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Cell-free DNA Results Lead to Unexpected Diagnosis

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39 Key Clinical Message: Maternal cell-free DNA (cfDNA) results that are discordant
40 with the diagnostic fetal karyotype should prompt further investigation. If deeper
41 analysis of the cfDNA results demonstrates a “saw-tooth” pattern characteristic of
42 genome-wide imbalance, maternal malignancy is suggested. Identifying the
43 maternal malignancy can, however, be difficult.

44

45 Key Words: Cell-free DNA, metastatic neoplasm, multiple monosomies, prenatal
46 diagnosis

47 **Introduction:**

48 Maternal plasma cell-free DNA (cfDNA) analysis has become a preferred
49 method chosen by patients to screen for common fetal trisomies. However, when
50 the results are discordant with follow-up diagnostic testing, there are limited
51 follow-up recommendations at present for practitioners and patients. Possible
52 explanations for discordant results include confined placental mosaicism, maternal
53 chromosomal mosaicism, co-twin demise, DNA copy number variants in mother or
54 fetus, maternal organ transplant from a male donor, and maternal malignancy.¹
55 Here, we report a patient who had plasma cfDNA test results suggestive of full or
56 partial monosomies for chromosomes 13, 18, 21, and X who was subsequently
57 found to have hepatic lesions on magnetic resonance imaging (MRI). Postpartum
58 the patient was diagnosed with stage IV colon cancer.

59 **Case History:**

60 The patient was a 37 year old G2P1001 woman who two years earlier had
61 undergone *in vitro* fertilization and preimplantation genetic testing for cystic
62 fibrosis as both she and her husband are carriers. This resulted in a full-term,
63 healthy female. During this first pregnancy, the patient had plasma cfDNA testing in
64 that pregnancy that was reported as low risk for fetal aneuploidy.

65 In the current pregnancy, the couple used their remaining frozen embryos to
66 conceive. Two embryos were transferred. A subsequent ultrasound scan
67 demonstrated a single, viable intrauterine pregnancy. Maternal plasma cfDNA test
68 results at 12 weeks suggested full or partial monosomies for chromosomes 13, 18,
69 21, and X. The patient then underwent diagnostic testing by amniocentesis at 18
70 weeks' gestation. The fetus had a 46, XX karyotype and a normal chromosomal
71 microarray.

72 The concern for a maternal malignancy as an explanation for the discordant
73 results between the cfDNA study and amniocentesis prompted a request for a
74 deeper analysis of the whole genome sequencing results by the original testing
75 laboratory (Figure 1). This showed multiple areas of genome-wide imbalance,
76 suggestive of malignancy. The patient was subsequently referred to the cancer
77 genetic counseling service for an oncologic evaluation at 21 weeks gestation. She
78 was clinically asymptomatic. Her general physical examination was normal and
79 laboratory studies were unremarkable. Her family history was not suggestive of a
80 hereditary cancer syndrome.

81 Following a discussion with multiple medical specialists, a full body MRI scan
82 without contrast was performed at 23 weeks' gestation to search for a possible
83 malignancy (Figure 2). The imaging identified multiple T2 hyperintense and T1
84 hypointense lesions in the liver: the largest measured 5.5 x 4.3 x 6.6 cm. The
85 differential diagnosis included hepatic adenomas, primary hepatocellular
86 carcinoma, or metastatic lesions. The patient was further counseled regarding these
87 new findings. The decision was made not to perform a liver biopsy.

88 The patient underwent another MRI scan at 27 weeks' gestation. This
89 demonstrated that the hepatic lesions had increased in size with the largest one
90 measuring 9.9 x 5.4 x 8.8 cm. Due to the concern that the lesions could become

91 hemorrhagic, at 28 weeks' gestation the patient underwent an invasive radiology-
92 guided embolization procedure. A repeat maternal plasma cfDNA analysis
93 continued to show multiple monosomies. Evaluation of the whole genome
94 sequencing results showed a similar but more exaggerated pattern of genome-wide
95 imbalance compared to the previous test (Figure 1). Because of a dropping
96 hematocrit and increasing right upper quadrant pain, a third MRI scan was
97 performed, which demonstrated that the largest lesion measured 10.5 x 5.4 x 9.7 cm
98 and the smaller lesions were reduced in size. Her liver enzyme values remained
99 normal.

