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ORIGINAL ARTICLE

First trimester screening for aneuploidy: may combined test and fetal DNA work together?

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ABSTRACT

Introduction: The purpose of the study was to evaluate the screening performance of combined test (based on the measurement of nuchal translucency, pregnancy-associated plasma protein A, free β-human chorionic gonadotropin, and maternal age) and fetal DNA screening (NIPS) for trisomies 21 (T21), 18 (T18), and 13 (T13).

Material and methods: Women who accepted screening had a first-trimester combined test (pregnancy-associated plasma protein A, free β-human chorionic gonadotropin, nuchal translucency interpreted with maternal age) and fetal DNA.

Results: Among 302 women screened (including 4 with affected pregnancies), our study demonstrated that DNA screening for trisomies 21, 18, and 13 achieved a detection rate of 100% with a false-positive rate of 0.02%, overcoming the traditional combined test with 75% of sensitivity and 4.7% of false-positive rate. In particular, fetal DNA may be useful in case of intermediate risk, in order to avoid invasive diagnostic procedures such villocentesis and amniocentesis. Because of fetal DNA costs, it can be used in clinical practice as a second step screening in case of intermediate or high risk at combined test.

Conclusion: Fetal DNA screening may be successfully implemented in routine care, achieving a high detection rate, low false-positive rate, and, consequently, greater safety with fewer invasive diagnostic tests than other methods of screening.

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Down syndrome; NIPS; prenatal screening; trisomy 13, 18, 21

Introduction

Before 1984, aneuploidy screening was primarily based on consideration of maternal age. In that year, Merkatz and colleagues observed a correlation between trisomy 18 and 21 and a decreased maternal serum alfa-fetoprotein at 15–21 weeks.1 Over the past 3 decades, aneuploidy screening options have continued to expand. Several paradigms now exist for aneuploidy screening that combines measurement of nuchal translucency (the fluid-filled space on the dorsal aspect of the fetal neck) with certain maternal analyte levels in the first trimester (serum-free or total beta-hCG, pregnancy-associated plasma protein A) and second trimester (alfa fetoprotein, dimeric inhibin A, unconjugated estriol). These screening paradigms differ with respect to detection rates, false-positive rates, and the times during which they can be performed. Detection rates for trisomy 21 and 18 now range from 88% to 96% and 85% to 95%, respectively [1].

In addition to screening options, diagnostic procedures including chorionic villus sampling (CVS) and amniocentesis are options for diagnostic testing during pregnancy, with loss rates of 1 in 500 and 1 in 1000, respectively [2]. CVS is typically performed at 10–13 weeks, and amniocentesis between 15 and 20 weeks.

With the discovery that male fetal DNA could be detected in maternal plasma, efforts have focused on the analysis of cell-free fetal DNA (cffDNA), more commonly known as noninvasive prenatal screening (NIPS) [3].

NIPS became commercially available in 2011 and has been rapidly incorporated into obstetrical care. The American College of Obstetricians and Gynecologists (ACOG), Society of Maternal Fetal Medicine (SMFM), and the American College of Medical Genetics (ACMG) support a pregnant patient's decision to choose NIPS regardless of the pretest risk,

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although cost-effectiveness data in the low-risk obstetric population are limited [4].

NIPS involves the sequencing of small fetal DNA segments (fewer than 200 base pairs) that are free-floating in maternal plasma. These fragments are derived from apoptosis of placental trophoblasts that are released into the maternal circulation continually during pregnancy. The origin of cffDNA is not fetal but placental, and as a placental test, clinical challenges may arise that will be discussed further. These fragments of cffDNA represent short sequences of the entire fetal genome that have an extremely short half-life of 16 min. Maternal and fetal cell-free DNA can be differentiated based on fragment length, with cffDNA having shorter fragments [5]. After delivery, most cffDNA is cleared from the maternal circulation within 2 h [6].

