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Clinical and Economic Evaluation after Adopting Contingent Cell-Free DNA Screening for Fetal Trisomies in South Spain

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Keywords
Cell-free DNA · Contingent screening · Combined test · Fetal trisomy · First-trimester screening · Prenatal diagnosis

Abstract
Introduction: Contingent cell-free (cf) DNA screening on the basis of the first-trimester combined test (FCT) results has emerged as a cost-effective strategy for screening of trisomy 21 (T21). ***Objectives:*** To assess performance, patients' uptake, and cost of contingent cfDNA screening and to compare them with those of the established FCT. ***Methods:*** This is a prospective cohort study including all singleton pregnancies attending to their FCT for screening of T21 at 2 university hospitals in South Spain. When the FCT risk was ≥1:50, there were major fetal malformations, or the nuchal translucency was ≥3.5 mm, women were recommended invasive testing (IT); if the risk was between 1:50 and 1:270, women were recommended cfDNA testing; and for risks bellow 1:270, no further testing was recommended. Detection rate (DR), false-positive rate (FPR), patients' uptake, and associated costs were evaluated. ***Results:*** We analyzed 10,541 women, including 46 T21 cases. DR of our contingent strategy was 89.1% (41/46) at 1.4% (146/10,541) FPR. Uptake of cfDNA testing was 91.2% (340/373), and overall IT rate was 2.0%. The total cost of our strategy was €1,462,895.7, similar to €1,446,525.7 had cfDNA testing not been available. ***Conclusions:*** Contingent cfDNA screening shows high DR, low IT rate, and high uptake at a similar cost than traditional screening.

Introduction

In singleton pregnancies, first-trimester combined test (FCT) for screening of trisomies 21, 18, and 13 using a combination of maternal age, fetal nuchal translucency (NT) thickness, and serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma

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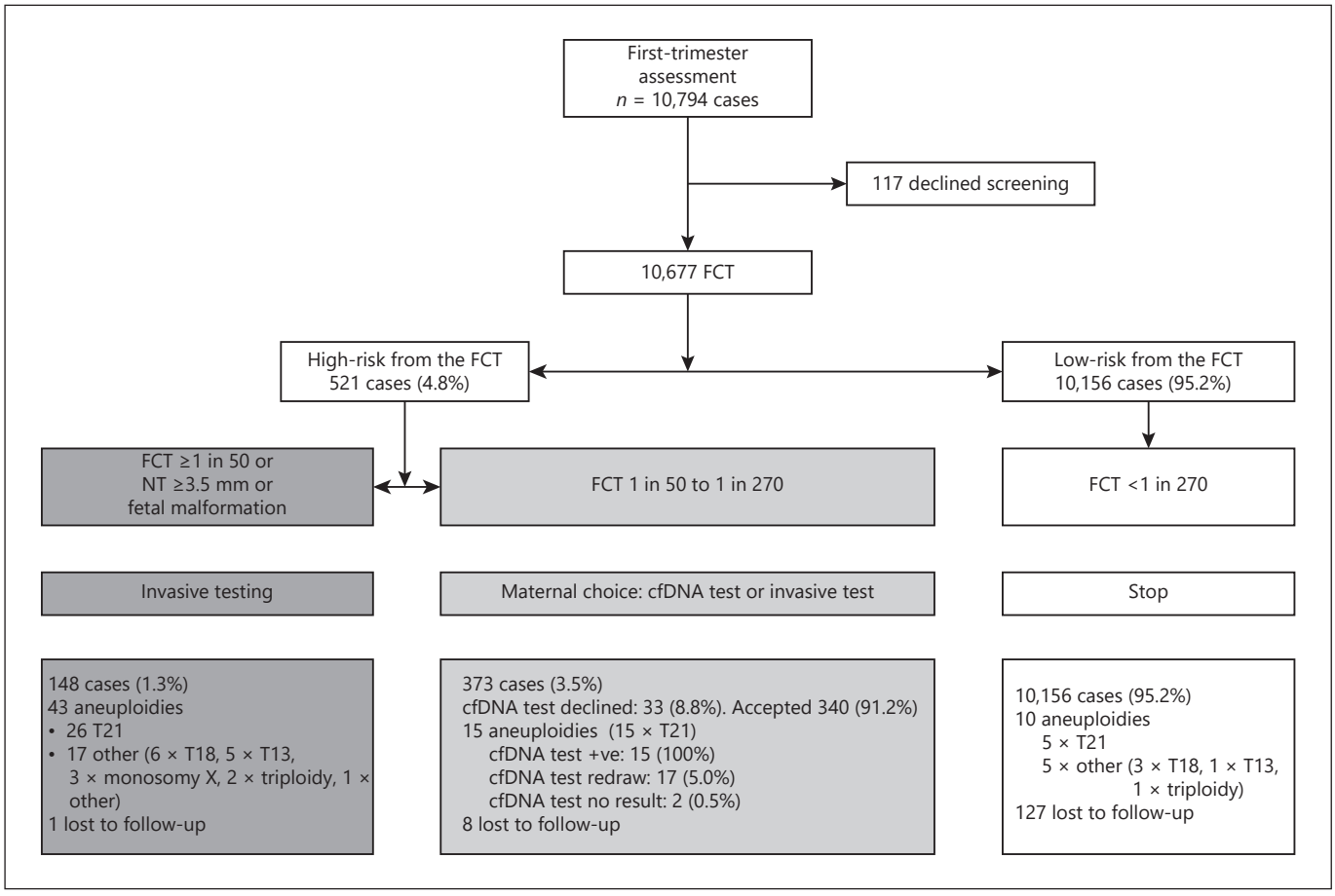

