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A contingent model for cell-free DNA testing to detect fetal aneuploidy after first trimester combined screening

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ABSTRACT

Objective: To assess the results of the first trimester combined test to design a prenatal protocol for the introduction of the cell-free fetal DNA test as a contingent screening model.
Method: An observational retrospective study in 12,327 singleton pregnancies to analyze the results of the combined first trimester screening, the nuchal translucency ≥97.5 percentile, their cytogenetic results and birth outcomes.
Results: A total of 533 (4.3%) pregnant women had a risk in combined first trimester screening above 1/300. In this group, sixty nine had an unbalanced karyotype. The abnormal/normal karyotype ratio was 1/28 in pregnant women with intermediate risk (1/51-1/300) for trisomy 21 and trisomy 18, 1/58 with intermediate risk just for trisomy 21 and 1/37 with intermediate risk just for trisomy 18. A 19.8% of the unbalanced karyotypes had chromosomal abnormalities other than trisomies 21, 18 and 13. Two false negatives cases at first trimester combined screening presented a nuchal translucency ≥ p97.5th.
Conclusion: We propose the introduction of the cell-free fetal DNA test when the risk of first trimester combined screening is intermediate (1/51–1/300) and when nuchal translucency is ≥ p97.5th with a low risk in the combined screening. This policy would allow us to continue to detect uncommon chromosomal abnormalities.
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Introduction

First trimester combined screening (FTCS) has reported detection rates for trisomy 21 (T21) of 85–90% with a false positive rate of 3–5% [1,2] and its influence in women's prenatal choices has already been demonstrated [3–5]. Pregnant women with a low risk at FTCS usually decline an invasive test because of the risk of miscarriage that is quoted in 0.1–1% [6,7]. Cell-free fetal DNA (cf-DNA) test in maternal blood for T21, trisomy 18 (T18) and trisomy 13 (T13) offers an alternative option with a high specificity and sensitivity for all pregnant women but especially as a contingent screening model in women at risk for fetal common aneuploidies.

T21, T18 and T13 represented approximately 70% of aneuploidies prenatally detected [8,9], but there are other chromosomal abnormalities that must be evaluate before the implementation of the cf-DNA test in order to avoid their undiagnosis [8,10–12].

The aim of this study is to assess the results of the FTCS to design a prenatal protocol in our hospital, with the introduction of the cf-DNA test as a contingent screening model to achieve a better selection of pregnant women at risk for fetal aneuploidies and to avoid the invasive test. We present the analysis of a population of pregnant women with their results at birth and propose a cut-off risk for the introduction of the cf-DNA test for T21, T18 and T13 as well as the inclusion of other criteria for the cf-DNA test.

Methods

We performed an observational retrospective cohort study, to evaluate the results of the FTCS and the prenatal and postnatal cytogenetic studies, during 2009–2014, on pregnant women who came to our hospital. The inclusion criteria were all singleton

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pregnancies who were undergone to FTCS and the exclusion criteria those with no FTCS, twin pregnancies or miscarriages.

All pregnant women underwent an ultrasound scan between 10 and 14 weeks' gestational for measuring crown-rump length and nuchal translucency (NT) following by a biochemical analysis of the pregnancy-associated plasma protein-A (PAPP-A) and β fraction of free human chorionic gonadotrophin at the same day. NT percentile was estimated with the scale of *Borrell* et al [13]. FTCS was performed and provided a risk assessment for T21 and T18 (SsdwLab V. 5.0. © SBP Software CB. Spain). Pregnancies with a FTCS risk greater than 1/300 for T21 or T18 were considered at risk for fetal aneuploidies. Pregnant women with advanced maternal age (AMA, ≥ 35 years old), abnormal ultrasound, FTCS at risk, family or personal history of aneuploidies or maternal anxiety were referred for prenatal counselling. An invasive prenatal testing by chorionic villus sampling or amniocentesis was offered to them and a rapid test by quantitative fluorescence-polymerase chain reaction (QF-PCR, Devyser Compact v3) for the most common aneuploidies and a karyotype study were performed. Pregnancies with risk at FTCS ≥ 1/50 for T21 or T18 were considered at high risk, with a FTCS risk between 1/51–1/300 for T21 or T18 were considered at intermediate risk and with a FTCS risk <1/300 for T21 and T18 were considered at low risk. In pregnancies with high risk or an abnormal ultrasound scan, subtelomeric and microdeletion syndromes were ruled out by multiplex ligation dependent probe amplification techniques (MLPA, Salsa P036, P070, P245, MRC Holland) or array comparative genomic hybridization (array-CGH, KaryoNIM 60K®prenatal), as long as the results of QF-PCR and karyotypes were normal. Birth outcome data were compiled and postnatal karyotypes were performed if newborn's physical examination suggested a chromosomal syndrome.

