Although cell-free nucleic acids were first described in the 1940s (1), it was not until tumor-specific DNA sequences were detected in the plasma of cancer patients (2) that they started to attract the interest of the wider scientific community. Because the placenta shares numerous features with malignant tissues, such as a high rate of cell turnover and the expression of certain protooncogenes, these seminal reports prompted Lo and colleagues to examine whether fetal DNA could be detected in an analogous manner in the plasma of pregnant women. This hypothesis indeed proved to be true, and in 1997 Lo et al. (3) described the presence of fetal DNA circulating in maternal plasma and serum. This observation has been one of the most important for those attempting to develop safe and efficacious methods for prenatal diagnosis of fetal genetic traits. The reason for this interest is that current methods of obtaining fetal material for prenatal diagnosis rely on invasive procedures such as amniocentesis or chorionic villus sampling, both of which are associated with a procedure-related fetal loss rate of almost 1% (4). Although concerted efforts had been made to develop noninvasive, and hence risk-free, alternative prenatal diagnostic methods using fetal cells enriched from the blood of pregnant women, this approach has been hampered by the extreme scarcity of these cells in the maternal circulation (5). By contrast, fetal DNA could be detected in as little as 10 μL of maternal plasma (3). Consequently, this approach was readily seized on by various research groups, who in rapid succession showed that circulating fetal DNA can be reliably used for the determination of fetal sex, rhesus D status, and inherited Mendelian disorders (reviewed in Ref. (6)].

The development of real-time quantitative PCR (7) introduced a new dimension to the study of plasma DNA. By these means it was shown that the concentration of circulatory fetal DNA constitutes 3% and 6% of the total circulatory DNA in maternal plasma in the first and third trimesters, respectively (8). Furthermore, by examining both serum and plasma samples, studies showed that circulatory fetal DNA exists mainly in a cell-free form and is not released from dead or dying fetal cells in the maternal circulation, as there was little difference in fetal DNA concentrations between the native plasma and clotted serum samples (8). By contrast, the amount of maternal circulatory DNA was significantly increased in serum samples compared with plasma because of the liberation of maternal DNA from maternal cells lysed during clotting (8).

It was, however, when researchers examined pregnancy-related disorders that this technology demonstrated its inherent potential. Significant increases in the concentration of circulatory fetal DNA were determined in pregnancies affected by preeclampsia (9, 10) and preterm labor (11) as well as those bearing aneuploid fetuses (12, 13). These studies also showed that under certain conditions, such as manifest preeclampsia, the amounts of both circulatory fetal and maternal DNA were increased (10).

The quantification of both fetal and maternal circulatory DNA concentrations, however, raised the question of how the plasma sample should best be prepared for extraction of circulatory DNA. From previous experiments using plasma or serum, it should be clear that the concentration of fetal DNA would not be influenced much by the procedure chosen. The contamination of the plasma sample by maternal cells or cell remnants would, however, affect the apparent concentration of maternal DNA in the sample. Another caveat to which we have alluded previously is the apparent fluctuation of these molecular analytes over time (14). We observed up to 13.5-fold variations in total cell-free DNA over a period of 3 days. More importantly, we noted a 2.2-fold fluctuation in the amount of circulatory fetal DNA over the same period, the magnitude of which is similar to the 2-fold increase noted in pregnancies with trisomy 21 fetuses (12, 13). These features were the source of considerable discussion at the 2nd International Symposium on Circulating Nucleic Acids in Plasma and Serum held earlier this year in Hong Kong [the abstracts from the symposium are listed in this journal (Clin Chem 2001;47:361–71)].

In an elegant study, Chiu et al. (15) have now reexamined some of these aspects more closely. Their work emphasizes the importance of the initial preparatory steps to separate the plasma from any maternal cellular remnants when considering studies in which circulatory maternal and fetal DNA concentrations are assessed. They examined both maternal and fetal DNA concentrations in plasma fractions obtained either by a single centrifugation of the sample (at 800g or 1600g) or by use of a discontinuous Percoll density gradient, as well as plasma fractions obtained by these means that were subsequently cleared further by high-speed centrifugation in a desktop microcentrifuge or filtration with a 0.2 μm filter unit. Their study shows that procedures with a single centrifugation step do not allow for measurement of circulating maternal DNA concentrations. This was most pronounced in the sample prepared by Percoll gradient. As expected, no significant difference was noted in the concentrations of circulatory fetal DNA, clearly indicating that this is mainly in an acellular form. In their analysis of the degree of fluctuation, Chiu et al. again observed that this is largely influenced by the mode of preparation and that, provided appropriate precautions are taken, these variations are not significant for the concentrations of either circulatory fetal or maternal DNA. The almost twofold variations in circulatory fetal DNA reported by these authors (15) and by our group (14) lead us to reiterate our view that caution should be exercised before interpreting small increments in circulatory fetal DNA as indicative of a pregnancy-related complication, such as a fetal aneuploidy. For those eager to use this new-found fetal genetic analyte for prenatal diagnostic purposes, the message can
be summarized as follows: If you are examining only circulatory fetal DNA, any of several initial processing steps appears adequate. If you are considering the analysis of both circulatory fetal and maternal DNA, however, care must be taken in clearing the plasma of any maternal cellular remnants. By analogy, these statements should, of course, also hold true for the examination of circulatory cancer or transplant-derived DNA and comparison with total circulatory patient DNA.

References

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