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Noninvasive Prenatal Testing and Detection of Maternal Cancer

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Noninvasive prenatal testing (NIPT) to detect fetal aneuploidy (an abnormal number of chromosomes) using massive parallel sequencing of cell-free DNA (cfDNA) from maternal blood is gaining rapid acceptance in obstetrics, given its high sensitivity and specificity for the detection of trisomies 21, 18, and 13. Placental DNA, a surrogate of fetal DNA, enters the maternal bloodstream via apoptosis of trophoblasts in the intervillous space of the placenta, where it mixes with cfDNA of maternal origin. The fetal fraction in this mixture usually varies between 3% and 15% in the late first and second trimesters. NIPT represents a form of advanced screening, and positive results consistent with aneuploidy require confirmation by a diagnostic test using material obtained via amniocentesis or chorionic villus sampling (CVS).^{1,2}

The sensitivity of NIPT for the detection of trisomies 21, 18, and 13 ranges between 91% and 99.2%; the false-positive rates range between 0.09% and 0.13%.³ False-positive results have been attributed to confined placental mosaicism, maternal chromosomal mosaicism, maternal DNA copy number variants, and maternal organ transplant from a male donor.⁴ Two case reports (in 2013 and 2015) suggested that the discordant results (between those of NIPT and amniocentesis) could be due to undiagnosed maternal cancer.^{5,6}

The first case described a 37-year-old pregnant woman with an uncomplicated pregnancy who had NIPT performed at 13 weeks of gestation. The findings were suggestive of fetal aneuploidy for chromosomes 13 and 18 in 2 different maternal blood samples. Yet the fetal karyotype was 46,XY without evidence of submicroscopic genomic imbalances with whole genome oligonucleotide microarray. After delivering a healthy infant, the patient reported

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pelvic pain found to be due to a pathologic fracture of the pubis; a biopsy showed metastatic neuroendocrine carcinoma of unknown origin. A separate lesion on the anterior vaginal wall proved to be a high-grade neuroendocrine small cell carcinoma. Tumor cells showed aneuploidy for chromosomes 13 and 18, consistent with the findings from the analysis of maternal blood assessed with NIPT.⁵

The second patient was a 27-year-old asymptomatic woman at 12 weeks of gestation whose NIPT results had abnormal genomic findings for chromosomes 8, 9, and 14 that were discordant with a normal fetal karyotype from amniotic fluid cells. Moreover, maternal lymphocytes did not have evidence of aneuploidy or mosaicism. Given the genomic imbalances, a malignancy was suspected, and the patient underwent a whole-body diffusion magnetic resonance imaging (MRI) scan, which showed an anterior mediastinal mass and enlarged lymph nodes in the cervical and retroclavicular regions. The diagnosis of nodular sclerosis Hodgkin lymphoma was made, based on a biopsy of the mediastinal mass. Hodgkin/Reed-Sternberg cells showed findings consistent with those observed with the analysis of cfDNA in maternal blood. Based on these observations, the authors investigated whether cfDNA could be informative in newly diagnosed cases of nodular sclerosis Hodgkin lymphoma in nonpregnant women ($n = 4$) and in nonpregnant patients who had a first relapse after therapy ($n = 5$). The results in 8 of the 9 cases showed genomic imbalances, and such findings were validated by fluorescent in situ hybridization on Hodgkin/Reed-Sternberg cells in tissue specimens.⁶

Common features of the 2 cases were that the patients were young asymptomatic pregnant women, had abnormal NIPT results discrepant with the fetal karyotype (“false-positive NIPT”), were subsequently diagnosed to have cancer, and the malignant cells showed evidence of multiple aneuploidies that mirrored the findings reported by maternal cfDNA analyzed for NIPT.

A recent study reported that among more than 4000 pregnant women undergoing NIPT, 3 patients were found to have aberrant genome representation profiles that were reminiscent of cancer-related copy number variation. These women underwent whole-body diffusion-weighted MRI and were found to have cancer—the first had a high-grade serous ovarian carcinoma (stage IV with multiple metastases); the second, a follicular lymphoma; and the third, Hodgkin lymphoma. Analyses of the genome representation profile of the tumors showed that the copy-number variations detected in maternal plasma were present in the tumors.⁷

The report by Bianchi et al⁸ in this issue of JAMA is the first large-scale study that strengthens the premise that a false-positive NIPT result could be due to the presence of maternal malignancy based on the results of a case series of pregnant women who had NIPT and were subsequently diagnosed with cancer. This work leverages the information of a laboratory that had processed 125 426 samples from NIPT over 2.5 years with a test to detect the presence of aneuploidy for chromosomes 21, 18, and 13 (testing for sex chromosome aneuploidy was optional). Of these samples, 3% (3757) had NIPT results consistent with aneuploidy. The practice of the laboratory was to discuss all abnormal NIPT results with the referring clinician and recommend a diagnostic test, and clinicians were

asked to inform the laboratory if the results were discordant. When such results were reported, the possible explanations for discordance were discussed by the laboratory staff (medical director and/or certified genetic counselors) with the referring clinician.

