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Unusual Prenatal Genomic Results Provide Proof-of-Principle of the Liquid Biopsy for Cancer Screening

Diana W. Bianchi, M.D.

National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

A best-selling book, entitled, “What to Expect When You’re Expecting,” has provided reassuring information to several generations of pregnant women (1). Filled with advice on topics such as nutrition and morning sickness, it lacks a chapter on being diagnosed with cancer during pregnancy. Why would it even have such a chapter? Pregnant women represent the epitome of good health and the promise of new life. No one expects to be diagnosed with cancer while pregnant. Yet, as Dharajiya and colleagues demonstrate (2), incidental detection of maternal neoplasia is possible while performing prenatal whole genome DNA sequencing to screen for fetal chromosomal aneuploidies. Not only is it possible; it is not uncommon. It is also creating new ethical and clinical dilemmas.

These investigators described 55 maternal plasma samples sequenced over a three-year period in a large-scale clinical laboratory that had altered genome profiles and were suspicious for maternal neoplasm. Of these, 43/55 (78.1%) had some clinical follow-up information available. Forty of 43 had confirmed maternal neoplasms; half were due to uterine leiomyomas and half were due to a variety of malignant tumors. Of these, 7/20 were already diagnosed, but 13 women who were expecting information about their fetus instead received the worrisome news that their plasma DNA profile put them at risk for cancer.

Starting in 2011, analysis of circulating cell free DNA (cfDNA) in the blood of pregnant women began to be offered clinically as a prenatal screen for trisomies 13, 18, and 21 (3). Due to its superior sensitivity and positive predictive values compared to serum biochemistry and ultrasound markers, the cfDNA test became rapidly incorporated into clinical prenatal clinical care. By 2013, however, reports began to appear that described examples of false positive test results (4). Experts questioned the methodologies used to sequence cfDNA and interpret the raw sequencing results. Furthermore, while some clinical laboratories issued reports of “test failure,” others called out results that would be incompatible with a normally developing fetus, such as monosomy 13 or multiple aneuploidies.

cfDNA analysis is performed on maternal plasma samples taken any time between 10 and 40 weeks of gestation. The maternal plasma contains circulating DNA from the placenta (which serves as a proxy for the fetus) as well as the maternal hematopoietic system. Clinical laboratories use different techniques to perform cfDNA analysis for fetal aneuploidies (3), including comparing single nucleotide polymorphisms in the cfDNA to reference (usually maternal leukocyte) DNA, using oligonucleotide arrays with expanded coverage of the

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chromosomes of clinical interest, and whole genome sequencing (WGS), in which the sample's cfDNA is compared to a euploid reference genome. To date, results suggestive of maternal neoplasm have only been reported following WGS.

In this study, the investigators used machine learning to create an algorithm from a training cohort of 31 known cases of neoplasia (2). They then applied the algorithm to prospectively interpret the sequencing results from pregnant women whose sequencing results demonstrated multiple, widespread areas of genome-wide imbalance. For women whose results suggested neoplasm, a discussion was held between the laboratory director and the referring physician. While, as the authors' state, "no diagnosis was offered," the conversation led to follow-up testing in 43/55 women. In 93% of the women who underwent additional testing, neoplasia was found.

While not yet approved by the FDA, prenatal genomic screening for aneuploidy has been extensively validated worldwide. Using the prenatal test as a means of screening for neoplasm raises questions. Should the incidental test results be disclosed, as was done here (5)? A review of consent forms for prenatal cfDNA sequencing showed that only a few mentioned that maternal DNA was concurrently being sequenced or the possibility that maternal diseases could be disclosed by the test (6).

Prior studies avoided this dilemma by studying the DNA profiles retrospectively, in which a clinical diagnosis of malignancy was already known. In the first case, described in 2013, a 37 year-old woman underwent NIPT at 13 weeks of gestation and received a test report of "aneuploidy detected, trisomy 13 and monosomy 18" (7). As per recommended clinical guidelines, an amniocentesis was performed, and showed a normal male (46, XY) karyotype. The patient delivered a healthy infant, but post-partum experienced pelvic pain. Her work-up demonstrated pathologic pelvic bone fractures due to metastatic neuroendocrine carcinoma. Similarly, Bianchi *et al.* reported on eight cases ascertained by the referring physician voluntarily reporting to the clinical laboratory that a diagnosis of cancer had been made (8). At the time of cfDNA testing these women were clinically asymptomatic; five of the eight had multiple aneuploidies (including sex chromosome aneuploidies) detected. The women were re-consented for re-analysis of the WGS results, revealing areas of the genome that were previously masked for clinical testing. Multiple areas of genome-wide imbalance were subsequently observed. Similar to the Dharajiya report (2), the most common malignancies were lymphomas, as would be expected in a relatively young adult population. Additional tumors included two cases of colorectal cancer, one case of acute T-cell lymphoblastic leukemia, and the previously mentioned neuroendocrine carcinoma.

With increased understanding of maternal malignancy as the underlying basis for unusual plasma DNA sequencing results, additional case reports have been recently published. These include a 27 year-old pregnant woman with stage IIA Hodgkin's lymphoma (9), a 25 year-old pregnant woman with deletions of the long arms of chromosomes 9 and 22 in her plasma DNA who was diagnosed with chronic myelogenous leukemia (10), a 37 year-old pregnant woman with monosomies of 13, 18, 21 and X that was due to stage IV metastatic colon cancer (11), and a 40 year-old pregnant woman with extensive copy number variation who

was diagnosed with multiple myeloma (12). Although there is no one uniform sequencing result that correlates with neoplasia, patterns are beginning to be recognized. As shown in the accompanying report (2), certain characteristics are sensitive and specific predictors for copy number alterations. Other groups have described a typical “saw tooth” pattern associated with malignancies when analyzing the DNA results across the genome (11).