100 The patient underwent a planned cesarean delivery at 32 weeks gestation to
101 facilitate her medical management. At the time of her surgery, fine needle biopsies
102 of four liver lesions were performed. Three lesions demonstrated necrotic type
103 material consistent with the patient's recent embolization. One biopsy
104 demonstrated poorly differentiated adenocarcinoma. Postpartum, she had a CT
105 scan that demonstrated cecal thickening. Subsequent colonoscopy revealed a
106 circumferential mass involving the cecum and proximal ascending colon. Multiple
107 biopsies were taken but did not reveal any evidence of malignancy. The patient
108 underwent a positron emission tomography (PET) scan that demonstrated a
109 fluorodeoxyglucose(FDG)-avid cecal mass consistent with colon cancer along with
110 FDG-avid pericecal lymph nodes consistent with metastasis. In addition, there were
111 FDG-avid right lobe hepatic lesions which were consistent with metastases. The
112 patient had a repeat biopsy of the hepatic lesion that demonstrated metastatic
113 poorly differentiated adenocarcinoma. The diagnosis was stage IV colon cancer and
114 systemic chemotherapy was initiated. There was no response, so she underwent a
115 right colectomy and partial hepatectomy. She then had a second round of
116 chemotherapy but did not respond and died approximately 10 months postpartum.
117 The infant is alive and well.

118 Given the abnormal cfDNA test results seen in the second pregnancy, the
119 genome-wide tracings from the first pregnancy were retrospectively reviewed and
120 were still considered to be unremarkable.

121 **Discussion:**

122 Fetal cfDNA is detectable in maternal serum as early as 5-7 weeks of
123 gestation.² In the first trimester, approximately 10% of cfDNA is fetal in origin and
124 is almost entirely derived from placental trophoblast cells. Several different
125 techniques exist to analyze cfDNA.² The technique used to analyze the DNA in the
126 patient's sample was massively parallel shotgun sequencing (MPSS). MPSS involves
127 identifying and counting DNA fragments. Both maternal and fetal DNA segments are
128 sequenced simultaneously. The segments are sequenced, aligned, and uniquely
129 mapped to sites from a reference human genome. Each individual laboratory
130 employs its own statistical method to determine when to call a sample monosomic
131 or trisomic for a specific chromosome. The test utilized here incorporated a
132 software program called bowtie to align the sequences to the 19th reference version
133 of the human genome sequence map.³ The clinical laboratory's proprietary
134 software then evaluated the target chromosomes (13, 18, 21, X, and Y) by
135 calculating a ratio between the normalized coverage on each target chromosome to
136 the sum of normalized coverage on a respective set of reference chromosomes
137 (typically two to six chromosomes). The software has upper and lower limits that it
138 applies to the test results in order to generate an aneuploidy classification status for
139 chromosomes 13, 18, and 21. These include aneuploidy detected, suspected, or no
140 aneuploidy. For sex chromosomes it includes aneuploidy detected or not. The
141 excess amount of circulating DNA sequences from the reference chromosomes,
142 particularly the chromosomes with peak sequences above the horizontal line in
143 Figure 1, resulted in abnormal ratios, thus generating the test results of monosomies
144 for 13, 18, 21, and X. The screening result of multiple monosomies is caused by a
145 bioinformatics artifact.

146 Because the patient's sample was analyzed by MPSS, the genome-wide data
147 were available and could be re-analyzed. These demonstrated an abnormal pattern
148 of multiple chromosomes across the genome that led to a "saw-tooth" pattern
149 (Figure 1). Given the multiple abnormalities across the genome, this pattern was
150 suspicious for a malignancy. However, genome-wide aberrations have also been
151 reported for benign, neoplastic lesions in pregnancy, such as uterine leiomyomas.⁴
152 If the patient's sample had been tested using the targeted sequencing method that

153 does not use ratios, the results from chromosomes 13, 18, and 21 would likely have
154 been normal and the suspicion for cancer may not have been raised.

155 CfDNA levels are frequently elevated in patients with cancer.⁵⁻⁸ Ongoing
156 research is addressing whether the increased cfDNA levels can be used for different
157 purposes in cancer screening and monitoring response to treatment.⁶ Several
158 studies have demonstrated that plasma cfDNA is increased in metastatic colon
159 cancer.⁸ Other, non-ratio approaches use detection and monitoring of a tumor-
160 specific oncogene such as *KRAS*. This was not done here, and in fact, would require
161 a separate test from the MPSS counting approach.

162 In 2013, the first case of a pregnant patient with discordant results
163 subsequently being diagnosed with metastatic cancer was published.⁵ The patient
164 was a 37-year old G2P1 woman with cfDNA test results that demonstrated fetal
165 aneuploidy for chromosomes 13 and 18. At two weeks postpartum the patient was
166 diagnosed with metastatic small cell carcinoma of vaginal origin. In June 2015 three
167 more patients diagnosed with cancer (ovarian carcinoma, follicular lymphoma, and
168 Hodgkin lymphoma) after discordant cfDNA results were reported.⁶ In July 2015, an
169 additional ten pregnant patients were reported to have a malignancy after
170 undergoing cfDNA testing (neuroendocrine of unknown origin, non-Hodgkin
171 lymphoma in three patients, colorectal, Hodgkin lymphoma, acute T-cell
172 lymphoblastic leukemia, and 2 patients critically ill with type of cancer not
173 reported).⁷

