Meeting the needs of Mediterranean nations: perinatal and pregnancy laboratory medicine

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Prenatal screening for chromosomal abnormalities: where do we stand today in Mediterranean countries?

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ABSTRACT

Over the last 4 decades the practice of prenatal screening has evolved from the second-trimester triple test to complex combinations of biophysical and biochemical testing for aneuploidy, testing of fetal DNA in the maternal circulation and development of screening tests for adverse pregnancy outcomes. Presently, combined test in the 1st trimester is the preferred multimarker screening protocol in most countries. Since 2010, cell-free fetal DNA (cffDNA) in maternal plasma, in combination with the next generation sequencing techniques, made a big breakthrough step in screening for Down Syndrome (DS) and other aneuploidies. It seems that the position of cffDNA in the current screening strategies is a secondary contingent use to combined test, at least as long as its price is still high and its use as a primary test is not cost effective. Concerning the situation in Mediterranean countries, at least with those who answered the questionnaire, screening in the 1st trimester is an established practice, reimbursed from social security organizations, and not compulsory. cffDNA is used in all countries and its average cost is about 500 €.

INTRODUCTION

Screening is the process of surveying a population with specific markers in order to identify those individuals with a higher risk for a particular disorder. For high risk individuals, a diagnostic test is applied to definitely diagnose the disorder. A successful screening program should be complemented with an accurate diagnostic test to identify those who are truly affected and also with a clear strategy of how to treat the affected individuals.

Over the last four decades, the practice of prenatal screening has evolved from the simple second-trimester maternal serum α-fetoprotein (AFP) test for open neural tube defects (NTDs) to complex combinations of biophysical and biochemical testing for aneuploidy. It continues to evolve with the testing of fetal DNA in the maternal circulation and the development of screening tests for adverse pregnancy outcomes such as pre-eclampsia. Diagnosis of major fetal chromosomal aneuploidies is done by karyotype of fetal cells, obtained through amniocentesis after the 15th week of pregnancy, or chorionic villus sampling (CVS) between the 12th and 14th weeks. There are several financial and ethical implications of how pregnancies with affected fetuses are treated in different countries and these differences are even bigger between Mediterranean countries with different economical, social and cultural status.

BRIEF HISTORICAL OVERVIEW

Screening for fetal aneuploidy in pregnancy began in the 1960-1970s with maternal age as the only available marker. As maternal age increases, the chance of delivering a child with Down syndrome (DS) or other major autosomal trisomies like trisomy 18 (T18; Edwards syndrome) or 13 (T13; Patau syndrome) increases. However, screening with maternal age alone (cut-off >35years), could detect about 30% of trisomies. The majority of babies with DS are born by women less than 35 years of age.

The first breakthrough in screening for fetal chromosomal abnormalities was done in 1988 with the introduction of a multiple marker screening test, based on a "risk" calculation for each pregnant woman using her age and the concentrations of 3 biochemical markers: human Chorionic Gonadotropin (hCG), AFP, and unconjugated Estriol (uE3) (Triple test) from blood samples in the 2nd trimester of pregnancy [1]. Such screening has led also to the diagnosis of a large proportion of the other common trisomies, like T18 and T13. In 1992, ultrasound fetal nuchal translucency (NT), by far the single best individual marker, was introduced [2] and in 1997, a new multiple marker screening test the Combined test, using NT, Fb-hCG and Pregnancy Associated Plasma Protein –A (PAPP-A) was started [3]. The following years, several complex screening protocols were introduced using both first- and second-trimester markers. Table 1 [4] shows the model predicted detection rate (DR) and positive predictive value (PPV) for a 1% or 5% false positive rate (FPR) for the traditional screening strategies described above.

Comparing the first and second trimester screening protocols, 1st trimester's Combined test has better DR than the Triple or Quadruple (Triple+inhibin) test in the 2nd trimester, for 5% FPR. Presently, the Combined test is the preferred multimarker screening protocol in most countries. Protocols combining 1st and 2nd trimester markers, in one step or contingently or using more biochemical or ultrasound markers gave better performance but made the screening more cumbersome, expensive and time consuming.

DNA SCREENING FOR ANEUPLOIDIES

The discovery that there is sufficient cffDNA in maternal plasma, in combination with the next generation sequencing techniques, made the

next breakthrough step in screening for DS and other aneuploidies [5]. In principle, the screening is based on counting a large number of DNA fragments (both maternal and fetal) using massive sequencing, assigning them to a chromosome and quantifying the proportion assigned e.g. to chromosome 21. The results are expressed as a *z* score computed by comparison to an expected proportion for a sample from a euploid fetus.