Since virtually all cancers have somatic genetic alterations that can be reflected in circulating cfDNA,^{9,10} and many of these derangements involve chromosomes 21, 18, and 13 (the focus of NIPT),¹¹ it is plausible that discordant NIPT results could be explained by the presence of a maternal malignancy, particularly if the results of NIPT show multiple aneuploidies.

Bianchi et al⁸ estimated that 20% to 44% of discordant NIPT results that included multiple aneuploidies could potentially be explained by maternal malignancies. This estimate was based on information available from 39 patients with NIPT results with multiple aneuploidies. Of those patients, 16 had discordant results between NIPT and fetal karyotype, 4 were concordant, and for the remaining 19, no information was available about concordance. Of the 16 cases with discordant results, 44% (7/16) were diagnosed to have maternal cancer. Assuming that the 19 cases without available information about fetal karyotype were all discordant (best-case scenario), then 20% (7/35) of discordant NIPT results with multiple aneuploidies could be explained by maternal cancer.

Is this estimate reasonable? The information provided by Bianchi et al⁸ reflect the best estimate, based on available data, and is an important advance from individual case reports and small case series. Ideally, these estimates should be derived from a cohort study; something easier said than done, because it requires participation and systematic ascertainment of a large number of patients undergoing a test that provides private and potentially sensitive information. Laboratories with similar information would provide a valuable service by sharing their experience with NIPT testing results because this may improve the accuracy of the estimates. Another step could be the establishment of a registry of cases with discordant NIPT results associated with multiple aneuploidies, and also with single aneuploidies, to determine maternal cancer status at the end of pregnancy and beyond. There is still much to be learned. Perhaps some instances of tumor-derived cfDNA in the bloodstream reflect active, and ultimately successful, control of the cancer by the maternal immune system. As the science evolves and prospective data collection occurs, physicians and researchers may need to consider how the consent process might need to be modified to ensure that the results from collection of NIPT data can be reported as research studies in scientific journals.

What are the implications of the report by Bianchi et al for obstetrical practice? Obstetricians have experienced the issues of incidental findings associated with the implementation of a screening test that led to the identification of maternal cancer. The case in point is maternal serum alpha fetoprotein (AFP), originally implemented to screen for neural tube defects. An unexplained elevated maternal serum AFP level has been reported in exceedingly rare cases of maternal liver cancer^{12,13} or endodermal sinus tumors,¹⁴ both neoplasms that produce this protein. Cell-free DNA, however, has the potential to be more informative because AFP is a biomarker for only a small fraction of tumors. To put things into perspective, the overall rate of abnormal NIPT results was approximately 3% and the

rate of false-positive results was only 0.05% to 0.1%.⁸ Moreover, the frequency of abnormal NIPT results with multiple aneuploidies was 0.03%; therefore, the constellation of findings that would increase the probability of detecting a maternal malignancy is present in only a small fraction of pregnant women. For the few patients who had discordant results and multiple aneuploidies, the probability of cancer appears to be considerable (20%–44%).⁸ Replication of these results is needed. Overall, however, based on the data in this study, the rate of occult malignancies was 0.008% (10 cases of maternal cancer among 125 426 NIPT results).

The question of whether incidental findings derived from genome sequencing should be reported to the patient has been discussed by the American College of Medical Genetics and Genomics.¹⁵ There is increasing emphasis on patient choice with appropriate discussion and informed consent. NIPT already challenges the ability of clinicians to have patients understand that NIPT is a screening test, not a diagnostic test, that focuses on a limited set of fetal aneuploidies. The possibility of uncovering information relevant to maternal cancer will be a further challenge, yet may have important therapeutic implications for the patient. At this time, there is insufficient evidence about the benefits, risks, and costs of reporting the incidental findings, as Bianchi et al mention.⁸ As the authors correctly recommend, the data emphasize the need for performing a diagnostic procedure to determine the fetal karyotype in all situations in which there is an abnormal NIPT result. Given that it is likely that NIPT will increase in the coming years, an active dialogue among stakeholders (obstetricians, patients, laboratories, ethicists, policy makers, etc) needs to take place to provide informed advice to potentially affected pregnant women and to guide the care of such patients.

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