Brief Report

Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing

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Over the past years, noninvasive prenatal testing (NIPT) for fetal aneuploidy has become a clinical reality. Most NIPT providers focus on the detection of only the most common aneuploidies, trisomies 21, 18, and 13. However, random genome sequencing not only enables the detection of the viable fetal trisomies but also other chromosomal aneuploidies and even segmental fetal imbalances. Applying similar large parallel sequencing approaches to plasma-derived DNA from patients with cancer has recently been shown to detect tumor-associated copy number profiles in selected tumors prone to copy number changes. We optimized a large parallel sequencing-based NIPT dataset and analysis, which not only interrogates the common trisomies but also allows the genomewide discrimination of fetal and maternal segmental aneuploidies. All patients undergoing NIPT consented to release of information for study purposes beyond trisomy 13, 18, and 21; this consent and the study protocol were approved by the University Hospitals, Leuven ethical board. Of the first 4000 prospective NIPT samples, we identified 3 profiles with an aberrant quality score and reproducible genomewide representation (GR) profiles reminiscent of cancer-related copy number variation. All 3 women (and only those 3 women) were referred for whole-body diffusion-weighted magnetic resonance imaging (WB-DWI), which revealed a tumorous mass in all 3 cases (eMethods and eFigure in the Supplement).

Report of Cases

Case 1
Bilateral ovarian carcinoma with diffuse peritoneal spread was detected in a pregnant woman who underwent NIPT, along with retroperitoneal lymphadenopathies and the presence of bilateral pleural fluid, consistent with ovarian cancer, FIGO (International Federation of Gynecology and Obstetrics) stage IV-A (Figure 1A). The pathological examination confirmed the presence of a high-grade serous ovarian carcinoma with multiple metastases to the omentum (14-mm), the paracolic peritoneum, and the appendix, as well as implants on the small bowel. In addition, 12 of 30 sampled lymph nodes tested positive for tumor cells.
To confirm that the abnormal GR profile was due to tumor-derived cell-free DNA (cfDNA), fluorescence in situ hybridization (FISH) was performed on tumor biopsy using probes for IRF4/6p24 (gained), TCRB/7q35 (gained), JAK2/9p24 (gained) and BCL2/18q21 (lost), which confirmed that the genomic imbalances identified in the cfDNA matched the gains and losses of the corresponding chromosomal regions in carcinoma cells (Figure 2).

Case 2
In a second woman who underwent NIPT, multiple supradiaphragmatic and infradiaphragmatic lymphadenopathies were revealed on subsequent WB-DWI, as well as diffusion restriction at the spleen and left tonsil, corresponding to Ann Arbor stage III-SE disease (Figure 1B).

An excision biopsy from the involved left tonsil indicated follicular lymphoma (FL), grade 3a (CD5-, CD10+, CD20+, BCL2+, and Ki67+). Cytogenetic analysis of the biopsy material detected an abnormal karyotype in 13 of 20 analyzed cells, described as follows: 48,XX,i(6)(p10),dup(7)(q11q22),+dup(7)(q11q22),+11,dup(12)(q13q15),du1p(13)(q21q34),t(14;18)(q32;q21). FISH testing with probe Vysis LSI IGH/BCL2 (Abbott Molecular) detected the IGH/BCL2 rearrangement resulting from the FL-characteristic t(14;18) in 49% of the analyzed interphase cells. Array comparative genomic hybridization (aCGH) analysis on DNA of the tumor biopsy confirmed the imbalances detected by cytogenetics (Figure 3), and most of the imbalances previously detected by cfDNA GR profiling strongly suggested that the GR profile was FL derived.

Case 3
In a third woman who had undergone NIPT, subsequent WB-DWI revealed a mass in the anterior mediastinum and multiple lymphadenopathies in the left neck, while excluding involvement of bone marrow, spleen, or visceral organs, corresponding to Ann Arbor stage II disease (Figure 1C).

A transthoracic computed tomography–guided punch biopsy of the anterior mediastinal mass was performed. Pathological examination indicated a nodular sclerosis form of Hodg-
kin lymphoma characterized by the presence of CD15+/CD30+ neoplastic Hodgkin and Reed-Sternberg (HRS) cells. FISH analysis of the available formalin-fixed, paraffin-embedded tumor biopsy specimens using probes for MYC/8q24, JAK2/9p24, and IGH/14q32 (eTable in the Supplement) confirmed the copy number alterations in the HRS cells, thus indicating that the cfDNA GR profile matched the genomic imbalances in the HRS cells (Figure 4).

Interestingly, following blood sampling after the first chemotherapy administration, the aberrant GR profile had “normalized” (eFigure, axis E, in the Supplement), and the profile remained within normal parameters for all successive samplings. This finding led to a pilot study of cfDNA in a series of patients with Hodgkin lymphoma.7

Discussion

In 2 previous reports,8,9 false-positive NIPT results have been attributed to metastatic cancer or uterine fibroids. Our report is the first to our knowledge of aberrant NIPT results prompting investigations that led to the diagnosis of cancer. The identification of 3 cancers in a prospective series of 4000 women is within the range expected from population cancer incidence, which is estimated at 1 per 1000 to 2000 person-years in 20- to 40-year-old women, suggesting that NIPT is a sensitive method to detect tumors characterized by chromosomal imbalances.10,11 Since all 3 tumorlike cfDNA-derived GR profiles were confirmed by FISH or aCGH
on biopsy specimens, the test is also specific. A systematic referral to the oncology unit is warranted for those women with cancerlike GR profiles observed during NIPT on repeated sampling.