Fig. 1. Protocol for pregnancy management according to the results of the FCT, 19–21 weeks' anomaly scan, and maternal blood cfDNA test. FCT, first-trimester combined test; cfDNA, cell-free DNA; NT, nuchal translucency; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13.

protein A (PAPP-A) has a detection rate (DR) of about 90% for trisomy 21 and about 95% for trisomies 18 and 13, at an overall false-positive rate (FPR) of 5% [1]. This method of screening is the first-line screening for aneuploidies in many countries in Europe, including Spain. Recently, analysis of cell-free (cf) DNA of maternal blood has been incorporated in clinical practice, providing effective screening for the major trisomies as early as 10 weeks' gestation [2]. A recent meta-analysis of clinical validation and implementation studies reported that the DR of cfDNA testing for trisomies 21, 18, and 13 is 99.7, 97.9, and 99.0%, respectively, at an FPR of 0.12 [3]. Therefore, since cfDNA testing is highly effective in screening for trisomies and it only involves the simple taking of a maternal blood sample, it could be argued that universal cfDNA screening should be introduced in routine clinical practice. However, such an approach is still limited by the higher cost of the test in comparison with the traditional FCT. Over the last few years, several studies have been published assessing the economic impact of different strategies for implementing cfDNA testing in health care systems [4–6]. An alternative strategy to universal screening is to offer cfDNA testing contingent on the results of another method of screening used as first-line screening, preferably the FCT. By this approach, only women at high and/or intermediate risk would be offered cfDNA testing and, therefore, it would still be possible to retain the main advantage of the test in terms of early results and high performance, but the cost of such a screening program would be considerably lower [7–9]. This strategy would also allow retaining the advantages of the first-trimester combined assessment such as pregnancy dating, early detection of major defects, and prediction and potential prevention of a series of pregnancy complications [10].

In Spain, like in many countries in Europe, screening for trisomy 21 is carried out by the FCT, both in private and public settings. However, unlike other European countries, screening for trisomy 21 is not yet part of the Spanish National Screening Programs and, therefore, there is no regulation to coordinate and monitor it. Recently, the Spanish Society of Gynecology and Obstetrics (Sociedad Española de Ginecología y Obstetricia; SEGO) has updated its guidelines to incorporate cfDNA testing as a screening option, and current recommendation is universal screening by the FCT followed by contingent cfDNA testing for risks between 1 in 50 and 1 in 250 [11]. The aim of this recommendation is to reduce the rate of invasive procedures without modifying DR or increasing the cost. Some Spanish public hospitals have already reported their experience with this approach [9] but, to the best of our knowledge, no economic evaluation after implementation has yet been published. The objectives of our study are first to analyze the influence of implementation of cfDNA contingent screening in the global performance of screening, second to assess patients' acceptability of the cfDNA test, and third to evaluate the difference in costs after implementing this new screening strategy.

