Changes of Cell-Free Fetal DNA in Maternal Plasma after Elective Termination of Pregnancy, Tuangsit Wata-

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Fetomaternal hemorrhage (FMH) during pregnancy or

subsequent isoimmunization, caused by a breach of the

placental barrier, could lead to an adverse perinatal

outcome (1, 2). However, concerns have been raised re-

garding the accuracy of the standard Kleihauer–Betke test

to quantify FMH (3). In a previously published study, we

explored the possibility of using cell-free fetal DNA (fDNA)
in maternal plasma as a novel marker of FMH after surgical

and medical elective first-trimester termination of pregnancy (TOP) (4).

In our earlier study, pre- and posttermination blood

samples were drawn at various time points relative to

TOP. To adjust for this confounder, we retrospectively

applied a mathematical model to the analysis of the data.

Projected pretermination fDNA values were calculated

based on the anticipated linear increase of fDNA during

pregnancy. Adjusted posttermination fDNA concen-

trations were calculated based on the reported 16-min clear-

ance rate of circulating fDNA after delivery (5). Adjusted

posttermination fDNA concentrations were then com-

pared with the projected pretermination concentrations.

In the present study, we specifically enrolled only

pregnant women in the first trimester undergoing elective

surgical TOP. In addition, the pre- and posttermination

blood samples were drawn on the same day with a

deliberated time interval between TOP and the posttermi-

nation blood collection. The concentrations of circulating

fDNA were then analyzed along with pertinent clinical

information to better understand the dynamics of circu-

lating fDNA after TOP.

This study was approved by the Institutional Review

Boards at Tufts-New England Medical Center and Boston

University School of Medicine. Pregnant women in the

first trimester of pregnancy undergoing elective surgical

TOP at Boston Medical Center were enrolled. Gestational

dates were ascertained by ultrasonography and were ex-

pressed as postmenstrual days. Paired blood samples

were obtained before and immediately after the termina-

tion procedure. The blood samples were centrifuged at

800g, after which plasma was isolated and frozen at

−80°C until analysis.

The patients received intravenous sedation with fenta-

nyl and midazolam, or local anesthesia. No patients

required bolus intravenous fluid. The products of concep-

tion (POC) were evacuated by use of manual vacuum

aspiration, followed by sharp curettage at the discretion

doctor. The times of suction initiation and blood
drawing were recorded. Products of conception were

frozen in saline for fetal gender identification.

Plasma (900 μL) was centrifuged at 11 500g for 10 min
to remove residual cells. We extracted DNA from 800 μL
of the supernatant by use of the QIAamp Blood Kit
(Qiagen Inc.) with the Blood and Body Fluid Spin Protocol
described by the manufacturer. The extracted DNA was

eluted into a final volume of 50 μL. We processed 500 μL
of the normal saline solution that contained DNA diffused
from the POC in the same fashion and used 400 μL of the

supernatant for DNA extraction.

We measured fDNA in maternal plasma and the POC
solution by real-time PCR amplification using a Perkin-

Elmer Applied Biosystems 7700 Sequence Detector (Ap-
Table 1. Comparison of clinical variables that could potentially affect DNA trafficking in women who had decreased or increased concentrations of posttermination fDNA.

