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Original Article

Cell-free DNA and contingent screening: Our first year

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ABSTRACT

Introduction: Analysis of cell-free DNA (cfDNA) from maternal blood has showed a great potential as a screening method for fetal aneuploidies. cfDNA can be used as a first line screening tool or in a contingent model, after the combined test.
Methods: Prospective study of women attending for first trimester combined screening in our Hospital, in the first year of contingent cfDNA screening. According to the combined screening test result patients were divided in high-risk (offered invasive test or routine follow-up), intermediate-risk (counselled for cfDNA, invasive or routine follow-up) or low-risk (routine ultrasound follow-up). Pregnancy outcomes and performance of screening were evaluated. A cost-effectiveness analysis was also done.
Results: The majority of the 1272 enrolled participants were Caucasian (82,6%), multiparous (51,7%) and the median maternal age was 30 years old. Thirty women screened high-risk and 83,3% of them opted for an invasive test. Forty-nine patients had an intermediate risk and 75,5% of them choose cfDNA testing. Our rate of invasive tests decreased from 3.5% to 2.4%.
Discussion: The cut-offs used to determine high and intermediate-risk are based on a compromise between detection rate, pregnancy lost rate and cost. Above a determined cut-off in the intermediate-risk group, the cost for each additional detected trisomy case is very high. One major benefit of this contingent model was the decrease in invasive testing.
Conclusion: The contingent cfDNA screening model can be easily implemented in a public hospital with a low-risk population. Since cost/benefit is an important issue, further studies are needed to determine the ideal cut-off for our country.

Introduction

First-trimester combined screening for fetal aneuploidies by a combination of maternal age, fetal nuchal translucency and serum levels of pregnancy-related proteins in maternal blood - β-human chorionic gonadotropin (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A) - can detect about 90% of fetuses with trisomy 21 and 95% of those with trisomies 18 or 13, at a false-positive rate (FPR) of about 5% [1–4].

The performance of this screening can be improved by the inclusion of other ultrasound first-trimester markers, as nasal bone, the index of pulsatility of the ductus venosus and the presence of tricuspid regurgitation, for a detection rate (DR) of 95% and a lower FPR [5].

After 10 weeks of pregnancy, about 3–13% of the total cell-free DNA in maternal circulation (cfDNA) has a fetal origin, mainly derived from the placenta. The concentration of fetal cfDNA increases with gestational age and is cleared from maternal circulation soon after delivery [6]. Noninvasive prenatal screening that uses cfDNA from the plasma of pregnant women has a tremendous potential as a screening method for fetal trisomies 21, 18 and 13 with a high sensitivity and specificity [1,2,7]. According to a recent meta-analysis of clinical validation studies the DRs for trisomies 21, 18 and 13 are 99%, 96% and 91%, respectively, with a FPR of 0.35% [3]. cfDNA is the most effective method of screening for trisomy 21. Since it is a screening and not a diagnostic test, the result is presented as low-risk (< 1: 10,000) or high-risk (>99%) [8]. A patient who screens high-risk should have a diagnostic test to confirm the result. A minimum amount of fetal cfDNA (3–4%) must be present in the sample of the maternal blood collected to obtain a viable test result [1]. An inadequate amount may occur in cases of obesity, suboptimal sample collection or early gestational age [9].

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The performance of screening for aneuploidies with cfDNA in multiple gestations has a lower DR and a higher failure rate than in singleton pregnancies [7,10].

Data on the performance of cfDNA testing in a low-risk population are similar to those previously published for a high-risk population [11,12]. However, given the lower prevalence of aneuploidies in the general obstetric population, the positive predictive value is lower and there will be more false positive results [13].

cfDNA screening is the most sensitive option and can be used potentially as a first line screening method for aneuploidies or in a contingent model, after the results of the combined test performed in the first trimester. Contingent screening has the advantages of combining a very high DR with a very low FPR and with a very low invasive testing rate, at a considerably lower cost than the universal application [2,3,7,8,14,15].