In order to decide the FTCS risk cut-off in which pregnant women could benefit from cf-DNA test for T21, T18 and T13 as an alternative option to an invasive procedure, we analyzed the prenatal and postnatal unbalanced karyotypes classifying them in three groups: high, intermediate and low risk just for T21, just for T18 and for both trisomies (T21-T18) at FTCS. Other risk cut-offs for T21 and T18 at FTCS have also been evaluated. False negative cases for T21 and NT percentile in our risk groups were evaluated. Other chromosomal abnormalities with relevant clinical significance which could not be detected by cf-DNA test were included into the analysis.

This study was approved by the research ethics committee of our hospital.

Results

During the period 2009–2014 a total of 12,327 singleton pregnancies came to our hospital for fetal screening of common aneuploidies by FTCS. Table 1 shows demographic and clinical data of our population.

Fig. 1 shows a schematic distribution of pregnant women. In 954 cases an invasive prenatal test was performed for fetal karyotype, which was normal or balanced in 878 cases (92%). There were 76 unbalanced prenatal karyotypes and 5 unbalanced postnatal karyotypes. The prevalence of chromosomal abnormalities in our cohort was 0.7%.

FTCS and unbalanced karyotypes

There were 533 (4.3% of 12,327) of all pregnant women with FTCS risk above 1/300. Only 12.9% of these pregnancies with FTCS at risk had an unbalanced karyotype. The results of FTCS and karyotypes studies in our risk groups are shown in Table 2. The detection rate of FTCS for T21 and T18 was 88.5% with a 4% of false positives. The detection rate only for T21 was 83.3% with a 2.3% of false positives. The positive predictive values were 10.1% and 10.9% for both T21-T18 and only for T21, respectively.

T21, T18 and T13 represented 80.2% of unbalanced karyotypes in our cohort. All T18 and T13 had a FTCS at risk. There were 39 cases of T21 prenatally detected, 35 by a FTCS at risk (R ≥ 1/300), 3 by abnormal ultrasound and 1 by AMA. In the remaining abnormal group, 8.65% of the cases were sex chromosomal aneuploidies (SCAs), 8.65% other uncommon chromosomal abnormalities and 2.5% were triploidy. In pregnancies with high risk (≥1/50) in the FTCS were detected 43% of the uncommon chromosomal abnormalities, and 57% were detected in the low risk group in the FTCS, all of them by karyotype. There were no cases in pregnancies with intermediate risk in the FTCS.

All the complementary studies by MLPA or array-CGH in pregnancies with high risk (R ≥ 1/50) in the FTCS or with abnormal ultrasound scan, as long as they had normal QF-PCR and karyotypes, were normal.

FTCS risk cut-offs

The Table 3 shows the distribution of several risk cut-offs at FTCS according to T21, T18 and others unbalanced karyotypes. Of the 81 unbalanced chromosomal abnormalities, 60% (49/81) were

Table 1
Demographic and clinical data in 12,327 pregnant women.

