Journal of Medical Diagnostic Methods
ISSN: 2168-9784

Yaron and Michaelson-Cohen, J Med Diagn Meth 2014, 3:2
DOI: 10.4172/2168-9784.1000156

Short Communication Open Access

The Evolution of Prenatal Diagnosis from Invasive Procedures to Non-invasive Prenatal Testing (NIPT)

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Received date: Feb 26, 2014; **Accepted date:** April 25, 2014; **Published date:** April 28, 2014

Introduction

Prenatal diagnosis of fetal aneuploidy, such as Down syndrome (DS), is usually based on a two-stage model: screening tests directed at the entire population of pregnant women and invasive diagnostic tests for the high risk population as determined by the aforesaid screening tests. Screening tests include different combinations of maternal serum biochemical markers and various ultrasound markers. Currently, the most prevalent screening test is the combined first trimester screening test, which includes ultrasound measurement of nuchal translucency in combination with maternal serum levels of PAPP-A and free beta-hCG [1,2]. This approach is gradually replacing *second trimester biochemical screening* which is based on maternal serum levels of AFP, hCG, uE3 (with or without inhibin A). Additional approaches include various combinations of first- and second-trimester screening markers, such as the integrated test [3] and contingent screening [4]. Results of these screening tests are reported as risk figures: a patient is determined to be at high risk for Down syndrome if the calculated risk exceeds a predetermined cutoff (such as 1: 270 at the time of screening). Different screening tests have Down syndrome detection rates ranging from 65% (2nd trimester triple test) to as high as 94% (fully integrated test). However, this relatively high rate is achieved at a cost of a 5% False Positive Rate (FPR) and at a relatively low Positive Predictive Value (PPV) of 2% - 4%. Thus most of the screen positive patients who ultimately undergo invasive testing are subsequently found to carry chromosomally normal fetuses.

Diagnostic tests are offered to patients determined to be at high risk, as determined by such screening tests; family or personal history of chromosomal anomalies or abnormal ultrasound findings. Diagnostic tests rely on chromosomal analysis (karyotype) of fetal cells which must be obtained by an invasive procedure such as Chorionic Villus Sampling (CVS) or amniocentesis. While diagnostic tests provide definitive results, they are associated with a slight but troubling risk of pregnancy loss [5].

Over the last two decades numerous attempts have been made to find noninvasive techniques for diagnosing fetal aneuploidy. Initial research focused on the isolation of fetal cells from the maternal blood [6]. However the isolation of such cells proved to be technically difficult and inconsistent. In recent years the focus has shifted to cell free fetal DNA (cfDNA) in maternal circulation.

What is cfDNA? As a result of cell turnover, short (~200 bp) fragments of cfDNA are released into the bloodstream. During pregnancy, about 10% of cfDNA is of fetal origin (cffDNA) [7]. Cell free DNA was first used for non-invasive prenatal diagnosis of fetal sex using PCR amplification of Y-chromosome specific fragments [8]. Subsequently, cfDNA was used to determine fetal Rhesus (Rh) status in Rh-negative women and to exclude paternally-derived mutations [9-11].

Recently cfDNA has been used to detect fetal chromosomal anomalies, by a technique generally referred to as Non-Invasive Prenatal Testing (NIPT). Most of these tests are based on sequencing and quantification of cfDNA in the maternal plasma. Non-invasive prenatal testing is based on the assumption that when the fetus has a normal constitution of 46 chromosomes, there is a constant ratio between the number of fragments derived from each chromosome. In contrast, when the fetus is affected by a chromosomal numeric aberration, there is a deviation from the expected ratio. For example, if the fetus has trisomy 21, more fetal cfDNA fragments from chromosome 21 are released into the maternal circulation. While the absolute increase in chromosome 21-derived fragments is quite low, sequencing and counting of numerous fragments provides statistical significant results [12,13]. This approach only became feasible with the introduction of shot-gun or Massive Parallel Sequencing (MPS), which allows the simultaneous sequencing and counting of millions of cfDNA fragments [14,15].

NIPT was first offered for trisomy 21 [16], but soon expanded to include trisomy 18 and 13 and sex chromosome anomalies such as Turner syndrome (45,X), Klinefelter syndrome (47,XXY), and others [17-19]. Recently, NIPT has been reported as efficient in the detection of sub-chromosomal anomalies that are usually detected by chromosomal microarrays such as Velo-cardio-facial syndrome (VCF) caused by a microdeletion in chromosome 22q [20]. An alternative to MPS employs targeted sequencing of only a few chromosomes of clinical interest. An example of this approach based on quantification of pre-selected non-polymorphic loci by digital analysis of selected regions (DANSRTM) [21-25]. Another targeted approach is based on sequencing of polymorphic loci on chromosomes of interest, which is then compared with the expected allele distribution based on maternal, and occasionally parental, genotypes [26,27]. Since such targeted approaches sequence only specific fragments of interest, the cost is expected to decrease.