Materials and Methods

The data for this study were derived from prospective screening for trisomy 21 at 11–13 weeks' gestation by contingent cfDNA testing on the basis of the results from the FCT. All women with singleton pregnancies attending to their first-trimester hospital visit at one of 2 university hospitals in South Spain (Hospital Universitario de Valme in Seville and Hospital Juan Ramón Jiménez in Huelva) from March 2016 to March 2018 were included. Ethics approval was obtained from the Local Research Ethics Committee (0109-N-16).

Clinical Implementation of cfDNA Contingent Screening for Trisomy 21

In the 2 participating hospitals, the FCT is routinely performed at 11–13 weeks' gestation. During the first-trimester scan, we confirm number of fetuses, check viability, diagnose major fetal defects, and measure crown-rump length (CRL) for pregnancy dating [12] and fetal NT. These measurements are combined with maternal age and maternal serum concentrations of free β-hCG and PAPP-A measured at 9–12 weeks' to calculate the patient-specific risk for trisomy 21 [1]. If the risk is >1 in 270, the mother is explained that her risk for trisomy 21 is low and she is booked for another scan at 19–21 weeks' gestation to examine fetal anatomy. If the risk is between 1 in 50 and 1 in 270, she is classified as high risk and given the options of invasive testing (chorionic villus sampling or amniocentesis) or cfDNA testing. Finally, if the risk is >1 in 50 or if there is any major fetal malformation or the fetal NT is ≥3.5 mm, the mother is explained that not only the risk of trisomy 21 is increased but also that of other chromosomal and subchromosomal abnormalities and, therefore, she is advised to have an invasive test with array analysis (Fig. 1).

Table 1. Maternal and pregnancy characteristics of the study population

Maternal characteristics	
Maternal age in years; median (IQR)	32.0 (28.5–35.1)
Gestational age in weeks; median (IQR)	12.6 (12.2–13.1)
Maternal weight in kg; median (IQR)	66.74 (59.0–74.4)
Body mass index in kg/m^2; median (IQR)	25.0 (23.4–29.1)
Racial origin	
Caucasian ethnicity; *n* (%)	9,950 (93.2)
North African; *n* (%)	611 (5.73)
Afro-Caribbean; *n* (%)	80 (0.73)
East Asian; *n* (%)	36 (0.32)
Cigarette smoking; *n* (%)	3,064 (28.7)
Diabetes mellitus on insulin; *n* (%)	1,494 (1.4)
Assisted conception; *n* (%)	4,057 (3.8)
Pregnancy characteristics	
CRL, mm; median (IQR)	61.1 (56.4–66.1)
NT, mm; median (IQR)	1.3 (1.1–1.7)
Delta NT; median (IQR)	0.9 (0.8–1.1)
Free β-hCG (MoM); median (IQR)	1.2 (0.85–1.58)
PAPP-A (MoM); median (IQR)	1.1 (0.75–1.45)
cfDNA test results	
Fetal fraction in %; median (IQR)	10.9 (6.8–14.5)
cfDNA test with no result from first draw; *n* (%)	17 (5.0)
cfDNA test with no result after second draw; *n* (%)	2 (0.5)

CRL, crown-rump length; NT, nuchal translucency; free β-hCG, serum free β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein A; cfDNA, cell-free DNA.

Women opting for cfDNA testing provided written informed consent, and maternal blood (20 mL) was collected into Roche Cell-Free DNA Collection Tubes (Roche, Pleasanton, CA, USA). The tubes were shipped without any processing to the cfDNA laboratory in Madrid, Spain. Targeted cfDNA testing for fetal trisomy was performed using the Harmony® prenatal test. In brief, Harmony® uses digital analysis of selected regions (DANSR) assays targeting sequences on chromosomes 13, 18, and 21 for chromosome quantitation and single nucleotide polymorphisms on chromosomes 1–12 for fetal fraction measurement. Products of the DANSR assays are quantified using a custom microarray. The FORTE (Fetal fraction Optimized Risk of Trisomy Evaluation) algorithm is used to include fetal fraction in data analysis and provide patient-specific risk assessments for trisomy [13]. A risk of ≥1% is considered to be high probability. In the study sites, women receiving a low-risk result are reassured that trisomies are unlikely and they are booked for anomaly scan at 19–21 weeks. However, women receiving a high-risk result are advised to consider invasive testing for prenatal diagnosis. For the cases where the cfDNA test does not provide results, women are offered a second draw and for those cases without results from second analysis, they are advised to have invasive testing (Fig. 1).