Population data n = 12,327	Risk ≥1/50 n = 147	Risk 1/51–1/300 n = 386	Risk <1/300 n = 11,794
Maternal age	36.3 ± 4.7	36.9 ± 4.4	31.1 ± 5.4
Weight	65.9 ± 12.2	71.6 ± 15.2	62.8 ± 11.1
Size	161.6 ± 6.6	163.3 ± 6.5	162 ± 6.8
Smoker	24 (16.3)	71 (18.4)	1,432 (12.2)
Origin			
Caucasian	108 (73.5)	275 (71.2)	7,587 (64.3)
Afro-Caribbean	2 (1.4)	5 (1.3)	516 (4.4)
Asian	1 (0.7)	9 (2.3)	410 (3.5)
Arabs	4 (2.7)	9 (2.3)	304 (2.6)
South-American	30 (20.4)	79 (20.5)	2,769 (23.5)
Others	2 (1.4)	9 (2.3)	208 (1.8)
Nuchal translucency (mm)	3.5 ± 2	1.5 ± 0.6	1.3 ± 0.3
Crown-rump length (mm)	57.6 ± 8.8	61.6 ± 8.3	60.4 ± 8.1
[a]Free-βHCG (MoM)	1.4 ± 1.3	1.3 ± 1.2	1.2 ± 0.9
[b]PAPP-A (MoM)	0.69 ± 0.5	0.63 ± 0.5	1.3 ± 0.7

[a] **Free-βHCG:** β fraction of free human chorionic gonadotrophin.
[b] **PAPP-A:** Pregnancy-associated plasma protein-A. Data are given as frequencies and percentages (%) for qualitative variables and as mean and standard deviation for quantitative variables with symmetric distribution.

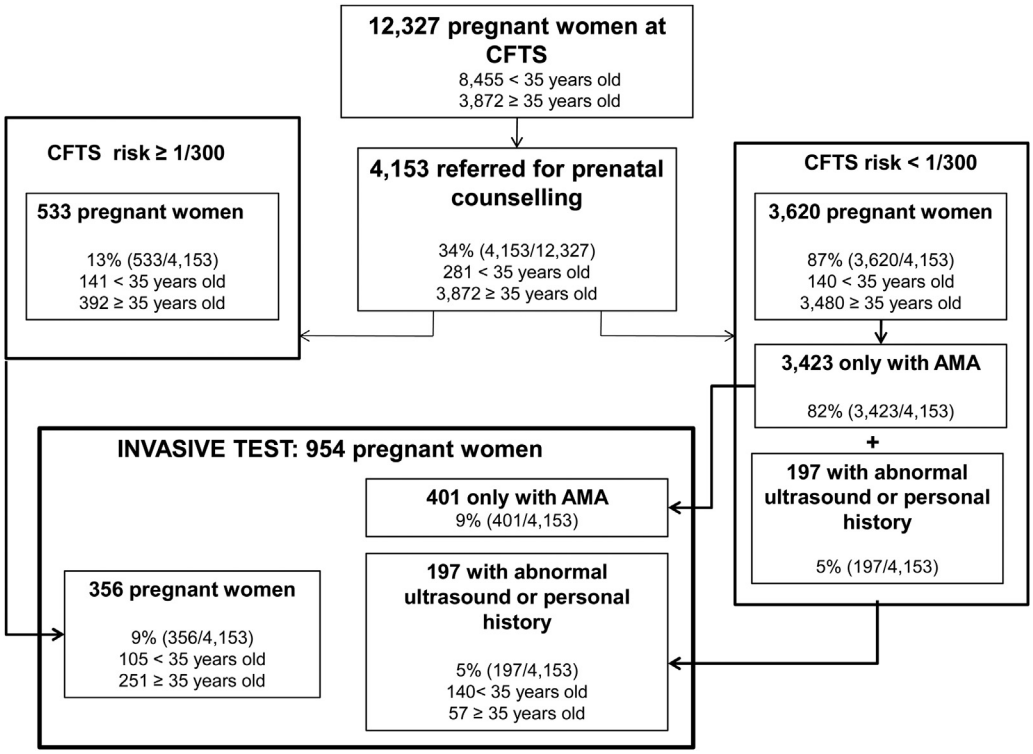

Fig. 1. Distribution of pregnant women referred for prenatal counselling.

Table 2
Results of FTCS and karyotype studies performed in pregnant women by risk group during 2009–2014.

