Editorial

Non-invasive Prenatal Testing — The Pride and Prejudice

Introduction

In modern obstetrics, prenatal screening and diagnosis of fetal aneuploidies are regarded as one of the integral parts of antenatal care. Invasive tests such as chorionic villous sampling (CVS) and amniocentesis offer an accurate diagnosis close to 100% but are associated with a small but definite risk of miscarriage. The clinical approach has long been assessing the risk of aneuploidy for each particular pregnancy, and invasive prenatal diagnostic tests are only offered to the high-risk ones. At present, the screening method most commonly used in Hong Kong is the combination of maternal age, sonographic measurement of the fetal nuchal translucency (NT), and measurement of maternal serum biochemical markers, namely, pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (HCG)[1]. In a large local prospective audit[1], this approach was confirmed to achieve a detection rate of 91.2% for trisomy 21, at a false-positive rate of 5.1%.

The use of fetal or maternal serum markers for screening will always carry shortfalls because these markers merely reflect an association with fetal aneuploidy but do not represent the underlying chromosomal anomalies. As a result, there will always be false-positive and false-negative results. An ideal test will be the one which allows the determination of the fetal genetic makeup from sampling the maternal blood. The presence of fetal cells in the maternal circulation was first discovered as early as 1969[2]. However, due to their low levels in the maternal circulation, non-invasive prenatal diagnosis focusing on analysis of these fetal cells has not yet been possible[3]. The presence of cell-free fetal DNA in the maternal circulation was discovered in 1997[4] and its quantity is 25 times higher than that available from fetal nucleated blood cells extracted from a similar volume of maternal blood[5]. Thus, research has diverted the focus to the use of cell-free fetal DNA for the purpose of non-invasive prenatal diagnosis.

Non-invasive Prenatal Testing for Trisomies 13,18 and 21

With the development of sequencing technologies, the precise determination of a relatively small difference in DNA concentrations is made possible, so that the presence of extra fetal DNA material within the maternal plasma, as in the cases of trisomy pregnancies, can be detected in a robust and reliable manner. Consider Down syndrome as an example: the total amount (i.e. fetal and maternal) of chromosome 21 in maternal plasma is slightly higher than that of other chromosomes, compared with euploid pregnancies, because there are three, rather than two copies of fetal chromosome 21. With the use of massively parallel sequencing (MPS), the maternal plasma DNA molecules are sequenced, and the chromosomal origin of each molecule is identified by comparison with the human genome. In trisomy 21 pregnancies, the number of molecules that are derived from chromosome 21, as a proportion of all sequenced molecules, is higher than that in euploid pregnancies[6]. Large prospective studies[7-9] using this approach have shown a detection rate of >99% for trisomy 21, at a false-positive rate of 0.1%. In addition to trisomy 21, the same method can be used, with similar efficacy, for screening for trisomies 18 and 13[10-11]. This method is commonly referred to as 'non-invasive prenatal testing' (NIPT) rather than 'non-invasive prenatal diagnosis' because it is not a 'diagnostic' test. It has been available for clinical use since late 2011. Apart from the MPS approach[7-11], other approaches such as targeted sequencing approach[12-14] and single-nucleotide polymorphisms (SNP)–based method[15,16] have also been reported, with similarly high sensitivity and specificity for trisomies 13,18, and 21.

Non-invasive Prenatal Testing — More than just Screening for Trisomies 13, 18, and 21

Sex chromosomal aneuploidies, including X chromosomal monosomy (Turner Syndrome), XXY syndrome (Klinefelter syndrome), XYY syndrome, and triple X syndrome can also be picked up by NIPT using both the MPS[17,18] and SNP-based approaches[19]. The test performance for sex chromosomal aneuploidies might be lower due to inherent sequencing bias associated with genomic guanine cytosine composition of the X chromosome, the marked sequence similarity between the X and Y chromosomes, the small size of the Y chromosome that leads to large variations in its measured representations,

and the presence of maternal or fetal mosaicism that can alter interpretation[18]. In one large study using the MPS approach, the sensitivity for sex chromosomal aneuploidies was 96.2%, at a false-positive rate of 0.3% and test failure rate of 5%[18].

More recently, the NIPT has also been extended to screen for microdeletion syndromes, including the 22q11.2 deletion syndrome (DiGeorge syndrome), Cri-du-chat syndrome, Angelman syndrome, Prader-Willi syndrome, and 1q36 deletion syndrome. The sensitivity for detection of microdeletion is affected by the size of such deletion, with a higher sensitivity for larger deletions. Using the MPS approach, a recent study[20] has shown the feasibility of detecting 3-Mb microduplication and deletion on a whole genome survey, at a sensitivity of 99%. The SNP-based approach also has a great potential for detecting microdeletion syndromes with high sensitivity and specificity. Data from prospective studies will likely further confirm this in the near future.

Non-invasive Prenatal Testing — Limitations

The current NIPT, regardless of whether it is the MPS approach, the targeted sequencing approach or the SNP-based method, requires a certain concentration of fetal DNA within the maternal plasma for it to be accurate[21]. It has been shown that the higher the fetal fraction, i.e. the percentage of fetal DNA concentration compared with the total DNA concentration in the maternal plasma, the higher the accuracy of the test because it is easier to detect an abnormality in case of aneuploidy[21]. Similarly, low fetal fractions (4% in some laboratories) are related to false-negative test results. As fetal fraction increases with gestational age, NIPT is usually arranged from 10 weeks of gestation onward, to avoid test failure and false-negatives due to low fetal fraction in the early gestation. Fetal fraction is also affected by other parameters such as increased maternal weight and obesity which are associated with low fetal fraction[21]. It is, therefore, important that the fetal fraction is measured to ensure validity of the results.