Table 2. Clinical management of aneuploidies according to the study protocol of contingent cfDNA screening

	Results from contingent cfDNA screening					
	group with FCT risk ≥1/50 or NT ≥3.5 mm or fetal malformation		high-risk group in the FCT (1 in 50 to 1 in 270) without increased NT or fetal malformation		low-risk group in the FCT (<1 in 270) without increased NT or fetal malformation	
Aneuploidies	trisomy 21	others	trisomy 21	others	trisomy 21	others
N	26	17	15	0	5	5
Description		6× trisomy 18, 5× trisomy 13, 3× monosomy X, 2× triploidy, 1× other	cfDNA test +ve: 15 cases	–	–	3× trisomy 18, 1× trisomy 13, 1× triploidy
Identified by FCT >1 in 50	13	4 (2× trisomy 18, 1× trisomy 13, 1× triploidy)				
Identified by NT >3.5 mm	9	6 (2× trisomy 18, 3× monosomy X, 1× triploidy)				
Fetal malformation	4 (2 atrioventricular septal defects, 1 posterior fossa malformation, 1 ventricular septal defect + hypoplastic left heart + club foot)	7 (2 trisomy 18: 1 posterior fossa malformation, 1 omphalocele + ventricular septal defect; 4 trisomy 13: 1 holoprosencephaly + clenched hand, 1 cleft lip + posterior fossa malformation, 1 hypoplastic right heart + holoprosencephaly, 1 hypoplastic left heart + hydrops fetalis), 1 trisomy 16 (fetal micrognathia + complex cardiac malformation)	–	–	1 atrioventricular septal defect	5 (3 trisomy 18: 1 ventriculomegaly + clenched hand, 1 IUGR + ventricular septal defect + clenched, 1 hypoplastic left heart + hydrops fetalis), 1 trisomy 13 (1 spina bifida + ventricular septal defect), 1 triploidy (IUGR + ventricular septal defect)
Evolution	26 TOP	17 TOP	15		1 TOP, 4 live births	5 cases TOP

cfDNA, cell-free DNA; IUGR, intrauterine growth restriction; TOP, termination of pregnancy; NT, nuchal translucency; FCT, first-trimester combined test.

Performance of Screening

DR and FPR with their confidence intervals (CIs) were calculated for both, cfDNA contingent screening and traditional FCT. Different cutoffs were explored to estimate performance of the contingent strategy when the group offered cfDNA testing is increased.

Pregnancy Outcome

Pregnancy outcome was ascertained by 2 methods: first, prenatal or postnatal karyotyping, and second, neonatal examination by a qualified physician within the first 3 days of the newborn's life. Cases raising any suspicion were followed up at least 6 months after birth. Cases lost to follow-up, including those ending up in miscarriage or stillbirth without karyotyping, were excluded.

Economic Assessment

We performed short-term economic analysis including all procedures carried out until delivery. For this analysis, we took into account only direct costs, including tests and procedures performed during pregnancy and delivery, as established by the public health system of Andalucía, Spain [14], except for the case of the cfDNA test which was externalized to a private laboratory.

Statistical Analysis

Descriptive data were expressed as median and interquartile range (IQR) and in proportions (absolute and relative frequencies). Comparisons between treatment groups were performed by the Mann-Whitney U test or 2-tailed χ^2 test as appropriate.

The statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses.

Results

Study Population

During the study period, a total of 10,794 women attended their first-trimester hospital visit at one of the 2 participating hospitals. 10,677 (98.5%) of those accepted FCT for screening of trisomy 21,117 (1.1%) declined screening, and 136 were lost to follow-up. Maternal and pregnancy characteristics as well as results from the FCT and cfDNA test when performed are shown in Table 1. In the study population, there were 68 (0.62%) chromosomal abnormalities, including 46 cases of trisomy 21 (0.43%).