From these cases it is clear that the analytic validity of noninvasive prediction of maternal neoplasia is improving. However, this has led to inconsistencies regarding next steps, and whether or not there is clinical utility in disclosing incidental findings. In a survey of over 300 genetic counselors, 95% were aware of the fact that noninvasive prenatal testing could detect maternal cancer (13). Yet only 29% regularly informed their patients of this possibility during pre-test counseling. Over 91% of counselors felt that professional guidelines were needed to guide subsequent patient management. Currently, no guidelines exist, except in Belgium, where noninvasive prenatal testing is currently provided as a first tier screening test to all pregnant women, and all results with clinical action ability are disclosed (10). Furthermore, in Belgium, following a cfDNA screening result that suggests neoplasia, it is recommended to perform maternal whole body magnetic resonance imaging (MRI) (14). In three pregnant women, MRI studies illuminated the source of the abnormal DNA: bilateral metastatic ovarian cancer, follicular lymphoma, and nodular sclerosing Hodgkin lymphoma (14). Based on this one report, some groups recommend follow-up imaging studies. In the United States, however, insurance rarely pays for these. Additional clinical research is urgently needed to provide evidence that can inform future professional guidelines.

Disclosure of results also needs further ethical reflection and discussion. There is evidence to suggest that early diagnosis led to a beneficial change in clinical management (9, 10), but it also precipitated a general anxiety disorder in one woman (12). There are also data to suggest that a delay in the maternal diagnosis due to uncertainty regarding the significance of the cfDNA results may have contributed to a worse prognosis (7, 11). Dr. Vinay Prasad, an oncologist, argues that the downstream testing needed to follow-up on the abnormal cfDNA results in “discomfort, anxiety and harm,” without evidence that the earlier diagnosis changes clinical outcome (5).

Another aspect of the present study is that it validated the laboratory’s prior demonstration that leiomyomas can be associated with abnormal cfDNA results (15). Here, twenty cases of abnormal cfDNA results were associated with the presence of known uterine leiomyomas, but since they are very common, especially in African-American women, one might wonder whether the ones that are detected via cfDNA sequencing have biological differences? Furthermore, how does the presence of a known leiomyoma affect pre-test counseling for cf DNA sequencing and post-test clinical management of abnormal results?

In summary, prenatal genomic testing provides proof-of-principle that liquid biopsy works as a screen to detect neoplasia, even when it was not designed to do so. This report shows that the clinical sequencing laboratory can find the women who are potentially at risk, with the limitation that there is no follow-up on the screen negative women. Now it is up to the

clinician-investigators to design a study that determines the best approach for clinical follow-up and determine if earlier diagnosis and treatment improves outcomes.

References

1. Murkoff H, Mazel S. What to Expect When You're Expecting. 5th Edition New York: Workman Publishing Co., 2016.
2. Dharajiya NG, Grosu DS, Farkas DH, et al. Incidental detection of maternal neoplasia in non-invasive prenatal testing. Clin Chem 2017; in press.
3. Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genet 2016; 17: 643–656. [PubMed: 27629932]
4. Mennuti MT, Cherry AM, Morrissette JJ, Dugoff L. Is it time to sound an alarm about false-positive cell-free DNA testing for fetal aneuploidy? Am J Obstet Gynecol 2013; 209:4150419.
5. Prasad V. Non-invasive, serum DNA pregnancy testing leading to incidental discovery of cancer: a good thing? Eur J Cancer 2015; 51:2272–2274. [PubMed: 26278647]
6. Bianchi DW. Pregnancy: Prepare for unexpected prenatal test results. Nature 2015; 522: 29–30. [PubMed: 26040879]
7. Osborne CM, Hardisty E, Devers P, et al. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. Prenat Diagn 2013; 33: 609–611. [PubMed: 23559449]
8. Bianchi DW, Chodova D, Sehnert AJ, et al. Noninvasive prenatal testing and incidental detection of occult malignancies. JAMA 2015; 314:162–169. [PubMed: 26168314]
9. Vandenberghe P, Wlodarska I, Tousseyn T, et al. Non-invasive detection of genomic imbalances in Hodgkin/Reed-Sternberg cells in early and advanced stage Hodgkin's lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study. Lancet Haematol 2014; 2(2): e55–e65.
10. Janssens K, Deiteren K, Verlinden A, et al. Detection of a case of chronic myeloid leukaemia with deletions at the t(9;22) translocation breakpoints by a genome-wide noninvasive prenatal test. Prenat Diagn 2016; 36: 760–765. [PubMed: 27293081]
11. Smith J, Kean V, Bianchi DW, et al. Cell-free DNA results lead to unexpected diagnosis. Clin Case Reports 2017; 5:1323–1326.
12. Imbert-Bouteille M, Chiesa J, Gaillard JB, et al. An incidental finding of maternal multiple myeloma by non-invasive prenatal testing. Prenat Diagn 2017; 10 12 Doi: 10.1002/pd5168.
13. Giles M, Murphy L, Krstic N, Sullivan C, Hashmi SS, Stevens BK. Prenatal cfDNA screening results indicative of maternal neoplasma: survey of current practice and management needs. Prenat Diagn 2017; 37:126–132. [PubMed: 27878832]
14. Amant F, Verheevke M, Wlodarska I, et al. Presymptomatic identification of cancers in pregnant women during noninvasive prenatal testing. JAMA Oncol 2015; 1(6): 814–819 [PubMed: 26355862]
15. Dharajiya NG, Namba A, Horiuchi I, et al. Uterine leiomyoma confounding a noninvasive prenatal test result. Prenat Diagn 2015; 35: 990–993. [PubMed: 26058600]