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Non-invasive prenatal screening: A 20-year experience in Italy

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ABSTRACT

Over the past two decades, there has been a rapid evolution in prenatal screening for fetal chromosome abnormalities. Initially, testing was focused on the identification of affected pregnancies in either the first, or, the second trimester (e.g. the Combined test or the triple test). This was replaced by sequential modalities (e.g. contingent screening) that have enhanced detection while reducing the need for invasive testing. More recently, the introduction of technologies based on cell-free DNA (cfDNA) in maternal plasma and enrichment of fetal cells in maternal circulation have further refined the concept of sequential screening. In this review, we document our experience with serum and ultrasound-based contingent screening where we were able to achieve a detection rate of 96.8%, a false-positive rate of 2.8% and an odds of being affected given a positive result of 1:11. We also describe our initial experience with a novel sequential protocol that includes the analysis of fetal cells in maternal blood.

Methods for enrichment for fetal cells cfDNA and cfDNA technologies offer the possibility of greater sensitivity and specificity as well as expansion in the scope of genetic disorders detectable. As costs decline, these technologies will become increasingly used as primary screening tools. In the meantime, sequential use offers a practical approach to maximizing the benefits of prenatal testing.

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Introduction

Non-Invasive-Prenatal Testing (NIPT) has been a goal in obstetric care since the 1990s [1–3]. NIPT includes maternal serum screening tests, ultrasound markers associated with aneuploidy, maternal plasma cell-free DNA analysis, and analysis of fetal cells in maternal blood. The need for NIPT arose because Invasive Prenatal Diagnosis (IPD) (chorionic villus sampling (CVS) and amniocentesis) was largely reserved for pregnant women aged 35, or more, with a primary focus on the identification of Down syndrome (DS) which is the most common clinically significant genetic disease (1/600-800 newborns) [4]. However, using maternal age as a criterion for IPD allowed only 30-40% of DS livebirths to be prenatally identified [4–6]. IPD was not offered to all women because of the cost, the risk of miscarriage, and anxiety associated with the testing [7,8]. NIPT potentially overcomes these problems because it can be offered to all the pregnant women regardless of their age and if the results are negative women can avoid IPD.

Maternal serum screening can be applied in the first trimester (11-13 weeks), the second trimester of gestation (15-21 weeks), or both, through the integration of the data from the first and second trimester into a single result [9–13]. These serological screenings aim to carefully and rigorously select those pregnant women that have the highest risk and to offer them IPD. The tests must have a high detection rate (DR), a low false positive rate (FPR) and be acceptable to pregnant women. This approach can lead to a significant reduction of IPD, a high rate of trisomy 21 prenatal diagnosis and reduction in health service costs [14–17]. Non-directive genetic counseling by a knowledgeable healthcare professional is an essential component of the service.

Fetal and neonatal outcomes were obtained from review of clinical records. Neonates underwent clinical evaluation, and neonatal karyotyping if a chromosomal abnormality was suspected. Follow-up also included cases that resulted in a fetal demise or abortion that underwent fetal karyotype and post-mortem evaluation. We assumed that no further cases of trisomy went undetected in our population because our unit is the regional referral center for all clinical genetic consultation, prenatal and postnatal cytogenetic diagnoses.

Our experience with NIPT began in 1998 with the second trimester triple test (maternal serum alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE3)). Successively, we introduced the first trimester Combined test (ultrasound measurement of nuchal translucency with maternal serum pregnancy associated plasma protein-A (PAPP-A) and hCG) and sequential provision of both tests. This led to a significant reduction of IPD analyses. Such reduction also led to a decrease in costs for the diagnosis of DS [18]. These serological tests have also proved useful for the screening of trisomy 13 and 18, Turner syndrome and triploidy. We have observed a DR of 95-97% for trisomy 18, 80-90% for trisomy 13, and 92-95% for Turner syndrome [18–21].

In 2011 and 2012 the first reports appeared for the use of maternal plasma cell-free DNA (cfDNA) as a noninvasive screening test [22–24]. Although the cfDNA test had a specificity of approximately 99.9% and a DR of 99% for DS, it was still classed as a screening test because there are false-positive (FP) and false-negative (FN) cases and confirmation of test-positive cases through IPD is required [25,26]. cfDNA analysis has been established as the most effective current form of non-invasive screening. Recent studies have reported use of fetal-cells present in the maternal peripheral blood to identify fetal genetic disorders [27–29].

In this review, we describe the maternal serum screening tests in detail and our experience with these serological tests from 1998 to 2017. Results will be compared with those reported in the literature. We also describe our initial experience with a novel sequential protocol that includes the analysis of fetal cells in maternal blood.