In a recent review and metanalysis [6], the DR of cffDNA for DS was found 99.4% for 0.1% FPR (results from 148344 tests); for T18, 97.7% for 0,1% (results from 146940 tests); and for T13, 90,6% for 0,1% (results from 134691 tests). The authors concluded that "cffDNA based non-invasive prenatal testing (NIPT) can be diagnostic for fetal sex and rhesus D, but only screening test in aneuploidy".

Table 1 Model predicted DR and PPV for different policies for Down syndrome screening according to FPR

Policy	FPR = 1%		FPR = 5%	
	DR (%)	PPV [a]	DR (%)	PPV [a]
Second trimester				
Quad	50	1 in 16	71	1 in 54
First trimester				
Combined	72	1 in 12	85	1 in 46
Both trimesters				
Serum integrated	58	1 in 14	76	1 in 51
Integrated	83	1 in 10	92	1 in 42
Contingent [b]	83	1 in 10	91	1 in 42
Improved				
Combined plus NB	88	1 in 10	94	1 in 41
First trimester contingent [b]	86	1 in 10	90	1 in 43
Quad plus facial profile	83	1 in 10	93	1 in 42
Combined plus PlGF and AFP	77	1 in 11	89	1 in 44

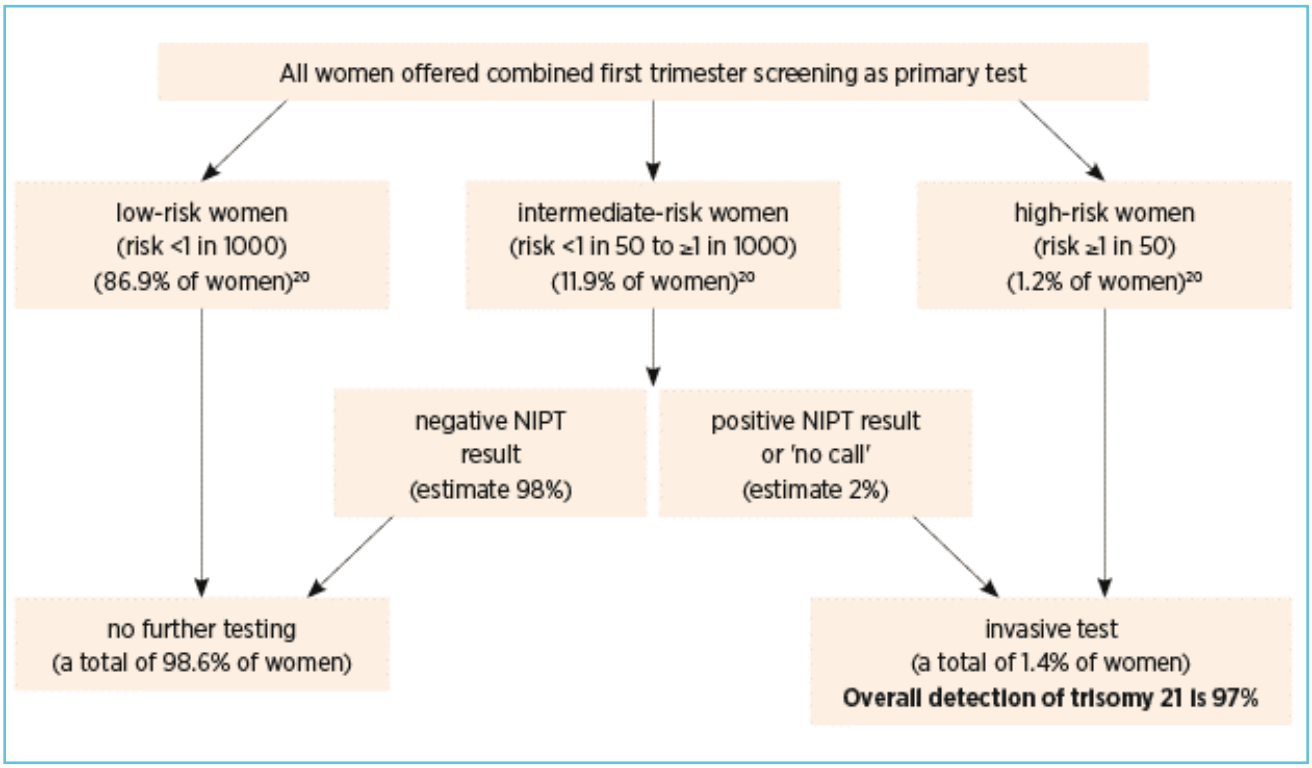
[a] *At term*
[b] *First stage cutoff risks 1 in 50 and 1 in 2000 at term*

Concerning the position of cffDNA based NIPT in the established screening strategies, two main approaches have been suggested:

a) as "primary" testing replacing the conventional screening; and
b) as "secondary" testing offered after the 1st trimester' s Combined test.

