetal aneuploidy screening using cell- free DNA analysis. Some thought leaders propose that positive predictive value (PPV) may be a useful concept for both education and counseling. It is important to recognize that PPV is a statistical measure within the context of a population and that there are limitations to applying it individually. Theoretical PPV and NPV calculations based on estimated pre-test risk and published test performance may provide a foundation for discussion and setting expectations regarding the significance of screening results and may assist patients in decision-making regarding diagnostic testing. However, this information should be interpreted with caution and an understanding of its limitations. Ultimately, all risk assessment must be considered within the broader clinical picture.

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The Harmony non-invasive prenatal test is based on cell-free DNA analysis and is considered a prenatal screening test, not a diagnostic test. Harmony does not screen for potential chromosomal or genetic conditions other than those expressly identified in this document. All women should discuss their results with their healthcare provider who can recommend confirmatory, diagnostic testing where appropriate.

The Harmony prenatal test was developed and its performance characteristics determined by Ariosa Diagnostics, Inc. a CLIA-certified and CAP-accredited clinical laboratory in San Jose, CA USA. This testing service has not been cleared or approved by the US Food and Drug Administration (FDA).