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Opinion

Genome-wide cfDNA testing of maternal blood

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Measurement of cell-free(cf) DNA in maternal blood has been shown to provide effective prediction of fetal trisomy 21 and, to a lesser extent, of trisomies 18 and 13, both in singleton and in twin pregnancy[1,2]. This has led to clinical implementation of the test in several countries, usually in women identified through prior screening by the first-trimester combined test to have a high or moderate risk for trisomy 21. In Belgium and The Netherlands, however, cfDNA testing is being offered to all pregnant women, as an alternative to the first-trimester combined test, and the test is based on genome-wide (GW) analysis rather than being confined to screening for the three major trisomies. The rationale for such a policy is that GW testing has the potential to diagnose clinically significant rare autosomal trisomies (RATs) and rare additional fetal segmental imbalances (SIs).

The results from the first year of GW-cfDNA testing in The Netherlands (TRIDENT-2 study) included 56 818 women who underwent GW-cfDNA testing, from an initial cohort of 73 239 women who had a cfDNA test; in 207 (0.4%) of these women, the test was positive for RATs ($n = 101$), SIs ($n = 95$) or complex abnormal profiles ($n = 11$)[3]. Among the 101 RATs, six were confirmed but only one of these was associated with an abnormal phenotype. Among the 95 SIs, 29 were confirmed but the number with abnormality not discoverable through ultrasound was not defined. In another seven cases consistent with maternal malignancy or premalignancy, the benefit of the discovery was not demonstrated. An abnormal test result inevitably leads to anxiety and, in some cases, to termination, as well as the need for both fetal and maternal testing; however, even when the fetal karyotype is found to be normal after a positive RAT result, uncertainty persists as to whether there are true mosaicisms in crucial fetal tissues and organs. When an invasive procedure confirms a true fetal mosaicism after a positive RAT result, it is impossible to predict clinical outcome and, in case of confined placental mosaicism (CPM), except CPM for trisomy 16, there is evidence that the incidence of adverse pregnancy outcome in an unselected population is not different from that in pregnancies with normal karyotype at chorionic villus sampling (CVS)[4]. Therefore, TRIDENT-2 shows that, at present, the benefits of screening for all genetic imbalances do not seem to outweigh the potential harms and that clinical implementation, even in a research setting, may be questionable ethically.

A study in Belgium, involving 3373 women, reported that GW-cfDNA testing identified additional findings beyond the common trisomies in 28 (0.8%) cases; these included four sex-chromosome aneuploidies, six RATs and one rare autosomal monosomy, none of which was confirmed in the fetus or the neonate, as well as 17 large or sub-microscopic SIs, of which three were confirmed in amniocytes[5]. In all 28 cases, the clinical follow-up was normal. Benn *et al.* reviewed the types of RAT identified following CVS, as reported in 10 recently published cfDNA studies, and found that the clinical outcome of cases with cfDNA analysis positive for RATs mostly involved the birth of an apparently normal baby (40%) or a miscarriage/fetal loss (27%), for which screening tests are not recommended[6]. There was a weak association between RATs and pregnancy complications, such as fetal growth restriction and fetal abnormalities, in the tested population.

There are several points of concern that arise from GW-cfDNA testing.

1) Increase in the screen-positive rate of a test that was initially meant to reduce it, and increase in the rate of invasive testing for conditions of unknown clinical significance that remain of unknown significance even after an invasive procedure.
2) There is uncertainty as to the clinical significance of a heterogeneous set of chromosomal abnormalities and how best to manage a positive result. Consequently, no professional society currently recommends this test[7–11].
3) There is heterogeneity of home-brew massively parallel shotgun sequencing protocols.

4) There are ethical and legal challenges to overcome regarding how best to counsel parents before they give their informed consent, since accurate information is lacking. In fact, women are already undergoing GW-cfDNA screening without clear information about its limitations and drawbacks, and clinical decisions are already being made based on results of uncertain clinical significance[12–14]. There are also ethical concerns regarding increased voluntary termination of pregnancy due to positive RAT results even after a normal karyotype and normal ultrasound scan.
5) The test violates World Health Organization screening principles[15].

In conclusion, although research should always be encouraged, the benefits *vs* harms of implementation of GW-cfDNA screening must be weighed carefully. Healthcare providers and grant-awarding bodies have a responsibility to ensure that more robust data and management strategies are available before endorsing studies or strategies incorporating GW-cfDNA testing into nationally reimbursed screening programs.

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