According to recent reviews a contingent screening model using the first trimester combined screening and cfDNA can have a DR of about 98% for fetuses with trisomy 21 and 96% for trisomy 13 and 18 and approximately 0.1% of FP, with an overall rate of <1% invasive diagnosis [1,5,7]. A contingent screening approach would also retain the advantages of the first trimester ultrasound examination: accurate pregnancy dating, early detection of major fetal defects and screening for obstetric complications [1,5,12]. Furthermore, most of the false positive and negative results obtained can be avoided if the "a priori" risk provided by the combined screening is taken into account in the interpretation of the individual risk [1,8,14,16].

Therefore, the objective of our study was to assess the performance of the introduction of cfDNA testing contingent on the results of the combined first trimester test that is currently done, one year after the implementation of a new screening protocol in our Hospital

Material and methods

Study design and participants

This is a prospective study that included all the women attending for first trimester combined screening in our Hospital, in the first year of implementation of contingent screening for aneuploidies using cfDNA (between March 2017 and February 2018).

At the first appointment, around 9–11 weeks, data was collected from all the pregnancies: demographic maternal characteristics, obstetric and medical history, mean arterial pressure (MAP) and plasma levels of β-HCG and PAPP-A. Subsequently, on a second appointment, all women had a first trimester scan, including the combined screening for aneuploidies, with assessment of nuchal translucency, nasal bone, ductus venosus flow and tricuspid regurgitation and their combination with the data collected at the first visit. Women diagnosed with a multiple pregnancy or a major fetal abnormality were excluded.

The risk for trisomy 21, 13 and 18 was calculated, using the software available in our Hospital (ViewPoint Version 5.6.12.601). Post-test counseling was undertaken.

Our protocol divided patients in high, intermediate and low-risk. Patients were classified as high-risk if the estimated risk was greater or equal to 1:100 in the combined screening. This group was counselled for invasive test or, if refused, routine ultrasound follow-up. Patients with an intermediate risk (defined as a risk between 1:100 and 1:500) had to choose between cell-free DNA test, invasive test or routine ultrasound follow-up. Low-risk patients (defined as a risk less than 1:500) were informed that fetal trisomies were unlikely and recommended to have routine ultrasound follow-up. The cut-offs were chosen based on the risk of fetal loss after an invasive test and a cost/benefit analysis.

Patient characteristics, results from combined, invasive or cfDNA testing and pregnancy outcomes were assessed and compiled in to a database. The performance of aneuploidies screening and the rate of invasive tests performed was evaluated. The outcomes were divided into normal or abnormal karyotype, according to prenatal or postnatal karyotyping in chorionic villi, amniotic fluid or neonatal blood. The cases without karyotyping were assumed normal if the neonate had a normal phenotype.

A cost-effectiveness analysis was done comparing the two screening strategies: before (first-trimester combined screening) and after the implementation of the new protocol (contingent screening with cfDNA). The invasive tests have 3 major associated costs: procedure (medical and nursing appointment, ultrasound monitoring), the hospitalizations due to complications of invasive testing and the cost of the karyotype analysis. The price associated with the first two was not available in our setting but were extrapolated from a previous publication from Spain (the National Health Service organization and costs are similar). [17]. Thus, we considered the procedure price 340 euros and 1500 euros the cost of hospitalization for 1 week due to complications of the invasive procedures. In our institution the price of the karyotype analysis is 525 euros and the cfDNA tests costs 370 euros. Regarding the rate of hospital admissions associated with invasive testing we used an estimation of 1%, inferred from a previous publication. [17].

Statistical analysis

Descriptive data were presented as mean and median (interquartile range (IQR)) for continuous variables and as n (%) for categorical variables. Comparisons between outcome groups were performed using $\chi 2$ test. Statistical significance was accepted at the level of $p < 0.05$.

Table 1
Maternal and pregnancy characteristics.