First trimester tests

Various combinations of screening tests can be performed in the first trimester of pregnancy between 11-13 weeks, of which the Combined test is the gold standard although the Contingent test actually provides better results and use of cfDNA testing offers even better prenatal screening for DS [14,22].

Combined test

The Combined test consists of the assay of PAPP-A and free-beta-hCG together with an ultrasound scan that measures the gestational age and nuchal translucency (NT) thickness. Relative to unaffected pregnancies, PAPP-A is usually lower, hCG higher, and NT is increased. The combined test has a DR of 90% for DS and a FPR of 3-7%. The test also helps to identify other chromosomal aberrations, such as trisomies 13, 18 and triploidy, some cardiac abnormalities and other disorders [9,30–39].

In our study of 7292 pregnant women submitted for the combined test, 6981 had a negative result and 311 a positive result. This testing had a DS detection rate of 17/21 (81%), and a FPR of 294/7271 (4%). The odds of having an affected pregnancy given a positive result (OAPR) was 1:17 [30]. Approximately 2300 pregnant woman needed to be tested to achieve 100 positive tests (Table 1).

Second trimester tests

Second trimester tests are performed between 15-21 weeks of gestation but optimally 15-18 weeks. The most well-known tests are the triple test and quadruple test.

Triple test

In 1988 Wald et al. developed the triple test, consisting of serum markers AFP, hCG and uE3 combined with maternal age [1]. In DS pregnancies AFP and uE3 levels are generally lower than normal, while hCG is often higher. In trisomy 13 and trisomy 18 pregnancies, the values of all three hormones are significantly reduced [14,19,20]. In triploidy, the analytes are usually extremely elevated or low, depending on whether the extra set of chromosomes is paternal (diandric) or maternal (digynic) in origin

Table 1
Trisomy 21 screening actual performance for various screening approaches.

Type of test	DR	FPR	PTR	OAPR	DS/100PT	WS
Combined	81%	4%	4.20%	1:17	5	2300
Triple	82%	7%	8%	1:39	3	1400
Crosstrimester	100%	3.40%	4%	1:13	4	2800
Contingent	97%	2.80%	3%	1:11	9	3300

DR = Detection Rate; FPR = False Positive Rate; PTR = Positive Test Rate; OAPR = Odds of being Affected given a Positive Result; DS/100 PT = Down Syndrome Cases per 100 Positive Tests; WS = number of women screened for 100 positive results.

[40]. The DR of the triple test for DS is about 70%. An additional ultrasound, can be performed to evaluate numerous fetal parameters such as the length of the femur, of the humerus, the cranial and the abdominal circumferences, the hyperechogenic intestine, and cardiac defects. This can increase the DR to 80-85% for a FPR of 5-7% at a cut-off of 1:250 [41–43].

In one of our studies involving 22,504 pregnant women tested for triple test only at the cut-off risk 1:250, there were 1632 positive tests (1 in 14 tests) (7%) yielding a DR for DS of 41/50 (82%) with a FPR of 7% and the OAPR 1:39. Approximately, 1400 pregnant women were tested to achieve 100 positive tests (Table 1) [44].

Quadruple test

The quadruple test consists of a triple test with an additional assay of Inhibin A, a hormone that is often increased in the maternal circulation in DS pregnancies. Literature indicates that the addition of the Inhibin A in maternal serum screening increases the DR [3,38,41].

Integrated and sequential first and the second trimester tests

These are tests that combine ultrasound parameters and serological assays from the first and second trimester, providing the pregnant woman either a single result in the second trimester or more than one risk estimate as additional tests are performed [14,44,45]. Among the best-known tests we can mention the integrated, the crosstrimester, the stepwise sequential and the contingent tests.

Integrated test

Wald et al. developed the integrated test in 1999 [42]. It is comprised of the first trimester Combined test and the second trimester triple test with all results combined and presented to woman as a single risk in the second trimester. Based on a cut–off of 1/250, this test shows a DR of 94% and FP 1% [16,14,44,45].

Crosstrimester test

The crosstrimester test is similar to the integrated test but includes an additional ultrasound performed around 14-15 weeks of gestation (i.e. before the triple test) [44]. In our studies on 5060 pregnant women receiving this combination of tests, at a risk cut-off of 1:250, there were 183 DS positive tests (1 in 28 tests) (4%), while in 10 cases the screening was positive for trisomy 18. Nineteen pregnant women had a fetus with an abnormal karyotype consisting of 13 DS cases and 6 cases of trisomy 18. The DR for DS was 100%, FPR 3.4% and OAPR 1:13. Approximately 2800 pregnant women were tested to achieve 100 positive tests (Table 1). This test was also particularly useful for trisomy 13, 18 and triploidy detection since the values of all serum analytes in these cases were below the normal value. The additional ultrasound was important to identify other abnormalities, including intrauterine growth retardation [14,44].