The proportion of cffDNA in the maternal circulation that is derived from the placenta is known as the fetal fraction. CffDNA may be detected as early as 4 weeks' gestation and is reliably detected at 10 weeks' gestation when the fetal fraction typically is 10% [7]. At less than 10 weeks, NIPS is less likely to provide a result [8]. From 10 to 21 weeks, the fetal fraction remains relatively constant, with an approximate rate of increase of 0.1% per week, and after 21 weeks, this rate increases substantially to 1% per week [9]. At about 30 weeks, the fetal fraction is twice that observed at 20 weeks [10]. Maternal weight is inversely related to the fetal fraction [11]. Decreasing fetal fraction with increasing maternal weight is likely due to a higher concentration of maternal cfDNA as a result of the turnover of adipose cells. Most laboratories require a minimum fetal fraction of 4% for reliable analysis. Among women weighing more than 250 lb (113.4 kg), about 10% will have a fetal fraction of less than 4% [12]. A low fetal fraction is a primary cause of test failures, also known as no-call results. If a test failure is encountered in a patient with obesity, consider waiting to retest until after 21 weeks, when the fetal fraction begins to increase [12,13]. Although turnaround times vary among the different laboratories that offer NIPS, results usually are available in about 7 days. In addition to maternal weight and gestational age, a low fetal fraction may be associated with fetal aneuploidy, particularly trisomy 13 or 18 [14]. Thus, as part of pretest counseling in patients with obesity, discuss the possibility of a no-call result, highlighting that maternal weight typically is the main reason for a low fetal fraction, but that aneuploidy also is a possibility. Fetal fraction does not appear to be influenced by maternal age, prenatal screening result, or nuchal translucency measurement [15].

The objective of this study was to examine how combined test and NIPS work for first-trimester screening. The secondary objectives of the study were to determine the uptake of NIPS in women with intermediate risk at the combined test and to determine the perinatal outcomes in women who participated in the study.

Material and methods

This was a prospective cohort study conducted between January 2019 and April 2020 at the Operative Unit of Obstetrics of the State Hospital of the Republic of San Marino, that offer a publicly funded first trimester screening test for Down syndrome, consisting of combined test for aneuploidies and noninvasive cell-free prenatal DNA screening (NIPS) to detect trisomies 13, 18 and 21. this study was approved by the ethics committee. Women were provided with leaflets at booking and prior to screening that described Down syndrome, the screening options, the follow-up testing options should their pregnancy be considered high risk (HR).

The examination is composed of the Fetal Medicine Foundation risk evaluation based on nuchal translucency evaluation, mother's age, presence of risk factors, presence of the nasal bone and Doppler of the ductus venous in addition to biochemical analysis of pregnancy-associated plasma protein A (PAPP-A) and beta-human chorionic gonadotropin (βhCG) markers. The cutoff point for high risk for aneuploidies was defined as greater than 1:100, with intermediate risk defined between 1:100 and 1:1.000, and low risk defined as less than 1:1.000. The variable aneuploidy was considered as a result not only of trisomy of chromosome 21 but also trisomy of chromosomes 13 and 18. The fetal crown-rump length (CRL) was measured, and if it was between 45 and 84 mm, we evaluated the following fetal ultrasound parameters: nuchal translucency (NT), nasalbone (NB), and ductus venosus(DV) flow.

After that, the PAPP-A and freeβ-hCG levels in the maternal serum were determined. The sample was analyzed by means of a fluoroimmunometric assay using an automated AutoDelfia system (Perkin Elmer, Wallac, Turku, Finland). Analysis of NT thickness, PAPPA-A and freeβ-hCG was performed using the algorithm provided by the FMF, in London, UK, and was calculated using the Astraia software (Astraia Software Gmbh, Munich, Germany). The ultrasound

Table 1. Patient's characteristics.