Maternal and pregnancy characteristics	n = 1272
Maternal age (years)	
Mean +/− SD	30,05 +/- 5,9
Median [range]	30 [14 – 46]
< 25 years – no. (%)	214 (16,8%)
25–35 years – no. (%)	725 (57%)
≥ 35 years – no. (%)	333 (26,2%)
Maternal BMI (Kg/m^2)	
Mean +/− SD	25,06 +/- 5,31
Median [range]	24 [15-53]
Underweight (< 18.5) - no. (%)	64 (5%)
Normal BMI (18.5–25) - no. (%)	612 (48,1%)
Overweight (25–30) - no. (%)	380 (29,9%)
Obese (30–40) – no. (%)	192 (15,1%)
Morbid obese (≥ 40) – no. (%)	24 (1,9 %)
Racial origin – no. (%)	
Caucasian	1051 (82,6%)
Afro-Caribbean	161 (12,7%)
South Asian	31 (2,4%)
East Asian	4 (0,3%)
Mixed	25 (2,0%)
Parity – no. (%)	
Nulliparous	614 (48,3%)
Multiparous	658 (51,7%)
Previous pregnancy with a chromosomal abnormality – no. (%)	3 (0,2%)
Conception – no. (%)	
Spontaneous	1256 (98,7%)
Ovulation drugs or IVF	16 (1,3%)

Results

During the study period, 1297 pregnant women were screened. Twin pregnancies (22 cases) and pregnancies with major abnormalities (3 cases) were excluded. Maternal and pregnancy characteristics are summarized in Table 1.

Table 1 describes the detailed maternal and pregnancy characteristics of this population.

Of the 1272 enrolled participants, the majority were Caucasian (n = 1051, 82.6%) and the median of maternal age was 30 years (range 14–46). In the study population 333 women (26,2%) were 35 years or older. The mean maternal Body Mass Index (BMI) was 25.06 Kg/m^2 which corresponds to normal weight, but 192 women (15.1%) were obese and 1.9% (n = 24) had morbid obesity (BMI $\geq$ 40 Kg/m^2). As to parity, 658 women (51.7%) were multiparous and conception was spontaneous in 1256 cases (98.7%). Only 3 women (0.2%) had a previous pregnancy with a chromosomal abnormality.

Most patients (93.8%) had a low-risk combined test, 2,4% had a high-risk combined test and 3,8% had an intermediate risk (Fig. 1).

Table 2 describes pregnancy outcomes.

Table 3 describes detailed cases of chromosomal anomalies.

Thirty patients were classified as high-risk: 26 cases high-risk for trisomy 21 and 4 cases for trisomies 13/18. In 25 cases (83.3%) of this high-risk group the patients chose to have an invasive test. The other 5 high-risk cases decided to have routine ultrasound follow-up. The fetal karyotype was abnormal in 7 cases (28%): four cases of trisomy 21, one trisomy 18, one mosaicism 18/21 and one triploidy.

In terms of outcome, in the 7 cases with an abnormal karyotype 6 decided for termination, and one case of trisomy 21 the patient opted to continue the pregnancy, resulting in a live birth. In pregnancies with a normal karyotype, there were 16 normal live births, one miscarriage at 16 weeks (fetal hydrops and suspected neural tube defect) and a termination (fetal hydrops with post-natal diagnosis of Lethal multiple pterygium). The 5 cases that decided for routine follow-up all were live births, one newborn with trisomy 21 and the other four without aneuploidies identified.

In the intermediate risk group (49 patients), women opted for a cfDNA test in 37 cases (75.5%), 6 cases opted for an invasive test and the other 6 patients choose routine ultrasound follow-up. In all the cases that cfDNA test was performed there were no failed results and all results were low risk (< 1: 10,000). All cases resulted in a normal live birth except one termination at 23 weeks by agenesis of the corpus callosum with a normal karyotype. In the patients who chose invasive testing there was one abnormal karyotype (69, XXX), and the pregnancy ended as a miscarriage at 17 weeks. In the other five cases, all with normal karyotype, the outcomes were normal live births. The 6 cases that decided for routine follow-up, one pregnancy ended with a fetal death at 27 weeks (severe fetal growth restriction and preeclampsia) and the other cases resulted in a normal live birth.

In the low risk group (1193 cases), there were 1167 normal live births, 2 cases of newborns with trisomy 21 (without pre-natal diagnosis), 3 miscarriages, one fetal death at 39 weeks (without known cause) and 4 terminations of pregnancy (two for preterm premature rupture of membranes previous to viability, one at 24 weeks for cerebellar hypoplasia and one at 21 weeks for fetal bilateral renal agenesis and anhydramnios). There were 16 cases lost to follow-up.

