OBSTETRICS

Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities

Mary E. Norton, MD; Rebecca J. Baer, MPH; Ronald J. Wapner, MD; Miriam Kuppermann, PhD; Laura L. Jelliffe-Pawlowski, PhD; Robert J. Currier, PhD

BACKGROUND: Sequential and cell-free DNA (cfDNA) screening are both tests for the common aneuploidies. Although cfDNA has a greater detection rate (DR) for trisomy 21, sequential screening also can identify risk for other aneuploidies. The comparative DR for all chromosomal abnormalities is unknown.

OBJECTIVE: To compare sequential and cfDNA screening for detection of fetal chromosomal abnormalities in a general prenatal cohort.

STUDY DESIGN: The performance of sequential screening for the detection of chromosome abnormalities in a cohort of patients screened through the California Prenatal Screening Program with estimated due dates between August 2009 and December 2012 was compared with the estimated DRs and false-positive rates (FPRs) of cfDNA screening if used as primary screening in this same cohort. DR and FPR for cfDNA screening were abstracted from the published literature, as were the rates of "no results" in euploid and aneuploid cases. Chromosome abnormalities in the entire cohort were categorized as detectable (trisomies 13, 18, and 21, and sex chromosome aneuploidy), or not detectable (other chromosome abnormalities) by cfDNA screening. DR and FPR were compared for individual and all chromosome abnormalities. DR and FPR for the cohort were compared if "no results" cases were considered "screen negative" or "screen positive" for aneuploidy. DR and FPR rates were compared by use of the Fisher exact test.

RESULTS: Of 452,901 women who underwent sequential screening during the time period of the study, 2575 (0.57%) had a fetal chromosomal abnormality; 2101 were detected for a DR of 81.6%, and 19,929 euploid fetuses had positive sequential screening for an FPR rate of 4.5%. If no results cases were presumed normal, cfDNA screening would have detected 1820 chromosome abnormalities (70.7%) with an FPR of 0.7%. If no results cases were considered screen positive, 1985 (77.1%) cases would be detected at a total screen positive rate of 3.7%. In either case, the detection rate of sequential screening for all aneuploidies in the cohort was greater than cfDNA ($P<.0001$).

CONCLUSION: For primary population screening, cfDNA provides lower DR than sequential screening if considering detection of all chromosomal abnormalities. Assuming that no results cfDNA cases are high-risk improves cfDNA detection but with a greater FPR. cfDNA should not be adopted as primary screening without further evaluation of the implications for detection of all chromosomal abnormalities and how to best evaluate no results cases.

Key words: aneuploidy screening, cell-free DNA screening, noninvasive prenatal screening, noninvasive prenatal testing, sequential screening

The introduction of cell-free DNA (cfDNA) screening has impacted prenatal testing for aneuploidy significantly as a result of the reported high sensitivity for trisomy 21 of >99% at a false-positive rate of ≤0.15%.[1-6] Because initial data were all obtained from high-risk populations, professional societies recommended that cfDNA screening be reserved for women who are at high risk for trisomy 21.[7-9]

Recent studies of cfDNA screening in low- and average-risk populations also have reported high sensitivities and specificities for trisomy 21, as well as other common aneuploidies,[10-12] which has led to consideration of the potential use of cfDNA as a primary screening test in all pregnant women. cfDNA screening, however, has limitations that require consideration, including the limited range of targeted aneuploidies, as well as the failure of some tests to provide a result.

Consideration of optimal primary screening policy requires a comparison of available screening options. Aneuploidy screening with serum analytes and nuchal translucency (NT) measurement has long been the mainstay of fetal aneuploidy screening. The most accurate approach is sequential or integrated screening, which is reported to detect 90−95% of Down syndrome cases at a 5% false-positive rate (FPR).[13-16] Current screening algorithms generally target trisomies 18 and 21. Because serum and NT screening are nonspecific, many pregnancies with "false-positive" results for trisomy 18 and 21 are found to be affected with other chromosomal abnormalities[17]; however, detection of these requires that 1 woman in 20 undergo diagnostic testing. Screening with cfDNA also targets trisomies 18 and 21, as well as trisomy 13 and the sex chromosomal aneuploidies, but is very precise and has a far lower screen positive rate. Because of this chromosome specificity, however, cfDNA screening does not detect nontargeted aneuploidies. Analyses of cfDNA test performance have excluded cases with aneuploidies other than those that are targeted. Despite being individually rare, these other aneuploidies also can be associated with significant disability and in total account for as many as one-third of chromosome abnormalities detected prenatally and are therefore important to consider.[18,19]

Cite this article as: Norton ME, Baer RJ, Wapner RJ, et al. Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities. Am J Obstet Gynecol 2016;214:727.e1-6.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2015.12.018

Related editorial, page 673

Studies of cfDNA screening also have largely excluded cases in which a result is not obtained. Test failure rates vary by laboratory but occur in approximately 3% of screened pregnancies.[1] Test failure has been found to be associated with an increased risk of aneuploidy[10,20,21] and is therefore an important component of test performance that becomes more significant as the test is more broadly applied for primary screening. Follow-up of all "no results" cases would increase significantly the effective screen positive rate if cfDNA screening were implemented on a broad scale.