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Patients with decreased post-TOP fDNA</th>
<th>Patients with increased post-TOP fDNA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (25th, 75th percentiles) pretermination fDNA, GE/mL</td>
<td>13.5 (10.3, 22.1)</td>
<td>11.8 (11, 15)</td>
<td>0.34</td>
</tr>
<tr>
<td>Sharp curettage, %</td>
<td>53</td>
<td>36</td>
<td>0.34</td>
</tr>
<tr>
<td>Preprocedure bleeding, %</td>
<td>12</td>
<td>14</td>
<td>1.0</td>
</tr>
<tr>
<td>Median (25th, 75th percentiles) time interval between TOP and post-TOP blood draw, min</td>
<td>23 (21, 28)</td>
<td>23 (19.5, 35)</td>
<td>0.89</td>
</tr>
<tr>
<td>Median (25th, 75th percentiles) gestational age, days</td>
<td>63 (54, 68)</td>
<td>68.5 (66, 75.8)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Overall, the median concentration of post-TOP fDNA among all patients was not significantly increased over the median pre-TOP fDNA concentration, but our data show an increase in fDNA after TOP only after 8 weeks of gestation. This increase occurred in 11 of 17 patients when pregnancy was later than 9.5 weeks of gestation (Fig. 1). The increase in fDNA at later gestations could be attributable to either an increase in placental size/mass or disruption of the fetomaternal circulation. Using Doppler studies, Jauniaux et al. (7) showed that placental blood flow is not established until 8–9 weeks of gestation. Thus, we would not necessarily expect to find FMH after TOP earlier than this gestational age. In support of this, Leong et al. (8) found no fetal cells in the blood samples of women after surgical TOP at <6 weeks menstrual age. We therefore suggest that the source of this DNA may be fetal hematopoietic cells within newly established placental blood vessels that are disrupted as a result of the TOP procedure and that the increased fDNA concentrations may indicate excessive FMH after 9 weeks of gestation.

Plasma fDNA concentrations in many patients, however, unexpectedly decreased after the TOP procedure. Because no intravenous fluid bolus was given to any patient before the procedure, a dilution effect is unlikely. This decrease might be explained instead by the physiologic differences in how fDNA enters the maternal circulation. It is generally believed that in pregnant women the source of cell-free fetal nucleic acids is placently derived apoptotic cells (9). Some fDNA sequences are detectable in membrane-bound apoptotic vesicles (10). This particle-associated form is believed to protect fetal nucleic acids from degradation by nucleases in maternal blood (11). After elective TOP, fDNA is liberated directly into the maternal circulation from the sudden disruption of the fetomaternal interface; thus it may not be protected in apoptotic bodies. We therefore suggest that unprotected posttermination fDNA sequences are vulnerable to destruction by maternal nucleases, leading to the rapid decrease in these sequences after elective termination in some patients.

Future study should define the association between post-TOP cell-free fDNA concentrations, measured by real-time PCR, and cellular trafficking, determined by the traditional Kleihauer–Betke test, in a large cohort of patients at various gestational ages. In addition, the correlation between the alteration in fDNA concentrations and triggering of the maternal immune response remains to be elucidated. Current measurement of fDNA is based on Y-chromosome-specific sequence detection, which is not applicable to women who carry a female fetus. Therefore, the continued development of fetal gender-independent markers, such as fetal/placental specific mRNA, in the maternal circulation is essential (12). Comparison of fetal globin gene expression with placental derived gene expression may allow us to definitively determine whether the increased fetal nucleic acids seen after 9 weeks of gestation in maternal plasma are from a placental or hematopoietic source (13).

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Biological Variation of Tumor Markers and Its Application in the Detection of Disease Progression in Patients with Non-Small Cell Lung Cancer, Jaume Trapé1,2, Joaquim Pérez de Olaguer,2 Josep Buxó,2 and Laura López2 (1 Clinical Laboratory and 2 Service of Oncology, Hospital Sant Joan de Déu, Althaia Xarxa Assistencial de Manresa, Barcelona, Spain; * address correspondence to this author at: Clinical Laboratory, Hospital Sant Joan de Déu, Althaia Xarxa Assistencial de Manresa, Dr Joan Soler, s/n, 08243 Manresa (Barcelona), Spain; fax 34-93-8743859, e-mail jtrape@yahoo.es)

Patients with advanced non-small cell lung cancer (NSCLC) may benefit from chemotherapy (1,2). This type of treatment is highly toxic and expensive, and patients should be monitored to detect those in whom the disease progresses despite treatment to avoid ineffective treatment and a loss of quality of life. Tumor markers are

References


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