Performance of Contingent Screening

Following our strategy, we detected 41 (89.1%; 95% CI: 77.0–95.3) of the 46 trisomy 21 cases and 58 (85.3%; 95%

CI: 75.0–91.8) of the 68 cases of other aneuploidies at 1.4% (146/10,541; 95% CI: 1.2–1.6) invasive testing rate in the first trimester of pregnancy. After including the results from the 19–21 weeks' anomaly scan, we increased the DR for trisomy 21–91.3% (42/46; 95% CI: 79.7–96.6) and for the other aneuploidies diagnosed before or after birth, to 94.1% (64/68; 95% CI: 85.8–97.7). Had cfDNA testing not been available, FCT alone would have also detected 89.1% (41/46; 95% CI: 77.0–95.3) of the trisomy 21 cases but at 4.3% (457/10,541; 95% CI: 4.0–4.7) FPR.

Performance of cfDNA Testing Alone

In total, we carried out 340 cfDNA tests. We did not get a result after the first draw in 17 (5.0%) cases, but after repeating the test in all 17 cases, only 2 (0.6%) cases were left without a result. The cfDNA test detected all 15 cases of trisomy 21 with no false positives.

Women's Preferences on Clinical Management (Table 2)

The screening protocol of our hospitals is shown in Figure 1. In 148 (1.4%) cases we detected any major fetal malformation, fetal NT was ≥3.5 mm or the FCT risk was ≥1 in 50. In this first group, there were 43 aneuploidies, including 26 cases of trisomy 21. 145 (98.0%) women chose to have an invasive test, but 3 (2.0%) opted against it and had cfDNA testing instead. Among the women having invasive testing, there was 1 miscarriage at 16 weeks' gestation. In 373 (3.5%) cases, the FCT risk was between 1 in 50 and 1 in 270 without major fetal malformations or increased NT. In this second group, there were 15 cases of trisomy 21. 340 (91.2%) women in this group chose to have cfDNA testing, 30 (8.0%) chose to have invasive testing, and 3 (0.8%) women decided not to have any further testing. In this group, there were 2 miscarriages at 13 and 20 weeks, respectively. In 10,156 (95.1%) cases, the FCT risk was <1 in 270 without major fetal malformations or increased NT. In this third group, there were 10 aneuploidies, including 5 cases of trisomy 21 (1 case of spontaneous miscarriage). In total, we performed 210 (2.0%) invasive procedures: 145 in the first group, 30 in the second group, and 35 in the third one.

Of the 17 cases that did not receive a result after the first attempt, all women decided to repeat the test. The two women that did not get a result from the second draw decided not to do more studies and follow the usual pregnancy care.

Cost Analysis

Cost of screening of trisomy 21 by our cfDNA contingent strategy was estimated in €1,462,895.7, and cost/

Table 3. Economic evaluation of the traditional strategy for screening of aneuploidies by the FCT and the new strategy by contingent cfDNA screening following the recommendations from the Spanish Society of Obstetrics and Gynecology

Screening strategy	Cases, *n*	Screen positive cases by the FCT	Cases having cfDNA testing	Cases having invasive testing	DR in the first trimester	DR after FCT and 19–21 weeks' anomaly scan	FCT cost (120 € per test)	Invasive testing cost (260 € per test)	cfDNA testing cost (310 € per test)	TOP cost (323.84 € per procedure)	Total cost	Cost/ effectiveness
FCT alone	10,677	521 (4.8%)	–	556*	58/68 (85.2%)	64/68 (94.1%)	1,281,240 €	144,560 €*	0 €	20,725.76 €	1,446,525.76 €	22,601.9 €
Contingent cfDNA	10,677	521 (4.8%)	343	210*	58/68 (85.2%)	64/68 (94.1%)	1,281,240 €	54,600 €*	106,330 €	20,725.76 €	1,462,895.76 €	22,857.4 €

cfDNA, cell-free DNA; FCT, first-trimester combined test; DR, detection rate; TOP, termination of pregnancy. * 35 extra cases of invasive testing following the 19–21 weeks' anomaly scan.

