leukemia after allogeneic hematopoietic-cell transplantation needs to be determined in prospective trials.

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DNA Sequencing versus Standard Prenatal Aneuploidy Screening

TO THE EDITOR: Bianchi et al. (Feb. 27 issue)[1] described noninvasive diagnosis of fetal aneuploidy from cell-free DNA (cfDNA) in maternal plasma with the use of an Illumina HiSeq massively parallel sequencing platform and compared this method with standard prenatal aneuploidy screening. They concluded that prenatal screening by assay of cfDNA had significantly lower false positive rates and higher positive predictive values for detection of trisomies 21 and 18 than did standard screening. We screened for fetal aneuploidy using cfDNA from 2275 pregnant women (each of whom carried a single fetus) by means of a benchtop semiconductor sequencing platform (Ion Proton) and obtained similar results.[2] We then expanded the study to analyze plasma samples from 5010 women and discovered five false positive results from cfDNA owing to fetoplacental mosaicism. We had access to the karyotype status of 522 fetuses, 5 of whom had a false positive result. The remainder (4488 fetuses) tested negative on cfDNA testing. After delivery, these 4488 infants were examined clinically and followed for at least 6 months. None had clinical symptoms consistent with aneuploidy, and karyotyping results obtained after delivery confirmed these findings. Therefore, cfDNA testing yielded a false positive rate of approximately 1 in 1000 (Table 1). We confirmed fetoplacental mosaicism of aneuploidies by assaying chromosome-specific markers[3] with the use of fetal cells (amniotic cells or cord-blood cells), newborn placental tissue, and peripheral blood from the mother and father (Table 1).

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Table 1. False Positive Results of Cell-free DNA (cfDNA) Testing for Fetal Aneuploidy.*

Test	Case 1	Case 2	Case 3	Case 4	Case 5
Sequencing of cfDNA in maternal plasma	Trisomy 21	45,XO	Trisomy 21	45,XO	Trisomy 16
Karyotyping (source)	46,XY (amniotic cells)	46,XY (amniotic cells)	46,XY (cord-blood cells)	46,XX (amniotic cells)	46,XX (amniotic cells)
QF-PCR assay of fetal cells (source)	46,XY (amniotic cells)	46,XY (amniotic cells)	46,XY (cord-blood cells)	46,XX (amniotic cells)	46,XX (amniotic cells)
QF-PCR assay of placental tissue	Mosaicism of UPD-21/trisomy 21	Mosaicism of 45,XO/46,XY	Trisomy 13/46,XY	45,XO	Trisomy 16/46,XX

* QF-PCR denotes quantitative fluorescent polymerase chain reaction, and UPD uniparental disomy.

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TO THE EDITOR: Bianchi et al. compared the false positive rates of cfDNA testing with the average of five types of standard screening. However, there are substantial differences in the performance of these five types of screening.[1] Indeed, in Table S1 in the Supplementary Appendix of their article (available with the full text of the article at NEJM.org), false positive rates differ significantly among the five types of standard screening ($P=0.02$ for trisomy 21). In addition, the results of cfDNA testing were presented for all trimesters and for the first and second trimesters only (<27 weeks of gestational age). We would be interested in a comparison of the false positive rates between standard screening and screening by cfDNA for the subgroup of women whose blood samples were obtained before the end of the first trimester. In the event of a positive result, first-trimester screening permits early diagnosis on the basis of karyotype and a timely decision regarding pregnancy termination after genetic counseling.[2]

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THE AUTHORS REPLY: Liao and colleagues described detection of aneuploidies of chromosomes 13, 18, 21, X, and Y in maternal plasma with the use of a benchtop semiconductor sequencing platform,[1] which has potential to reduce DNA sequencing costs. In contrast to our study, in which 100% of the patients had reported clinical outcomes, Liao et al. had follow-up karyotype information on only 23% of patients (522 of 2275). In an expanded series of 5010 pregnant women, they observed a rate of false positives — entirely consequent to fetoplacental mosaicism — of approximately 0.1%. This is consistent with a previously reported false positive rate of 0.2%[2] and underscores the point that cfDNA sequencing is not a diagnostic test.

We agree with Vrachnis et al. that there is variation in test performance among the different types of prenatal screening for aneuploidy. However, as we showed in Table S1 in the Supplementary Appendix of our article, for each of the different standard screening approaches currently used in the United States, the false positive rate was consistently lower with cfDNA sequencing than with standard screening tests. Of 736 patients who underwent first-trimester combined screening, 32 (4.3%) had a false positive result for trisomy 21. In contrast, maternal plasma cfDNA sequencing yielded a false positive result in 2 of 759 pregnant women (0.3%) who had their blood drawn during the first trimester. We agree that first-trimester testing allows more time for subsequent management considerations, but a substantial number of pregnant women in the United States seek aneuploidy screening in the second trimester, and some undergo DNA testing in the third trimester, as shown by our study and our clinical laboratory experience.[3]

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