We calculated the total cost of the two strategies considering a population of 10,000 pregnant women (Fig. 2). According to our data the rate of invasive tests was 3,52% with the first strategy and 2,44% with the contingent screening model.

Based on these results we can estimate that the contingent model has an increment cost of 1,25 euros for each women screened.

Discussion

Our obstetric population is considered low-risk for fetal aneuploidies, since we are not a referral hospital, in contrast to the majority of the published studies [8,12,13]. However, several studies have shown that fetal cfDNA performance is similar in the low and high-risk obstetric population, and continues to show higher sensitivity and specificity when compared with other screening tests. The positive and negative predictive values will depend on the prevalence of aneuploidy in the population [4,11,18].

The risk cut-offs used in our study were based in previously published reports. In the terms of the high-risk group, the cut-off most commonly used is 1:100, comparable to the traditionally

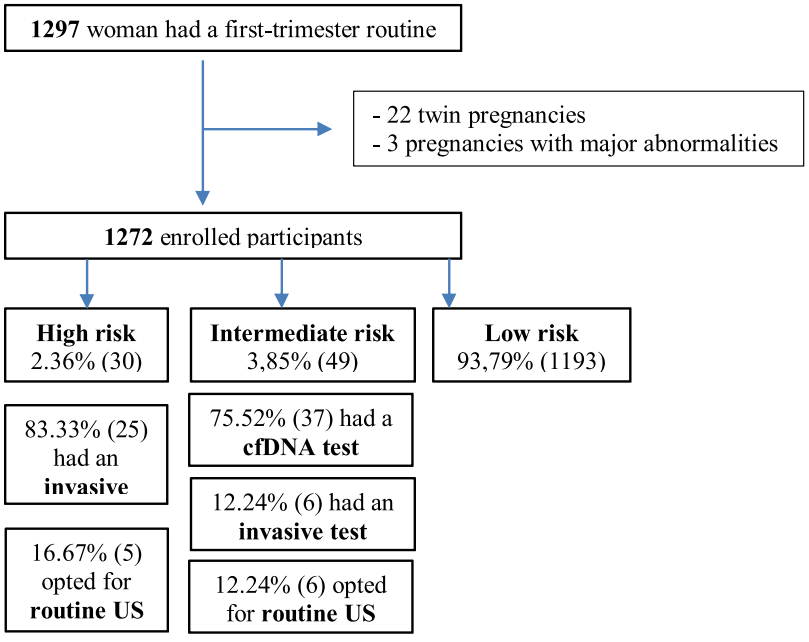

Fig. 1. Population selection and screening results.

Table 2
Pregnancy outcomes.

	High risk (n = 30)	Intermediate risk (n = 49)	Low risk (n = 1193)
Invasive tests	25	6	-
Abnormal Karyotype	**8**	**1**	**2**
Miscarriage	0	1	2
Termination	6	0	0
Live birth	2	0	2
Normal/Unknown Karyotype	**22**	**48**	**1191**
Miscarriage	1	0	3
Termination	1	1	4
Live birth	20	46	1167
Fetal death	0	1	1
Lost outcome	–	–	16

Table 3
Detailed cases of chromosomal anomalies.

	Risk of Trisomy 21	Risk of Trisomy 13/18	Invasive procedure	Prenatal karyotype	Pregnacy outcome	Postnatal karyotype
High risk group						
Case 1	1:23	1:1380	Yes	47, XX, +21	TOP	
Case 2	1:3	1:193	Yes	47, XX, +21	TOP	
Case 3	1:2	1:18	Yes	47, XY, +21	Live birth	
Case 4	1:2	1:5	No	No	Live birth	47, XX, +21
Case 5	1:2	1:2	Yes	47, XY, +21	TOP	
Case 6	1:16	1:2	Yes	47, XX, +18	TOP	
Case 7	1:110	1:40	Yes	69, XXX	TOP	
Case 8	1:9	1:134	Yes	48, XX, +18, +21(16) /47,XX,+21	TOP	
Intermediate risk group						
Case 1	1:4813	1:218	Yes	69, XXX	Miscarriage	
Low risk group						
Case 1	1:555	1:2052	No	No	Live birth	47, XY, +21
Case 2	1:5397	1:11010	No	No	Live birth	47, XX, +21