Table 4. Evaluation of performance and costs of possible cfDNA testing contingent models based on different first-trimester combined test risk cutoffs (10,541 cases)

Screening strategy	High-risk cases (FCT ≧1/50 or NT ≧3.5 mm or major fetal malformation)	Intermediate-risk cases	Low-risk cases	FPR, %	1st trimester DR of T21	1st trimester DR of other aneuploidies	Combined 1st and 2nd trimester DR of T21	Combined 1st and 2nd trimester DR of other aneuploidies	Total cost	Cost/effectiveness	Increase in total cost of the strategy
FCT alone	521 (4.3%)	–	10,020 (95.2%)	4.8	41/46 (89.1%)	58/68 (85.2%)	42/46 (91.3%)	64/68 (94.1%)	1,446,525.76 €	22,601.9 €	–
Contingent cfDNA: group 1 in 50 to 1 in 270	148 (1.3%)	373 (3.5%)	10,020 (95.2%)	1.4	41/46 (89.1%)	58/68 (85.2%)	42/46 (91.3%)	64/68 (94.1%)	1,462,895.76 €	22,857.5 €	1.2%
Contingent cfDNA: group 1 in 50 to 1 in 500	148 (1.3%)	694 (6.5%)	9,699 (92.2%)	7.8	43/46 (93.4%)	63/68 (92.6%)	43/46 (93.4%)	65/68 (95.5%)	1,564,899.60 €	24,075.3 €	8.1%
Contingent cfDNA: group 1 in 50 to 1 in 1,000	148 (1.3%)	1,473 (13.9%)	8,920 (84.8%)	15.2	43/46 (93.4%)	63/68 (92.6%)	43/46 (93.4%)	65/68 (95.5%)	1,796,159.60 €	27,633.2 €	24.1%
Contingent cfDNA: group 1 in 50 to 1 in 1,500	148 (1.3%)	1,868 (17.7%)	8,525 (81.0%)	19.0	44/46 (95.8%)	65/68 (95.5%)	44/46 (95.8%)	66/68 (97.0%)	1,914,603.44 €	27,633.2 €	32.3%
Contingent cfDNA: group 1 in 50 to 1 in 2,000	148 (1.3%)	2,252 (21.3%)	8,141 (77.4%)	22.6	45/46 (97.8%)	67/68 (98.5%)	45/46 (97.8%)	67/68 (98.5%)	2,029,007.28 €	29,009.1 €	38.8%
Contingent cfDNA: group 1 in 50 to 1 in 2,500	148 (1.3%)	2,626 (24.9%)	7,767 (73.8%)	26.2	45/46 (97.8%)	67/68 (98.5%)	45/46 (97.8%)	67/68 (98.5%)	2,140,607.28 €	31,949.3 €	47.9%
Contingent cfDNA: group 1 in 50 to 1 in 3,000	148 (1.3%)	3,213 (30.4%)	7,180 (68.3%)	31.7	46/46 (100%)	68/68 (100%)	46/46 (100%)	68/68 (100%)	2,315,771.15 €	34,055.4 €	60.1%

Cost evaluation includes (1) cost of the FCT (120 € per test); (2) cost of invasive testing (310 € per procedure); (3) cost of cfDNA testing (310 €); and (4) cost of termination of pregnancy (323.84 € per procedure). cfDNA, cell-free DNA; FCT, first-trimester combined test; NT, nuchal translucency; DR, detection rate; FPR, false-positive rate.

effectiveness was estimated in €22,857.4 (Table 3). If cfDNA testing had not been available, the estimated cost and cost/effectiveness would have been similar (€1,446,525.7 and €22,601.9, respectively). Therefore, implementation of cfDNA testing contingently after the FCT only resulted in a marginal 1.1% increase in the total cost of the program.

Performance of Different Strategies of Contingent cfDNA Testing

We finally evaluated performance and associated costs of contingent screening at different cutoffs from FCT, and results are reported in Table 4.

Discussion

Main Findings of the Study

In this study, we found that first, within our public health system, a strategy in which cfDNA testing is implemented contingently after the FCT is accepted by 91.2% of the women; second, our contingent strategy allows reducing the invasive testing rate from 4.2 to 1.4% for the same DR of about 90%; and third, this strategy can be implemented at a similar cost than traditional screening.