In both approaches, a confirmatory invasive prenatal diagnosis by CVS or amniocentesis is necessary for positive results. As a primary test, cffDNA has a much higher screening performance, at least for Down syndrome, than any of the conventional policies summarized in Table 1. However, the major concern of this approach is the cost. With the cost for a Down syndrome birth avoided to be almost 10 times higher for cffDNA than the conventional screening [7], this screening approach could be an unaffordable burden for every health care system. Another consideration is the test failure rate of cffDNA testing. The reported rates for "no-call" results from the commercial companies vary between 2-6%. The main reason for the test failures is the low fraction of fetal DNA in the total amount of free DNA in the maternal circulation. The fetal fraction has to be higher than 10% optimally, and today all the main commercial companies include the fetal fraction in their results' report. As a secondary test, a contingent use of cffDNA test is more cost effective than the use as a primary test. With this approach, conventional 1st trimester

Figure 1 **Example model for contingent screening for Down syndrome**



NIPT: non-invasive prenatal tets, "no call": test could not be reported
Non-invasive prenatal testing may be best used in a contingent approach. In the example, combined first trimester screening is offered to all women as an initial screening tool. From this, women are stratified by risk to determine further management. Women with a high risk are offered an invasive test (chorionic villus sampling or amniocentesis). Women with a low risk are reassured and advised that no further testing is needed. Women with an intermediate level of risk are offered a non-invasive prenatal test. Contingent screening allows highest detection rate (in the example 97%) while reducing the false positive rate (in the example 1.4%).

Table 2 Brief summary of the responses received from some Mediterranean countries

Question	Slovenia	France	Greece	Turkey	Israel	Albania
Is screening regulated by low?	Yes	Yes	No	Yes	Yes	No
Is screening compulsory?	No	No	No	No	No	No
Is screening reimbursed?	Yes	Yes	Yes	Yes	Yes	No
Screening strategies						
1st trimester only	No	Yes	No	No	No	No
1st or 2nd trimester	Yes	-	Yes	Yes	Yes	Yes
cfDNA testing	Yes	Yes	Yes	Yes	Yes	Yes
cfDNA testing reimbursed?	No	No	No	No	No	No
Primary or secondary	Sec		Sec	Sec		
Cost of cfDNA testing (€)	~ 450		350-650	400-600	-	600-800
Invasive cytogenetic diagnosis						
Woman's age only	Yes	No	Yes	Yes	Yes	Yes
Screening results	Yes	Yes	Yes	Yes	Yes	Yes
Other indications (US, family)	Yes	Yes	Yes	Yes	Yes	Yes
US monitoring	Yes	Yes (3)	Yes (3)	Yes	Yes (3)	Yes

combined screening is offered to all women. To those women with a very high risk for all type of aneuploidy (e.g. >1:50), invasive prenatal diagnosis is offered.

To all other women, the result of the combined test could be used for counseling, giving them the choice of selecting either:

a) no further action with a result lower than e.g. 1:1000;
b) proceed with cffDNA testing or
c) having invasive diagnosis.

An option for this approach is depicted in Figure 1. (https://www.nps.org.au/australian-prescriber/articles/non-invasive-prenatal-testing-for-down-syndrome)

RECENT GUIDELINES FOR SCREENING

In recently published recommendations of the American College of Obstetrician and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine for screening for fetal aneuploidy (https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Genetics/Cell-free-DNA-Screening-for-Fetal-Aneuploidy), among others it is mentioned that:

- A discussion of the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients.
- *Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.*
- The cell-free DNA test will screen for only the common trisomies and, if requested, sex chromosome composition.
- Given the potential for inaccurate results and to understand the type of trisomy for recurrence-risk counseling, a diagnostic test should be recommended for a patient who has a positive cell-free DNA test result.
- Cell-free DNA screening is not recommended for women with multiple gestations.
- If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered rather than cell-free DNA screening.
- Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy.

THE SITUATION IN MEDITERRANEAN COUNTRIES

Trying to imprint the situation of prenatal screening for chromosomal abnormalities in the different Mediterranean countries, a questionnaire in cooperation with MZ Congressi, was send to the members of Scientific Committee and was uploaded as a survey (https://docs.google.com/forms/d/e/1FAIpQLSdu6i2dvWbeCbObkGToJV51V8C0A6vA7LYTu0JBPtr4UrL_TQ/viewform). Unfortunately, a limited number of responses was received (Slovenia, France, Greece, Turkey, Israel and Albania) (Table 2).

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