described rate of miscarriage associated with invasive testing [19]. However, more recent reports found a procedure-related risk of miscarriage following invasive testing around 0,1%- 0,2%, much lower than previously described [20–23]. One of the potential advantage of increasing the cut-off to offer invasive test, for example to all cases with a risk more than 1:300, is the detection of other chromosomal abnormalities that would not be diagnosed if women choose to have cfDNA. In our series there was a triploidy in the intermediate risk group. However, this advantage is countered by the possible doubling of the number of invasive procedures performed to obtain the diagnosis of only one case of chromosomal anomaly. This might result in an increased rate of miscarriage and higher cost [24–26].

The upper limited in the cut-off used to determine the intermediate-risk is a compromise between DR and cost. In some of the most cited studies, the upper limited to consider intermediate-risk and offer the cfDNA was 1 in 2500. The authors estimated that with such policy they would offer cfDNA testing in about 25% of the population and would improve the DR of trisomy 21 to about 97% of cases and of trisomies 13 and 18 to about 95% [3,8,12,27].

In our data the performance of the combined screening test was similar to previous studies [2,3]. In Table 4 we can see de distribution of the abnormal cases, according to the screening risk. Considering high-risk at a cut-off more than 1:100 6 of the 8 cases of T21 would be identified, which corresponds a DR of 75% with a FPR of 1,5%. The improvement of the DR to 87,5% would only be possible using a cutt-off of 1:1000, but at the expense of a high FPR of 8%.

With cost/benefit in mind, we decided in our study protocol to set the intermediate risk between 1:100 to 1:500. It has been noted that, above a determined cut-off, the cost for each additional detected case of trisomy is high. As evidenced by previous publications on this topic, extending the limit for intermediate risk from 1:500 to 1:1000 provides an improvement in test sensitivity but decreases test specificity and increases the rate of false positive, with a lower positive predictive value for an equal negative predictive value [13,27]. Another study demonstrated that to increase the DR of

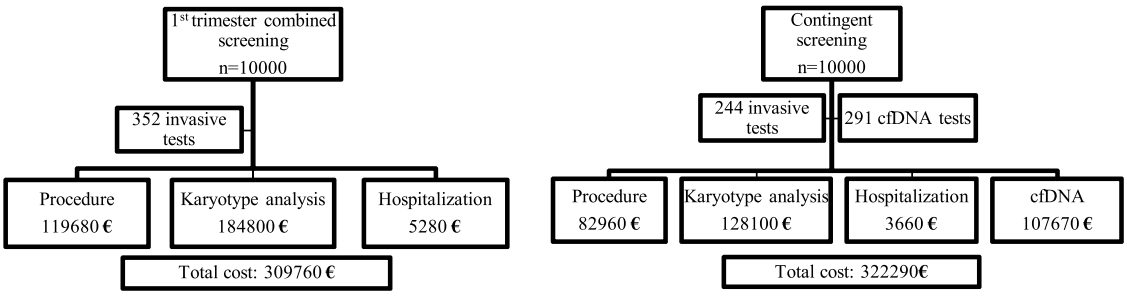

Fig. 2. Total costs of the routine first trimester combined screening and contingent screening with cfDNA.

trisomy 21 it would be necessary to increase the intermediate risk cut-off to grater or equal than 1:3500 to identify a new case of trisomy 21 [28]. The increment of the cut-off to 1:1000 or to 1:3500 would mean that we would probably need to offer cfDNA to around 8% or 35% of the population screened, respectively, in contrast to 4% (cut-off of 1:500) [13,27,28].

As such, we considered that our cut-off has a good cost/benefit ratio and the additional cost implied in higher cut-offs was not justified in our low risk population.

