OBSTETRICS

Chromosomal abnormalities not currently detected by cell-free fetal DNA: a retrospective analysis at a single center

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BACKGROUND: Cell-free fetal DNA analysis is used as a screening test to identify pregnancies that are at risk for common autosomal and sex chromosome aneuploidies.

OBJECTIVE: The purpose of this study was to investigate the chromosomal abnormalities that would not be detected by cell-free fetal DNA in a single medical center.

STUDY DESIGN: This was a retrospective cohort analysis of 3182 consecutive invasive diagnostic procedures that were performed at Montefiore Medical Center's Division of Reproductive and Medical Genetics from January 1, 2009 to August 31, 2014. All patients underwent cytogenetic analysis; one-third of the patients (1037/3182) went through chromosomal microarray analysis.

RESULTS: Clinically significant chromosomal abnormalities were detected in 220 of 3140 cases (7%) after we excluded multiple gestation pregnancies (n = 42). Of these 125 cases (57%) were diagnosed with the common autosomal trisomies that involved chromosomes 21, 18, and 13 and with sex chromosome aneuploidies. There were 23 mosaic karyotypes; 8 of them involved trisomy in chromosomes 21 and 13; 5 of them were sex chromosome mosaics, and 10 of them were other mosaic cases. Five cases of triploidy were detected. Additionally, 19 unbalanced chromosomal rearrangements, a rare autosomal trisomy, and 47 clinically significant findings on chromosomal microarray analysis were diagnosed. Based on the published detection rates of cell-free fetal DNA testing and considering the "no-results" rate, we calculated that 99 of 220 chromosomal changes (45%) could not have been detected by cell-free fetal DNA testing: 16 of the 125 common aneuploidies and sex chromosome aneuploidies, 1 of the 5 triploidy cases, 15 of the 23 mosaic cases, all cases of unbalanced chromosomal rearrangements (n = 19), rare autosomal trisomy (n = 1), and 47 clinically significant chromosomal microarray abnormalities.

CONCLUSIONS: Current cell-free DNA testing could not detect up to one-half of the clinically significant chromosomal abnormalities that were found, which included clinically significant chromosomal microarray abnormalities. Among the 99 abnormal karyotypes that were not identified by cell-free DNA screening, 79% were from women with abnormal screening or abnormal ultrasound finding; 21% were from women who underwent invasive testing simply for advanced maternal age/concern, with no other risk factors or ultrasound findings. This information highlights the limitations of cell-free DNA screening and the importance of counseling patients about all prenatal screening and diagnostic procedures and about the added gain of invasive testing with karyotype and microarray.

Key words: cell-free fetal DNA, detection rate, diagnostic tests

Cell-free fetal DNA (cffDNA) testing is a screening test that shows unsurpassed sensitivity for the detection of trisomy 21, both in the high-risk and the low-risk population.[1-8] CffDNA testing also shows good results in the identification of pregnancies that are at risk for other common autosomal aneuploidies (trisomy 18 and trisomy 13).[1,3,5,9-12] Detection of sex chromosome abnormalities is also improving, and recent studies have shown promising results.[3,13] Detection rates (DRs) for mosaics currently are undetermined, and the detection of triploidy with cffDNA depends on the method that is used and on whether it is a diandric or a digynic triploidy.[14-16] Many national organizations have set guidelines for the use of cffDNA for aneuploidy screening with a collective conclusion that patients who are at increased risk for aneuploidy can be offered cffDNA screening with appropriate pretest counseling.[17-23]

Amniocentesis and chorionic villus sampling (CVS) are invasive diagnostic procedures for the investigation of fetal chromosomal and subchromosomal abnormalities; both carry a risk for miscarriage. According to the recent metaanalysis,[24] the weighted pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95% CI, −0.04 to 0.26%) and 0.22% (95% CI, −0.71 to 1.16%), respectively.

The National Institutes of Health (NIH)−sponsored clinical trial investigated the accuracy of fetal diagnosis by comparing metaphase karyotype and chromosomal microarray analysis (CMA) and showed that there is an increase in the detection of clinically significant CMA abnormalities, even when the metaphase karyotype was normal.[25]

It was reported previously that 17.4% of pregnancies with a positive quadruple test result had karyotype other than the common trisomies (trisomy 21 or trisomy 18/13).[26] Additionally, among patients who underwent invasive prenatal diagnosis because of a positive first-trimester screening (FTS), nearly 30% of the patients were found to have a chromosomal abnormality on karyotype other than the common trisomies[27]; however, CMA abnormalities were not included in these studies.

It is of concern that the use of cffDNA to rule out trisomies 21,18, or 13 after

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a positive first- or second-trimester screening test might result in a diminution in the chromosomal abnormalities (microscopic and submicroscopic) that can be detected with the use of the current invasive procedures.

We aimed to ascertain the percentage of chromosomal abnormalities that would be missed if only cffDNA testing was performed in an underserved, high-risk population.

Materials and Methods

We report a retrospective cohort analysis of 3182 consecutive amniocentesis and CVS procedures performed at Montefiore Medical Center's Division of Reproductive Genetics from January 1, 2009 (the introduction of CMA in our center) to July 31, 2014.