Contingent test

The contingent test is a two-step sequential approach to maternal serum screening [45,46]. After the first step, women are classified in three groups, high-risk (IPD offered), intermediate risk (second step screening offered) and low risk (no additional screening necessary). The first and second trimester markers used are the same as for integrated screening. In our experience, for contingent screening performed on 24,408 women between 2011-2017, the first step cut-offs that provided the best results were >1/30 (high risk), 1/31 to 1/899 (intermediate risk) and <1/900 (low risk). For the second trimester testing a 1/250 cut-off was used [14]. At the first step, approximately 0.5% were deemed high-risk, 10% intermediate risk, and 90% low risk. This protocol had an overall 60/62 (96.8%) DR, with a 2.8% FPR, and OAPR 1:11. The first trimester positive tests were 1/300 (0.5%), while the overall positive tests were 3%. Around 3300 pregnant women were screened for 100 positive tests in the first trimester (Table 1) [46].

Overall impact on the numbers of IPD

The evolution of prenatal screening at our center has had a major impact on the utilization of IPD. Fig. 1 shows the numbers of IPD performed between 1998 and 2017 in women who received serological screening. We can see how IPD tests were reduced from nearly 800 units in 2004 to less than 100 in 2017.

Recently developed techniques

Fetal cfDNA in maternal plasma

Fetal trophoblast cell DNA can be detected in the maternal blood circulation as early as 5-7 weeks of gestation and by 9-10

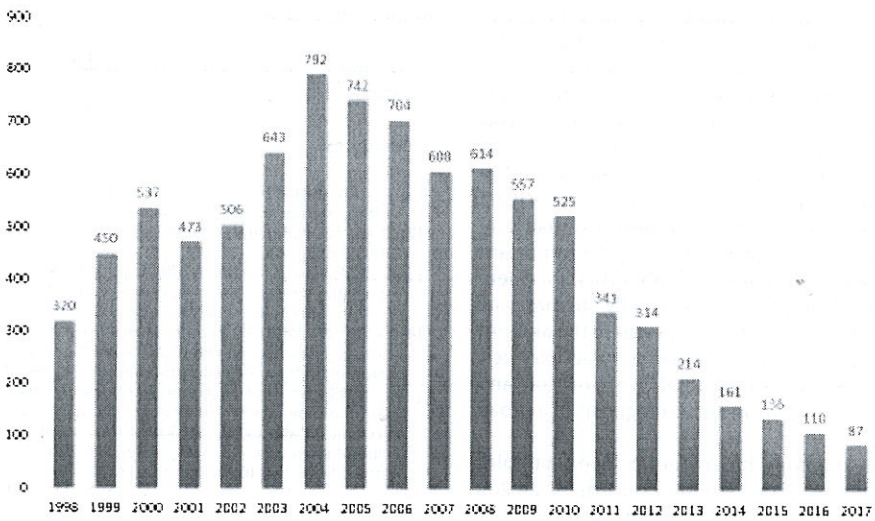

Fig. 1. Amniocenteses performed between 1998-2017.

weeks there is sufficient to provide prenatal screening for more than 95% of all pregnant women. In 2011, Palomaki et al. published the first validation series, supporting the value of this novel approach for DS screening and this was rapidly followed by additional studies [47]. Next generation sequencing (NGS), selective enrichment of cfDNA from chromosomes of interest, microarray technology, multiple polymerase chain reactions, single nucleotide polymorphism analyses, and other molecular analytic approaches combined with sophisticated biometrics are the technologies employed to screen for aneuploidy. Although different laboratories have developed entirely different technologies for cfDNA screening, all appear to be highly effective compared to traditional serum biochemical and ultrasound marker tests [23,24]. Based meta-analysis of multiple methods and study designs, this screening test approach has a DS DR of 99% and FPR of 0.04% [47–50]. The testing has been extended to trisomy 18, trisomy 13, and fetal sex chromosome abnormalities with overall DRs and FPRs approaching that achievable for DS. Triploidy and some microdeletions (notably 22q11.2 deletion syndrome) are also detectable by some approaches [50]. This analysis is considered the best in the field of the NIPT technologies and could potentially decrease IPD analyses by 98% [47–50].

However, some problems remain, such as the high cost, complexities in applying the test to twin pregnancies, and chromosome abnormalities that the cfDNA screening technologies are unable to detect (mosaicism, balanced translocations, and small copy number variations (CNVs)) [24–26]. Reasons for false-positive results include confined placental mosaicism, vanishing twins, benign CNVs, and maternal chromosome imbalances including those associated with maternal cancer [51,52]. Accurate measurement of the proportion of cfDNA that is fetal in origin (fetal fraction, FF) is important because in some cases this proportion is too low for a reliable result. Low FF is associated with an increased risk for digynic triploidy, trisomy 18 and trisomy 13 and pregnancy loss. For cases where there is no test result due to low FF, there needs to be a careful individual patient assessment to determine whether referral for ultrasound and IPD, repeating the test, or offering alternative screening tests is the optimal management [51–54].