In this study, in the pregnancies considered to be at intermediate risk for trisomies 21, 13 or 18 by the combined test, cfDNA were all true negatives, with a FPR of 0%.

The 2 cases of trisomy 21 with only post-natal diagnosis had a low risk in the combined first-trimester test (Case 1: Risk for Trisomy 21 1:555; Case 2: Risk for Trisomy 21 1:5397). These cases would also have been missed with the exclusive application of the combined test. If it's true that they could have possibly been detected with a larger intermediate risk cut-off, but to detect both cases it would be necessary to extended the intermediate risk group immensely. As previously discussed, the increased of the intermediate risk cut-off would lead to a higher rate of false positive, with an expected higher rate of invasive test and a much higher cost, probably too high to be accepted in our clinical setting.

One major benefit of this newly implemented practice was the decrease of invasive testing. It is well established that invasive tests have costs, risks (namely fetal loss) and add additional stress to pregnant women. Prior to the introduction of cfDNA testing in our hospital, high-risk was defined as a risk of aneuploidies greater or equal to 300, and invasive testing was offered to these women. Our rate of invasive tests was 3.52%. After the implementation of the contingent model, and even with the expanded limit off 1:500, 2.44% of participants decided to have an invasive test. The offer of cfDNA testing to the intermediate-risk pregnancies, although not statistically significant, was associated with a reduction in the rate of invasive testing (2.44% vs 3.52%, p = 0.086).

A screening test has to be cost effective and the high cost of cfDNA screening can be one of the greatest challenges of its routine use in clinical practice. Several reports showed that, when compared with first-trimester combined screening, contingent screening with cfDNA could improve the overall performance of prenatal screening without a significant increase in total cost [17,24–26]. We carried out a simplistic cost-effectiveness analysis, only considering the direct healthcare costs associated with screening. We compared an estimation of the cost of two screening strategies: before vs after the implementation of the new protocol. Considering that the cost of the screening program is the same in the two strategies, the major differences are the rate of invasive tests with its associated costs and the price of the cfDNA test. We found that, when pondering a screening population of 10,000 pregnant women, the contingent model has an increment cost of only 1,25 euros for each women screened. However, given the latest developments, this increase cost may eventually cease to be an issue. In the last years, the widespread use of this type of tests and the multiple offers by different companies have allowed a significant reduction on their price. On the other hand, the greater the number of women screened the less will be the increment. This is due to the reduction of the invasive diagnostic tests and its respective reduction in the total cost of the screening program [29,30].

Table 4
Distribution of risk from the combined test according to karyotype.

Risk cut-off point	Trissomy 21 n = 8	Trissomy 18 n = 1	Normal n = 1239
≥ 1:100	6	1	19
≥ 1:500	6	1	56
≥ 1:1000	7	1	99
≥ 1:2500	7	1	208
≥ 1:5000	7	1	401
≥ 1:10,000	8	1	659

Data are given as *n*.

The major strengths of our study are the fact that it is a prospective evaluation, the screening model is in line with the more recent evidence available and, as far as we know, is the first study assessing the performance of a contingent screening model for aneuploidies using cfDNA done in a public hospital in our country.

The major limitation of our study was the limited number of patients enrolled and the small number of trisomic pregnancies for significant conclusions. We aim to continue using this contingent model in the future, obviating this limitation. The inability to obtain karyotype confirmation for all the pregnancies that ended in spontaneous miscarriage, intrauterine fetal demise or termination, may have led to an underestimation of the total number of aneuploidies. The cost-effectiveness analysis done is limited because there is no reliable available data in our hospital on all the costs that need to be considered.

Conclusion

In conclusion, our study demonstrated that the introduction into clinical practice of a screening model by fetal cfDNA analysis of maternal blood, contingent on the results of the first trimester combined screening can be easily implemented in a public hospital with a low-risk population. This model improves the performance of screening for trisomies, decreased the rate of invasive tests and retains all the advantages of the first trimester combined test without a considerable increased in the total cost of screening.

Since cost/benefit is an important issue from a public health perspective, further studies are needed to know what is the ideal cut-off to offer cfDNA test in our country.

Conflict of interests

Authors have no competing interests to declare.

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