Comparison with Previous Studies

Our results are consistent with those from previous studies, which showed that contingent screening of aneuploidies by the FCT and cfDNA test is feasible and well accepted by patients [7–9, 15, 16]. The first study reporting on the performance of this contingent strategy used a cutoff of 1 in 2,500 from the FCT to offer cfDNA testing [15]. The authors reported that although this cutoff could potentially increase the DR up to 97% for trisomy 21 and up to 95% for trisomies 18 and 13, it would require that about 24% of the screened population had cfDNA testing [17]. Similarly, had we offered cfDNA testing to women with a risk of 1 in 2,500 or more, we would have detected 97.8% of the cases of trisomy 21 and 98.5% of the other aneuploidies by performing the test in about 26% of our population. In contrast, the SEGO proposal aims to ensure a DR of about 90% but only about 4% of the women to require cfDNA testing, as shown in our study. The main advantage of this strategy is the secondary reduction of invasive tests at a similar cost. During the study period, our invasive testing rate was 2.0% (210/10,541), which is considerably lower than our previously reported rate of 4.8% in 2005–2010 ($p < 0.0001$) [18]. Another Spanish study conducted in a public hospital in Madrid

region reported only 75% uptake of cfDNA testing within the high-risk group, defined as a risk of ≥1 in 250 at the time of screening [9]. However, this uptake increased from 8% in the very high-risk group (risks ≥1 in 10) to 100% in the less high-risk group (risks between 1 in 150 and 1 in 250), and the uptake of cfDNA testing in the women whose risk was between 1 in 50 and 1 in 250 was about 90% like in the present study [9].

Strengths and Weaknesses of the Study

The main strength of our analysis is the use of real clinical data, collected in fully funded public hospitals. Thus, these results reflect women's behavior in real life regarding uptake of trisomy 21 screening, cfDNA testing, and invasive testing regardless of economic status and, therefore, lead to real inputs for our model. However, although it was not the aim of our study, the small number of affected pregnancies included did not allow us to accurately assess the performance of neither the FCT nor the cfDNA test. Another limitation is that we only assessed short-term costs for economic evaluation, acknowledging that indirect costs, although difficult to quantify, are also of great importance. Additionally, we have not taken into account the costs related to personnel involved, but we believe that both, indirect costs and personnel costs, would be higher in the strategy without cfDNA testing, first, because sick leave is more likely to happen after invasive testing than after cfDNA testing and, second, because the cost of one or even two fetal medicine specialists performing an invasive procedure is higher than that of a nurse drawing blood for cfDNA analysis.

Interpretation

Essentially, there are 2 options for clinical implementation of cfDNA testing in screening of the major trisomies: first, universal screening and, second, contingent screening based on the results of first-line screening by another method. Universal screening would definitely lead to the best performance. However, the high marginal cost associated leaves this strategy out for most public health systems. Therefore, introducing cfDNA testing in a contingent fashion seems to be a reasonable alternative. Following cfDNA testing, the most accurate method for screening of trisomy 21 is the FCT and the results from our study prove that, only having a good-quality first-trimester scan and FCT, we can ensure high performance of any contingent screening proposal and keep the costs as previously determined. When the cost of the test decreases, current cutoffs may be replaced by lower ones and the proportion of women opting for cfDNA testing may be expanded; however, continuous audit and monitoring of performance and costs is necessary to keep them stable.

Conclusions

First, clinical implementation of contingent cfDNA screening following a high-risk result from the FCT as recommended by the Spanish Society of Obstetrics and Gynecology is feasible and shows similar DR and costs and lower invasive testing rate than traditional screening. Second, patients' uptake of such a strategy is high. Third, expanding the group of patients eligible for cfDNA testing would increase the DR but at the expense of an increase in the total cost of the program.

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Statement of Ethics

Ethical approval was given by the Biomedical Ethics Committee of the Junta of Andalucía (01-N-16), Spain (approval date: January 31, 2016). We have obtained written patient consent. The published research complies with the guidelines for human studies and includes evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

J.A.S., R.T., I.P., R.G., M.V., P.C., and J.A.G.M. participated in protocol and study development, data collection, data analysis, and manuscript writing and approval. B.S. and M.M.G. participated in data analysis and manuscript writing and approval.

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