	Patients ($n = 302$)
Median age (years) ±SD at estimated date of delivery (IQR)	32.5 ± 4.5 (22-41)
Median maternal weight (kg) (IQR	64 (57–63)
Median gestational age (weeks + days) at blood sample (IQR)	12 + 5 (11 + 6-13 + 1)
Nulliparous	152/302 (50.1%)
Spontaneous conception	267/302 (88.3%)
In vitro fertilization	35/302 (11.7%.)
Smoker	11/302 (3.4%)

SD: standard deviation; IQR: interquartile range.

parameters were evaluated only by experienced sonographers who had been certificated by the FMF for 11–13 weeks'scan. The measurements were taken using a transabdominal transducer (5 MHz curvilinear transducer, Voluson E10 [GE Healthcare, Milwaukee, WI, USA]). First-trimester risk assessment was provided for trisomy21, trisomy 18 and trisomy 13. The risk was calculated using a previously described algorithm [16].

The cutoff point for high risk for aneuploidies was defined as greater than1:100, intermediate risk was defined to be between 1:100 and1:1000 and low risk was defined as less than 1:1000 [17].

The variable aneuploidy was considered as a result not only of the trisomy of chromosome 21 but also the trisomy of chromosomes 13 and 18. Information about patient characteristics, chromosomal abnormalities and the pregnancy outcome was obtained by the personnel, hospital registry or the postpartum routine follow-up registry.

The statistical analysis was performed using the SPSS software version 20.0 (IBM Corp., Armonk, NY, USA). A descriptive analysis of the population was performed in the form of mean and median with standard deviation (SD) for quantitative variables and the proportions, percentages and ratios by calculating the 95% confidence intervals (95%CIs) for categorical variables. We calculated detection rates(sensitivity), specificity, positive and negative predictive values and percentage of false positive (FP) for aneuploidies. P-values < 0.05 were considered statistically significant. For quantitative variables, the Mann-Whitney test was used for comparison between two independent groups, and for categorical variables, we used the Chi-square test.

Results

During the study period, 302 women with a singleton pregnancy underwent first-trimester screening test. The mean maternal age was 32.5 years (standard deviation, SD = 4.5 years), 76 (25.3%) were ≥35 years of age at delivery, 152 (50.1%) were nulliparous, 267 (88.3%) had a spontaneously conceived pregnancy. These characteristics are reported in Table 1. There were 2 trisomy 21 (T219, 1 trisomy 18 (T18), and 1 trisomy 13 (T3) pregnancies (combined trisomy rate 0.46%).

The first-trimester combined test (based on the measurement of nuchal translucency, pregnancy-associated plasma protein A, free β-human chorionic gonadotropin, and maternal age) detected as high risk 1 T 21, 1 T18 and 1 T 13, while classified as low risk the other T 21. In addition, we reported 14 cases at intermediate risk for T 21. On the other hand, fetal DNA correctly identified all the affected pregnancies and also the intermediate risk. The combined test reporting an overall sensitivity of 75% with a false positive rate of 4.7%.

On the other hand, fetal DNA overcame these results, with a sensitivity of 100% and no false-positive cases. In particular, fetal DNA has reported a good application for the intermediate risk at the combined test. In Table 2 all these data are reported.

As an additional aspect, we analyze also the economic impact in our small cohort of patients. Compared to the conventional screening scenario, the addiction of NIPT (noninvasive prenatal testing) resulted in an efficient and more effective screening, reducing the number of invasive tests required to detect a trisomy by 92.8%, from 30.8 to 2.2, while improving the overall detection rate (from 81 to 99%) and reducing missed trisomies (from 77 to 5%). As a result, NIPT reduced the risk of procedure-related complications by 90.8%, avoiding an estimated 40 procedure-related miscarriages annually. At a cost of EUR 260 for the NIPT test, the difference in cost per trisomy diagnosed was estimated to be EUR 3,617 per trisomy diagnosed

Discussion

Prenatal screening for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome) using plasma (cell-free) DNA analysis offers substantial improvements over conventional screening methods, which are based on ultrasound and serum

Table 2. Performance of first-trimester combined test and fetal DNA screening for T21, T18 and T13.