174 **Conclusion:**

175 Management of the pregnant woman with discordant cfDNA results remains
176 a clinical dilemma, particularly when genome-wide sequencing results suggest
177 malignancy. The most common cancers that have been diagnosed in pregnant
178 patients include breast, cervical, Hodgkin lymphoma, leukemias, and malignant
179 melanoma. These are also the most common types of cancers seen in women of
180 reproductive age⁹. As more information becomes available, specific cfDNA test
181 result patterns may be helpful in guiding the subsequent evaluation. The extent of
182 the diagnostic work up may be limited by the pregnancy itself. Standard serologic
183 tumor markers are unreliable in a pregnant woman.

184 The current recommendations for evaluation of malignancy in the setting of
185 discordant cfDNA results are only based on expert opinions; these include obtaining
186 a complete blood count, chemistry panel, whole-body MRI scan without contrast,⁶
187 and referral to medical oncology. Similarly for women who are not pregnant, for
188 whom there is a suspicion of malignancy, there are no standard evaluations for
189 cancer of unknown primary cell type.¹⁰ Patients diagnosed with cancer typically
190 present with signs or symptoms that together with focused diagnostic testing lead
191 to an eventual diagnosis. Most of the pregnant women identified to date because of
192 abnormal cfDNA test results have been initially asymptomatic. A systematic multi-
193 disciplinary approach to cataloging additional cases of discordant cfDNA results,
194 and their associated diagnoses, is needed in order to better define patient-specific
195 risks and consistent recommendations for diagnosis and treatment.

196

197 Authorship List:

198 Jessica Smith-wrote the first draft, performed the literature search, and edited the
199 manuscript

200 Victoria Kean-edited the manuscript and obtained patient permission for
201 publication

202 Diana W. Bianchi-edited and critically reviewed the manuscript

203 Gerald Feldman-edited and critically reviewed the manuscript

204 Nancie Petrucelli-edited and critically reviewed the manuscript

205 Michael Simon-edited and critically reviewed the manuscript

206 Bernard Gonik-edited and critically reviewed the manuscript

207 References:

208 1. Bianchi DW. Prepare for unexpected prenatal test results. *Nature* 2015;522:29-
209 30.

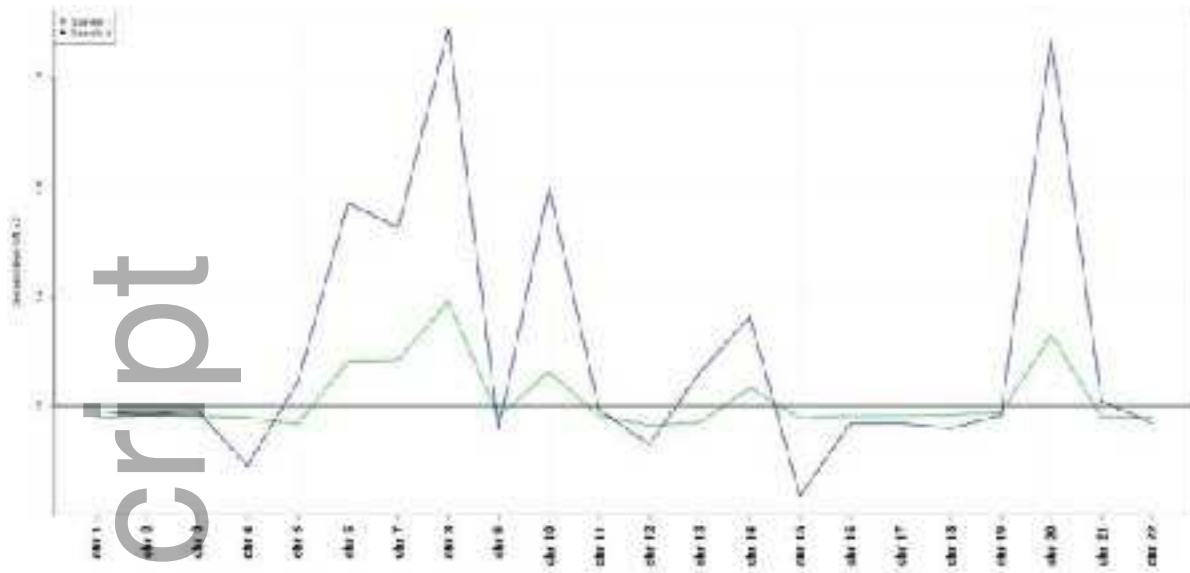
210 2. Rink BD, Norton ME. Screening for fetal aneuploidy. *Semin Perinatol* 2015; 40:35-
211 43.