Research data suggest that the so-called 'cell-free fetal DNA' originates from the cytotrophoblastic cells of the placenta[22]. Therefore, confined placental mosaicism, i.e. the presence of abnormal cell lines in the placenta but not in the fetus, can contribute to erroneous results at NIPT. In addition, as NIPT measures the total plasma DNA rather than the fetal (or placental) DNA, maternal conditions such as mosaicism and malignancy may also contribute to erroneous results[23,24].

While NIPT can be applied accurately to twin pregnancies[25], the issue of twin pregnancies is slightly complicated. If the genotype of both twins is the same, there is no problem with the NIPT as the fetal fraction would be double that of a singleton pregnancy and accurate results can be obtained. However, if the twins are discordant for aneuploidy, it requires adequate fetal fraction from each twin for the test to show representative results. For example, if the fetal fraction of a sample in a twin pregnancy is 10%, it is possible that each twin contributes 5% but it is also possible that twin 1 contributes 7% while twin 2 contributes only 3%. In the latter case, aneuploidy of twin 2 might not be picked up due to low fetal fraction from twin 2. In a recent publication on application of NIPT to 189 pair of twins, all trisomy 21 pregnancies were correctly picked up[26]. However, one rare case of discordant trisomy 18 in a monochorionic twin pregnancy was missed[26]. It is worth mentioning that antenatal ultrasound only allows determination of chorionicity in twin pregnancies but not necessarily zygosity. For monochorionic twins, it is most likely monozygotic, although there are exceptions. For dichorionic twins, the zygosity is unknown unless the twins have different genders. Non-invasive means for the determination of zygosity from maternal plasma are now possible[27]. Further research should assess its applicability for routine usage in twin pregnancies.

Non-invasive Prenatal Testing — Clinical Application

There are two ways of applying NIPT in clinical practice. First, it can be regarded as a secondary screening for the high-risk population, i.e. as an alternative to invasive prenatal procedures for women who are screened positive from the first-trimester combined NT and serum biochemistry screening, or the mid-trimester biochemical screening. Approximately 5% of all current screening tests for Down syndrome will yield positive results, and most of these pregnancies are not affected by Down syndrome[1]. Introduction of NIPT will help to reduce the potential risks of miscarriage related to invasive procedures. A local study[28] showed that the majority of pregnant women can accept NIPT as an alternative to invasive prenatal procedures provided that the test is 95% accurate in diagnosing the Down syndrome. While the safety of NIPT is appealing to pregnant women, it is important to highlight, during counselling, the difference in the information obtained from NIPT compared with that from invasive prenatal diagnostic tests. From CVS or amniocentesis, full karyotyping, instead of only chromosomes 13, 18, 21 and XY, can be obtained. Further tests such as array-based comparative genomic hybridisation can also yield

further information on sub-chromosomal aberration. In a retrospective analysis of 193,638 singleton pregnancies that had completed a first-trimester Down syndrome screening programme in Denmark, cytogenetic or molecular analysis was performed in 10,205 (5.3%) cases and 1122 of them had abnormal karyotypes[29]. Of these, 262 (23.4%) had chromosomal anomalies other than trisomies 13, 18, 21 or sex chromosomal aneuploidies, which would have been missed if NIPT had been offered[29]. This figure constitutes 2.6% of all cases screened positive in the Down syndrome screening programme. This prevalence of atypical abnormal karyotypes was increased in women above 45 years of age, in pregnancies with increased NT thickness (≥3.5 mm), and those with abnormal levels of free β-HCG (<0.2 or ≥5.0 multiples of the median [MoM]) or PAPP-A (<0.2 MoM)[29]. Hence, within the group that is screened positive, careful examination of the report may help to identify those who will benefit the most from invasive prenatal diagnosis or the NIPT.

The second way of applying NIPT clinically would be for primary screening due to its better sensitivity on autosomal aneuploidies. It is also beneficial in expanded screening for sex chromosomal aneuploidies and microdeletion disorders. At present, the relatively huge cost of the test poses difficulty for the implementation of this strategy on a national level. However, if the cost can be reduced to the level of our current Down syndrome screening test, a combination of NT screening and NIPT at 11 to 13 weeks would be the best first-trimester screening routine. There are concerns that the clinical performance of NIPT might not be as good in the low-risk population as most of the prospective studies were conducted in the high-risk population. In one study which stratified recruited subjects into high-, intermediate- and low-risk groups based on the first-trimester Down syndrome screening results, there was no difference in the fetal fractions among the three groups, suggesting that the test performance should not vary across populations at different risks[30]. Data from large prospective audits of NIPT among average-risk population have also shown reliable results[23,31].

This issue includes a local study[32] conducted in late 2012 that evaluates the attitude and knowledge about NIPT among pregnant women in Hong Kong. It was found that only 22.6% of pregnant women were aware of the test and, as expected, higher knowledge of the Down syndrome screening programme and NIPT were associated with higher education level, higher family income, and having antenatal care in both public and private sectors[32]. The development of this field is so fast that even clinicians might find it challenging to keep apprised of its progress. Counselling in pregnant women would therefore be even more important, especially if expanded screening for sex chromosomal aneuploidies and microdeletion syndromes are adopted.

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