		Affected		Unaffected[a]	
	Trisomy	Positive/total	DR (95% CI)	Positive/total	FPR (95% CI)
Combined test	21	1/2	50% (33–55%)	14/298	4.7% (4.1–4.9%)
	13	1/1	100% (63–100%)	0/298	0.00% (0.00–0.02%)
	18	1/1	100% (63–100%)	0/298	0.00% (0.00–0.02%)
	All	3/4	75% (56-81%)	14/298	4.7% (4.1-4.9%)
Fetal DNA screening	21	2/2	100% (63–100%))	0/298	0.00% (0.00–0.02%)
	13	1/1	100% (63–100%)	0/298	0.00% (0.00–0.02%)
	18	1/1	100% (63–100%)	0/298	0.00% (0.00–0.02%)
	All	4/4	100% (63–100%)	0/298	0.00% (0.00–0.02%)

CI: confidence interval; DR: detection rate; FPR: false-positive rate.
[a]Unaffected with any of trisomy 21, 18, or 13.

markers [1,2]. However, DNA analysis is complex and relatively costly, and has a technical failure rate of a few percent, particularly when the percentage of cell-free DNA from the placenta is low [4,18].

Our study demonstrated that DNA screening for trisomies 21, 18, and 13 achieved a detection rate of 100% with a false-positive rate of 0.02%, overcoming the traditional combined test. In particular, fetal DNA may be useful in case of intermediate risk, in order to avoid invasive diagnostic procedures such villocentesis and amniocentesis. Because of fetal DNA costs, it can be used in clinical practice as a second step screening in case of intermediate or high risk at combined test.

In particular, fetal DNA screening [19] can potentially achieve cost savings because of the reduction in the number of invasive diagnostic tests needed and the reduced need for patient counseling associated with the two-step approach. The main strength of our analysis was that we used actual clinical data, collected in a fully funded public sector maternity care system in units with a range of screening uptakes and modes of service delivery. These results thus reflect women's behavior in real life regarding uptake of Down's syndrome screening, NIPT, and invasive testing as inputs for our model.

There is an unmet need for a more accurate screening approach due to missed trisomies, high numbers of avoidable invasive tests, and resulting complications associated with conventional screening. By means of a case study of Belgium, which recently approved and funded NIPT as a first-line screening protocol, this analysis demonstrated that introducing NIPT for the general population is a cost-effective screening approach for public health systems. At a cost of EUR 260 per NIPT, effectiveness gains and reductions in adverse events come at a reasonable increase in cost. When NIPT is used as first-line screening in all pregnant women, the clinical benefits include very few unnecessary invasive tests, reduced false-positive results, more trisomies detected as early as at 10 weeks of gestation, and fewer missed trisomy diagnoses [20].

Several other studies have estimated the economic impact of NIPT first-line testing in prenatal screening programs. In the Netherlands, Beulen et al. [21] developed an economic model to compare the costs and outcomes of current clinical practice using conventional screening compared with NIPT first-line and second-line screening. At a cost of EUR 254 or less, NIPT first-line screening became the dominant approach, resulting in lower cost and improved clinical outcomes. In the US, Benn et al.'s [22] analysis of NIPT as a first-line test in the general population showed increased T21, T18, and T13 detection and can be economically justified at a break-even cost of USD 744. Fairbrother et al. [23] conducted a cost-effectiveness analysis of NIPT in the general population versus routinely FTS (first-trimester screening), assuming a societal perspective inclusive of both direct and indirect costs and inclusive of T21, T18, and T13. The study found that at a cost below USD 453 was cost saving and that a cost of USD 665 provided the same cost per trisomy detected as FTS. Similarly, an analysis by Walker et al. [24] reported a societal perspective break-even cost of USD 619, with first-line NIPT for all women the dominant approach. These analyses support an improved clinical performance at a reasonable cost per trisomy detected.

Based on our results, the benefits of fetal DNA screening arise mainly from the substantially lower false-positive rate compared with other methods of screening, the avoidance of recall-induced anxiety associated with contingent screening, and a detection rate similar to universal DNA testing. NIPT primary screening increases overall trisomy detection rates and provides more efficient referral to invasive testing, leading to a reduction in the number of procedure-related miscarriages and other procedure-related complications, at a similar cost per trisomy detected. These clinical benefits, together with the reduced cost compared with universal DNA testing, make the two-step approach (combined test + fetal DNA) a preferred method of screening.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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