212 3. Langmead B. Aligning short sequence reads with bowtie. *Curr Protoc*

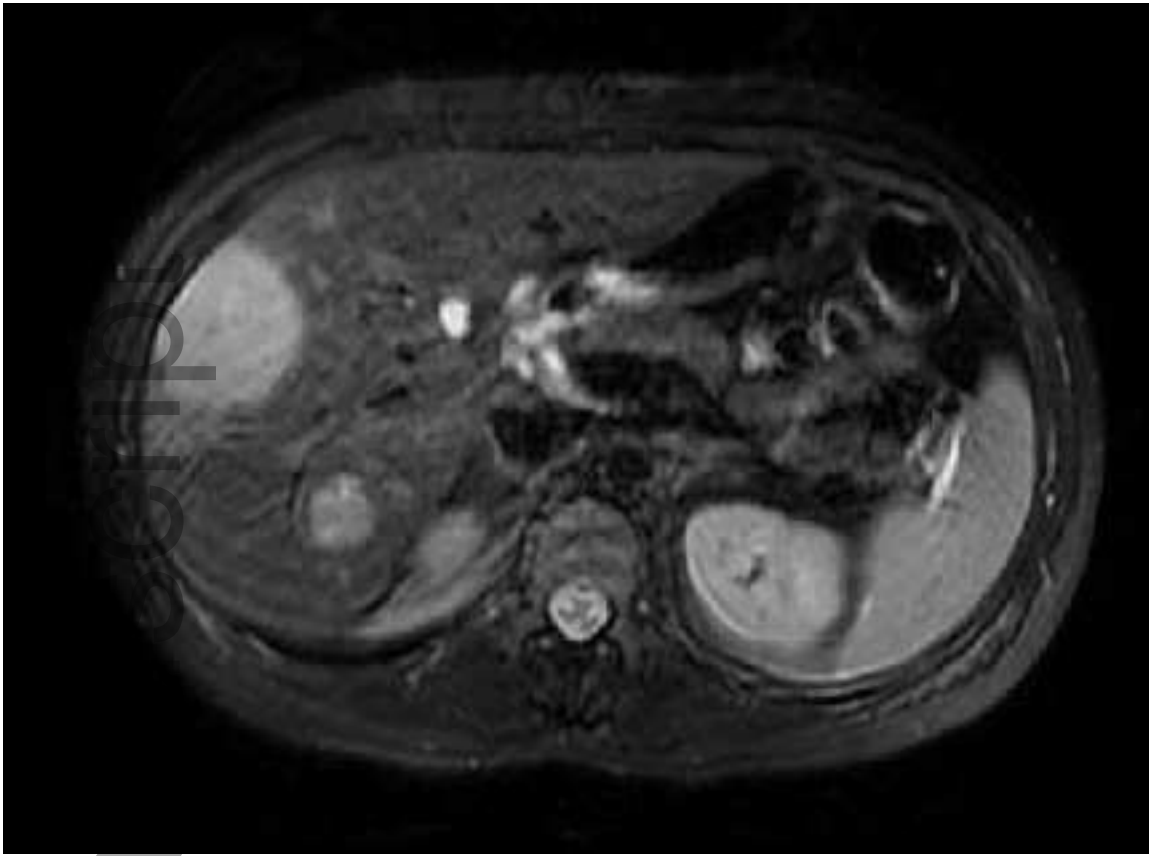
213 *Bioinformatics* 2010; Chapter11:Unit11.7. doi: 10.1002/0471250953.bi1107532.1.

- 214 4. Dharajiya NG, Namba A, Horiuchi I, *et al.* Uterine leiomyoma confounding a
215 noninvasive prenatal test result. *Prenat Diagn* 2015; 35: 990-993.
- 216 5. Osborne CM, Hardisty E, Devers P, *et al.* Discordant noninvasive prenatal testing
217 results in a patient subsequently diagnosed with metastatic disease. *Prenat Diagn*
218 2013; 33:609-611.
- 219 6. Amant F, Verheecke M, Wlodarska I, *et al.* Presymptomatic identification of
220 cancers in pregnant women during noninvasive prenatal testing. *JAMA Oncol* 2015;
221 1:814-819
- 222 7. Bianchi DW, Chudova D, Sehnert AJ, *et al.* Noninvasive prenatal testing and
223 incidental detection of occult maternal malignancies. *JAMA* 2015; 314:162-169.
- 224 8. Lecomte T, Ceze N, Dorval E, *et al.* Circulating free tumor DNA and colorectal
225 cancer. *Gastroenterol Clin Biol* 2010; 34: 662-681.
- 226 9. Pavlidis NA. Coexistence of pregnancy and malignancy. *The Oncologist* 2002; 7:
227 279-287.
- 228 10. Varadhachary GR. Carcinoma of unknown primary: focused evaluation. *J Natl*
229 *Compr Canc Netw* 2011; 9: 1406-1412.

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