Our objective was to compare the detection rate of sequential screening for all chromosomal abnormalities to the expected performance of primary cfDNA screening in a large, population-based cohort. We compared detection and FPRs for trisomy 21 and other individual common targeted aneuploidies in the cohort, as well as for all chromosome abnormalities if cases with "no result" were assumed to be euploid, and also if these cases were considered "high risk" and in need of follow-up.

Methods

Our cohort included participants in the California Prenatal Screening Program within the California Department of Public Health who underwent first- or first- and second-trimester (sequential) screening. California state regulations require that healthcare providers offer prenatal screening to all women seen before the 20th gestational week. MediCal (California's low-income health coverage) and almost all insurers cover the cost of this screening. Women found to be screen positive are offered follow-up services, with all costs covered by the Program. Covered services include genetic counseling, ultrasound, diagnostic procedures including chorionic villus sampling or amniocentesis, and karyotyping, through contracted Prenatal Diagnostic Centers. All participants are tracked, and results of diagnostic testing are recorded centrally. The Genetic Disease Screening Program California Chromosomal Defect Registry collects information about chromosome abnormalities and pregnancy outcome on all California births, regardless of whether prenatal testing was performed.[22]

Details regarding the Prenatal Screening Program, including screening algorithms and detection rates for first-trimester and/or sequential screening, were published recently.[16] To summarize in brief, since April 2009, the Program has provided first- and second-trimester serum screening with integration of NT ultrasound measurements into the risk algorithm. First-trimester serum screening uses maternal pregnancy-associated plasma protein-A and total human chorionic gonadotropin; patients who have an NT measurement performed are provided a first-trimester risk assessment for Down syndrome and trisomy 18. Second-trimester serum testing uses alpha-fetoprotein, total human chorionic gonadotropin, unconjugated estriol, and dimeric inhibin-A; these are integrated with first-trimester results for a final risk calculation Patients in whom an NT is not performed have serum sequential screening results calculated by the use of the results of the first- and second-trimester serum analytes.

We included data from all women with singleton pregnancies who underwent first-trimester only or first- and second-trimester sequential aneuploidy screening from April 2009 through December 2012. Karyotypes of fetuses or infants were categorized as normal or abnormal; abnormal results were further analyzed as to type of abnormality and whether the abnormality would be detectable by routine cfDNA screening. All abnormal karyotypes were included; although some karyotypes may be associated with a normal outcome, there is a range of potential outcomes with any chromosomal abnormality. The number of cases likely to have a completely normal phenotype (balanced translocations and confined placental mosaicism) was very small (<5% of total abnormalities). Infants with no karyotyping performed in their first year were presumed to be euploid. Although cfDNA laboratories provide somewhat different analyses, for the purpose of this study, nonmosaic trisomy 13, 18, or 21, or sex-chromosomal aneuploidy were considered detectable. Robertsonian translocations causing trisomy 13 or 21 also were considered detectable by cfDNA screening.[3,6] Other rare trisomies, triploidy, structural rearrangements, including unbalanced translocations other than Robertsonian translocation trisomy 13 or 21, duplications and deletions, and all forms of mosaicism were considered not detectable by cfDNA screening.

We compared the frequency of chromosomal defects detected with sequential screening to the frequency with which they would have been detected by primary screening with cfDNA. Detection by either method was defined as screen positive for any condition in a fetus or infant found to be affected by any chromosomal abnormality. Detection and FPRs of cfDNA were based on the recent meta-analysis by Gil et al.[1] The number of chromosomal defects of each type that would be detected by cfDNA was determined by taking into account the reported detection rate, as well as the percentage of each aneuploidy that is undetected as the result of failed cfDNA screening.[1-3,6,10,20] Because the failure rate varies by laboratory and method, a weighted average was calculated on the basis of the primary validation study reported by each of the major laboratories. For each aneuploidy, we calculated the number of affected infants in the cohort in which a result would be successfully obtained; the published detection rates were then applied only to the number of cases for which test results would be available. In a separate analysis, the detection and FPRs of sequential screening also were compared with the rates that would result from primary cfDNA screening if those cases with no result were considered to be screen positive and referred for follow-up testing.

In sum, the overall performance of sequential screening was compared with cfDNA under 2 different assumptions. In the first model, cases of aneuploidy with "no result" were presumed to be normal and therefore false negatives, and the detection rate was lowered accordingly, whereas the screen-positive rate reflected