All women who are treated at our medical center are offered a traditional screening test: FTS (nuchal translucency [NT] and analytes) when they initiate prenatal care early in pregnancy or quadruple screening (analytes alone) for patients who need prenatal care past the first trimester and up to 21 weeks gestation. A mid-trimester detailed anatomy scan is offered to all patients. High-risk patients and the patients who are interested in invasive testing are referred for genetic counseling.[28] If a patient chooses to have a diagnostic test, a CVS or amniocentesis (10 to 13 + 6/7 and 16-23 weeks gestation, respectively) is performed. Invasive procedures are performed on-site at our center; a standard metaphase cytogenetic analysis of cells that are obtained by amniocentesis or CVS is performed in one of the authorized diagnostic laboratories routinely used by our institute. Array-based comparative genomic hybridization (aCGH) has been used at our center since 2009 (2009-2010 as part of an NIH array study that used mainly oligonucleotide probes[25] and since 2010 have used a single nucleotide polymorphism [SNP] platform for most patients and oligonucleotide platform for the remainder of patients, depending on insurance coverage and referent laboratory). Until 2013, aCGH was offered only to high-risk patients who were having an invasive procedure (high risk includes advanced maternal age [AMA] and maternal age adjusted risk after screen positive test, women who had a previous fetus/child affected by autosomal trisomy, structural anomalies identified by ultrasonography and parental carrier of chromosomal rearrangement[28]). Beginning in 2014, aCGH was offered to all patients who would undergo an invasive procedure as per the American College of Obstetricians and Gynecologist recommendation.[29]

All results were recorded in the patient's electronic medical record and the department's log books by board-certified genetic counselors and were reviewed by medical geneticists. Results were categorized into common aneuploidies (involving trisomies in chromosomes 21, 18, and 13), sex chromosome aneuploidies (monosomy X, XXX, Klinefelter syndrome and XYY syndrome), triploidy, unbalanced chromosomal rearrangements (translocation, inversion and deletion/duplication), mosaics, and CMA abnormalities.

Statistical analysis

The Student *t* test and Pearson's chi-square test were used to evaluate the statistical significance of the comparison of the indication for procedure in the normal and abnormal results groups. A probability value of $<.05$ was considered to indicate statistical significance.

Calculation of detectability by cffDNA

We used the following weighted pooled DR and false-positive rates (FPR), based on the recent metaanalysis of studies of maternal peripheral blood cffDNA analysis[30]: For trisomy 21, 99.2% DR (95% CI, 98.5−99.6%) with 0.09% FPR (95% CI, 0.05−0.14%); for trisomy 18, 96.3% DR (95% CI, 94.3−97.9%) with 0.13% FPR (95% CI, 0.07−0.20%); for trisomy 13, 91.0% DR (95% CI, 85.0−95.6%) with 0.13% FPR (95% CI, 0.05−0.26%); for monosomy X, 90.3% DR (95% CI, 85.7−94.2%) with 0.23% FPR (95% CI, 0.14−0.34%); for sex chromosome aneuploidies other than monosomy X, 93.0% DR (95% CI, 85.8−97.8%) with 0.14% FPR (95% CI, 0.06−0.24%).

The DR of triploidy was calculated based on available publications at this time; aiming to identify fetal triploidy using cffDNA. Nicolaides et al[14] showed the correct identification of 4 of 4 diandric triploidy using a SNP-based cffDNA. That same method failed to detect 4 of 4 digynic triploidy. Others also demonstrated very low fetal fraction in digynic triploidy (fetal fraction <3%, no result reported on cffDNA)[15] and correct identification of diandric triploidy with the use of SNP.[16] Hence, a 100% DR for diandric triploidy with SNP-based cffDNA testing and 0% DR for digynic triploidy were assumed.

Clinical validation trials report a wide range of DRs of mosaics cases by cffDNA analysis[3,31,32]; 3 of 3 of mosaic trisomy 21 and 1 of 1 mosaic trisomy 18 were detected by cffDNA; the DR reported for monosomy X mosaic is 2 of 7 (29%)[3]. CffDNA test has not been reported to detect other complex mosaics.[3] Others have reported cffDNA analysis to detect only 1 of 2 cases of mosaic trisomy 21.[32] Also, cffDNA could not detect mosaic trisomy 13 and mosaic trisomy 21 superimposed with mosaic T18 (trisomy 21 was detected, but the mosaic T18 was not).[32] We conservatively calculated the common trisomies mosaic DR as the same as we calculated for the complete aneuploidies. This is probably an overestimation because the contribution of the fetal excess chromosome is partial; therefore, the DR is expected to be lower compared with the DR of the complete trisomies. For monosomy X mosaic, we calculated 29% DR.[3] For other sex chromosome aneuploidy mosaic, there is no published DR.

To date, there are no data available for the cffDNA DR of unbalanced chromosomal rearrangements or CMA abnormalities.

Data on the no-result rate because of assay failure or low fetal fraction varies dramatically from 0.5[2]-6.1%[33] and recently 3% in the United States[1] and 0.1% in China (with 2.18% required repeat blood sampling).[34] In pregnancies that are complicated with chromosomal aneuploidies, the rate of no-result is