Initial studies evaluated the clinical utility of NIPT in high-risk patients. Reported sensitivities for detection of trisomy 21 ranged from 98.6% to 100% and specificities from 99.7% to 100% (Table 1). Given the high sensitivity and the low false positive rates (<0.1% for trisomy 21), these tests are expected to reduce the number of unnecessary invasive procedures, while maintaining high Positive Predictive Values

(PPVs). However, as these tests are gradually used by low-risk patients, the PPV is expected to decrease. Morain et al. (2013) noted that in a patient population with a Down syndrome prevalence of 1:8, the PPV is as high as 97.94 %. However, in a low-risk population with a Down syndrome prevalence of 1:800, the PPV would theoretically drop to 29.42%. In a recent study [28] The PPV was significantly higher for NIPT compared to standard screening for trisomy 21 (45.5% vs. 4.2%). Nonetheless, NIPT has not been sufficiently validated in low risk patients and is still is not recommended by most professional societies as a primary screening tool for all pregnant women.

Study	Test results					Sensitivity	Specificity
	TP	FN	TN	FP	Total		
Chiu et al., [15]	86	0	143	3	232	100.0%	97.9%
Ehrlich et al., [17]	39	0	409	1	449	100.0%	99.8%
Palomaki et al., [16]	209	3	1468	3	1683	98.6%	99.8%
Bianchi et al., [18]	89	0	404	0	493	100.0%	100.0 %
Ashoor et al., [21]	50	0	297	0	347	100.0%	100.0 %
Norton et al., [23]	81	0	2887	1	2969	100.0%	100.0 %
Dan et al., [19]	139	0	2819	1	2959	100.0%	100.0 %
Nicolaides et al., [22]	8	0	1939	0	1947	100.0%	100.0 %
Sparks et al., [24]	39	0	252	0	291	100.0%	100.0 %
Sparks et al., [25]	36	0	123	0	159	100.0%	100.0 %
Futch et al., [39]	154	2	5515	1	5672	98.7%	100.0 %
Zimmerman et al., [26]	11	0	126	0	137	100.0%	100.0 %
Nicolaides et al., [31]	25	0	197	0	222	100.0%	100.0 %
Total	894	5	16462	7	17368	99.4%	99.96 %

Table 1: Clinical performance of NIPT in the detection of Down syndrome

The performance of NIPT for other aneuploidies is lower than that of trisomy 21. The combined detection rate for trisomy 18 is 97.4% and for trisomy 13 is only 83.3% [29]. These lower detection rates may be due to larger chromosome size and higher GC content of chromosome 13. Another explanation for the decreased sensitivity of NIPT for trisomies 13 and 18 may be due to the fact that in these cases there is a smaller placenta resulting in a lower concentration of cffDNA. As more conditions are tested (such as microdeletions), the cumulative false positive rates are expected to increase.

In addition to the limitations discussed above, there are a number of drawbacks that must be addressed before NIPT becomes common clinical practice:

False negative results: There have been several reports of false negative results, rates in the range of 0 to 1.4% [30]. This fact must be conveyed to patients during pretest counseling.

False positive results: These are more common in trisomy 13, 18 and sex chromosome aneuploidy. As more conditions are added to NIPT, cumulative false positive rates are expected to increase.

Cost: The current cost of NIPT is too high to offer to the entire pregnant population. However, it is possible that NIPT may be utilized as a secondary screen for women determined to be at-risk by standard screening test, using a contingent screening approach [31].

Technical difficulties in using NIPT: In rare instances, the test fails to provide a result. This is mostly due to a low fraction of fetal DNA in the maternal plasma and technology used. Test failure occurs in approximately 0.7-3.8% of tests [32,33]. However, redrawing blood from the patient will allow a result in the majority of these cases.

Because all NIPTs have potential false positive and false negative results, they are currently not considered diagnostic, but should be regarded as highly reliable screening tests. Therefore, abnormal NIPT results should be followed by invasive diagnostic testing (CVS or amniocentesis).

NIPT has rapidly been introduced into clinical practice, often with no regulation or clinical guidelines. To address this issue, consensus statements and clinical guidelines have been issued by several international and national professional societies such as the International Society for Prenatal Diagnosis [34] and the American College of Obstetrics and Gynecology (The American College of Obstetricians and Gynecologists Committee on Genetics, 2012) [35] the National Society of Genetic Counselors [36], and Israeli Society of Medical Genetics [37]. The use of NIPT in clinical practice should be an informed patient choice. Pre-test counseling should focus not only on the benefits but also on the limitations of NIPT. It should be made clear that NIPT does not replace invasive diagnostic testing. Post-test counseling is also of great importance. Patients receiving positive results are recommended to have definite diagnostic testing because of potential false positive results. Patients receiving negative results should be counseled regarding the residual risk for a chromosomal anomaly. In addition, it must be made clear that NIPT does not reduce the risk for chromosome anomalies that are not included in the test. Patients manifesting major structural anomalies should not be reassured given a negative NIPT result but should be referred for genetic counseling and invasive testing, including chromosomal microarray analysis [38].

Conclusion

In summary, NIPT has now become a reality. High sensitivity and specificity already mean that fewer patients will require unnecessary invasive procedures. If the sensitivity and specificity of NIPT improve NIPT may ultimately replace invasive procedures. However at this juncture, these tests are to be regarded as very good screening tests, as their performance characteristics are superior to other screening modalities. Commercialization of NIPT has to be taken into consideration and thus utilization of NIPT should adhere to professional guidelines.

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