Fetal cells in maternal blood

Fetal cells appear in the maternal circulation around the seventh week of pregnancy, with a maximum frequency around the 13th week [55–58]. Both nucleated red blood cells and cells of trophoblastic lineage are present. The former can be retained in maternal circulation for an extended period while the latter are thought to be subject to apoptosis much earlier [59,60].

FISH technology can be used to identify chromosome abnormalities such as trisomy 21, 13, 18 and triploidy [28,29]. This technology shows some restrictions because twin pregnancies are excluded, mosaicism is difficult to identify, and balanced chromosomal translocations and small CNVs cannot be identified. Due to small number of cases analyzed, robust estimates of sensitivity and specificity are not available [28,29]. An important point is that in cases with chromosomal abnormality, there appears to be an increased number of fetal cells in maternal circulation [28,29]. This differs from fetal cfDNA where the amount available for analysis can be reduced for some fetal aneuploidies. In the future, it is expected that single fetal cell isolation and amplification could facilitate the non-invasive diagnosis of a broad range of genetic disorders.

We have used FISH technology on maternal blood samples enriched for fetal cells. In one of our studies concerning 24,208 pregnant women screened with contingent test, 47 cases had positive screening results in the first trimester [46]. One woman preferred to proceed directly to CVS while 46 accepted the analysis of fetal cells prior IPD. In 39 of the 46 women, FISH analysis showed 2 normal signals for chromosomes 21 and 18, while in 7, a chromosomal abnormality was identified. This included 4 cases of trisomy 21, 2 trisomy 18 and 1 triploidy. Amniocentesis performed in all 46 cases, confirmed either the 7 chromosomal abnormalities or the 39 cases with normal FISH results,

Conclusions

Use of maternal serum markers provide simple, quick, and relatively inexpensive NIPT with a low test failure rate. A contingent approach to the testing is a favored strategy [14,45]. The study performed at our Centre on 24,408 pregnant women confirms the success of contingent test showing a DR of 96.8%, FPR of 2.8% and OAPR of 1:11 [46]. We have observed a reduction of invasive tests since 2004, particularly since 2011 (Fig. 1).

At the first step of the contingent test in the first trimester, a small proportion of women (0.5%) will have a positive test result. For these women, we favor the use of amniocentesis instead of CVS because of the risk of confined placental mosaicism [51,52]. Given the delay before an amniocentesis can be performed, these women could also receive a triple test [19,20,44]. Alternatively, the genetic counsellor could propose a cfDNA study or the analysis of fetal cells. The results can be available in 1-2 weeks. Our initial experience with fetal cell analysis, suggests that this can reduce the need for IPD as well as reduce patient anxiety. Additional clinical experience is needed with the fetal cell analysis, and there is a need for an analysis of cost and efficacy relative to cfDNA analysis. We anticipate that as costs for the newer technologies decline, these new approaches will become the primary protocols for NIPT.

In summary, increased sophistication in NIPT has resulted in a meaningful reduction in the need for IPD. In the future, we anticipate additional benefits arising through the application of newer technologies. This includes the detection of genetic disorders that previously could not be identified through prenatal screening.

Conflicts of interest

Peter Benn is a consultant to Natera, Inc., a provider of non-invasive prenatal testing. All other authors have no conflicts of interest.

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References

[1] Wald N, Cuckle H, Royston P. Antenatal screening for Down syndrome. Lancet 1988;2(December (8624)):1362.
[2] Wald NJ. Antenatal screening for Down syndrome. Prog Clin Biol Res 1995;393:27–42.
[3] Egan JF, Benn P, Borgida AF, Rodis JF, Campbell WA, Vintzileos AM. Efficacy of screening for fetal Down syndrome in the United States from 1974 to 1997. Obstet Gynecol 2000;96(December (6)):979–85.
[4] Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94(May (5)):387–402.
[5] Grati FR, Barlocco A, Grimi B, Milani S, Frascoli G, Di Meco AM, et al. Chromosome abnormalities investigated by non-invasive prenatal testing account for approximately 50% of fetal unbalances associated with relevant clinical phenotypes. Am J Med Genet A 2010;152A(June (6)):1434–42. doi: http://dx.doi.org/10.1002/ajmg.a.33370.
[6] Ong CY, Liao AW, Cacho AM, Spencer K, Nicolaides KH. First-trimester maternal serum levels of placenta growth factor as predictor of preeclampsia and fetal growth restriction. Obstet Gynecol 2001;98(October (4)):608–11.