Given the current large scale implementation of NIPT to screen for fetal aneuploidies, it is surprising that there are not more reports of maternal cancers presymptomatically revealed by NIPT.9 One explanation is that current NIPT analyses focus only on deviations of the viable trisomies 13, 18, and 21. However, our observations suggest that slight adaptations to NIPT analysis enabling the interrogation of (segmental) aneuploidies genomewide could not only avoid false-positive assignment of fetal aneuploidy due to the presence of a maternal cancer but, more importantly, enable identification of the imbalances as cancer-derived anomalies.

Cancer treatment, including chemotherapy, during pregnancy is an option without harming the fetus.12,13 The prognosis of cancer during pregnancy is similar to the prognosis in nonpregnant women if standard treatment during pregnancy is applied.14 Since cancer-related symptoms may be masked, especially during pregnancy, we consider the presymptomatic identification of maternal cancer as a potential added value of NIPT. Symptoms such as fatigue, nausea, abdominal pain, and vaginal blood loss can be misinterpreted as physiologic pregnancy-related symptoms.15

Of the 3 patients described herein, 2 (patients 1 and 3) underwent successful treatment. Patient 1 had treatment following delivery, and patient 3 underwent treatment during pregnancy without complications and subsequently gave birth to a healthy girl. Patient 2, diagnosed with follicular lymphoma, did not undergo treatment of the slow-growing entity of follicular lymphoma, which may not require treatment for many years. The limitations of this case series include a small sample size and the detection of only 3 different cancer types, of which 2 were hematological. To address these limitations, we aim to further investigate the potential of NIPT for cancer detection, not only in pregnant women, but also in the general population.

### Figure 3. Array Comparative Genomic Hybridization (CGH) Analysis, Chromosomal Abnormalities, and Genome Representation (GR) Profiles of Follicular Lymphoma (Patient 2)

<table>
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<th>Array CGH Overview</th>
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<tr>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y</td>
<td></td>
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<tr>
<td>+1</td>
<td>0</td>
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**A**, In the array CGH analysis, the column labels represent the numbered chromosomes plus X and Y; the y-axis, represents the log2 of the intensity ratios; each graphed point, an array probe; and the boxed areas highlight the chromosomes detailed in panel B. **B**, Illustrated chromosomal abnormalities (arrowheads) related to the genomic imbalances in the GR profile of follicular lymphoma: [i(6)(q10) (gain of 6p/loss of 6q), dup(7)(q1q22), +dup(7)(q1q22) (gain of 7 and extra gain of 7q1q22), +11 (gain of 11), dup(12)(q13q15) (gain of 12q13q15), and dup(13)(q21q34) (gain of 13q21q34)]. **C**, The GR profiles of the 5 relevant chromosomes. The partial loss of 6q (but not the entire 6q) and lack of trisomy 7 and 11 in the GR profile of cell-free DNA is likely related to a subclonal/subregional appearance of these aberrations. For an explanation of the graphic conventions used in a GR profile, see the caption of Figure 2. As evidenced by chromosome 11 GR profile, no abnormalities were found.
Conclusions

We show that maternal plasma cell-free DNA sequencing for the purpose of NIPT may enable the accurate presymptomatic detection of maternal tumors and treatment during pregnancy. However, the detection of cancer by genomic profiling need not be limited to pregnant women, and additional research on a large scale seems warranted.
Prenatal Testing for Presymptomatic Cancer in Pregnant Women

Brief Report Research

Study concept and design: Amant, Verheecke, Dehaspe, Brady, Moerman, Vergote, Putseys, Vandenberghe, Legius, Vermeesch. Acquisition, analysis, or interpretation of data: Wlodarska, Dehaspe, Brady, Brison, Van Den Bogaert, Dierickx, VandeCavey, Tousseyn, Vanderstichele, Vergote, Neven, Berteloot, Putseys, Danneels, Vandenberghe, Vermeesch. Drafting of the manuscript: Amant, Verheecke, Wlodarska, Dehaspe, Brady, VandeCavey, Vanderstichele, Vergote, Neven, Putseys, Vermeesch. Critical revision of the manuscript for important intellectual content: Wlodarska, Dehaspe, Brady, Brison, Van Den Bogaert, Dierickx, VandeCavey, Tousseyn, Moerman, Vergote, Neven, Berteloot, Danneels, Vandenberghe, Legius, Vermeesch. Statistical analysis: Dehaspe. Obtained funding: Amant, Vermeesch. Administrative, technical, or material support: Amant, Verheecke, Wlodarska, Brady, Brison, Van Den Bogaert, Dierickx, VandeCavey, Tousseyn, Moerman, Vergote, Neven, Berteloot, Danneels, Vandenberghe, Legius, Vermeesch. Study supervision: Amant, Verheecke, Brady, Vergote, Berteloot, Vandenberghe, Legius, Vermeesch. Collected biomaterial: Vanderstichele.

Conflict of Interest Disclosures: Dr Vermeesch reports being the founder of and stockholder in Cartagenia, which provides software for clinical analysis of genomics data. The analysis used in this study has been licensed to Cartagenia, for which Dr Vermeesch’s laboratory receives license fees. No other conflicts are reported.

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REFERENCES


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