FTCS Risk groups	[†]FTCS									
	Risk for T21-T18				Risk just for T21		Risk just for T18		Low risk	
	[‡]HR-T21		[§]IR-T21		HR-T21	IR-T21	[¶]LR-T21		LR-T21	
	[††]HR-T18	[‡‡]IR-T18	HR-T18	IR-T18	[§§]LR-T18		HR-T18	IR-T18	LR-T18	Total
Pregnant women on each risk group	6	20	2	28	92	173	27	185	11,794	12,327
Invasive test	6	20	1	19	81	108	18	103	598	954
Normal or balanced karyotype	0	5	1	18	49	105	12	99	589	878
Unbalanced karyotype	6	15	0	1	33	3	6	5	12	81
Trisomy 21		6		1	[a]26	2			[g]7	42
Trisomy 18	6	6					6	[e]1		19
Trisomy 13		3						1		4
Monosomy X					2					2
Triploidy					1			1		2
Sexual Chromosomal Abnormalities					[b]1	[d]1		[f]2	[h]1	5
Uncommon Chromosomal Abnormalities					[c]3				[i]4	7
Ratio between unbalanced karyotype and pregnant women on each risk group	*1:1*	*1:1.2*	*0:2*	*1:28*	*1:3*	*1:58*	*1:4.5*	*1:37*	*1:983*	*1:152*

[†] **FTCS:** First Trimester Combined Screening.
[‡] **HR-T21:** High Risk for T21 (1/2-1/50).
[§] **IR-T21:** Intermediate Risk for T21 (1/51-1/300).
[¶] **LR-T21:** Low Risk for T21(<1/300).
[††] **HR-T18:** High Risk for T18 (1/2-1/50).
[‡‡] **IR-T18:** Intermediate Risk for T18 (1/51-1/300).
[§§] **LR-T18:** Low Risk for T18(<1/300).
[a] 25 Prenatal and 1 postnatal karyotypes.
[b] 1 mosaic XXY/XY Trisomy.
[c] 1 Mosaic Trisomy 22, 1 Partial Duplication 5q and 1 Partial Trisomy 14q with Partial Monosomy 17q.
[d] XXX Trisomy.
[e] 1 Postnatal Karyotype.
[f] 1 XYY Trisomy and 1 Mosaic Monosomy X.
[g] 4 Prenatal and 3 postnatal karyotype.
[h] XXY Trisomy.
[i] 1 Mosaic Tetraploidy, 1 Mosaic Trisomy 20, 1 Mosaic 13q Deletion and 1 Supernumerary marker chromosome 15.

Table 3
Distribution of the FTCS risk cut-offs according to T21, T18 and others unbalanced karyotypes.

Risk cutt offs at [†]FTCS	Trisomy 21 cases (%) n = 42	Trisomy 18 cases (%) n = 19	Others unbalanced karyotypes cases (%) n = 20	Unbalanced / total cases
R ≥ 1/10	27 (64.3)	13 (68.4)	9 (45)	49/86
R ≥ 1/50	32 (76.2)	18 (94.7)	10 (50)	60/147
R 1/11-1/50	5 (11.9)	5 (26.3)	1 (5)	11/150
R 1/11-1/300	8 (19)	6 (31.6)	6 (30)	20/447
R 1/51-1/300	3 (7.1)	1 (5.3)	5 (25)	9/386
R 1/301-1/1000	2 (4.8)	_	2 (10)	4/1048
R 1/1001-1/2000	3 (7.1)	_	1 (5)	4/1215
R 1/2001-1/3000	1 (2.4)	_	_	1/926
R 1/3001-1/6000	1 (2.4)	_	1 (5)	2/2130
R < 1/6001	_	_	1 (5)	1/6475

[†] **FTCS:** First Trimester Combined Screening. When there were high or intermediate risks for both T21-T18, the highest risk of the two was considered to stratify the results of the karyotypes. Dates are given as frequencies and percentages.

detected when the risk was greater than 1/10 and 74% (60/81) when it was greater than 1/50. The 97.7% of the pregnancies in the intermediate risk group (1/51-1/300) had a normal outcome. In this intermediate risk group there were 9 unbalanced karyotypes (9/386): three T21, one T18, one T13, one triploidy and three SCAs cases. In the low risk group (<1/300) there were 25% (5/20) of the chromosomal abnormalities others than T21 and T18. These 5 unbalanced karyotypes are shown in Table 2, and only the partial deletion of 13q chromosome mosaicism detected by a Dandy-Walker malformation on second trimester ultrasound had clinical relevance.

False negative cases for T21

The seven false negatives cases of the FTCS for T21 are shown in Table 4. Two of them (numbers 1 and 5) had a FTCS risk between 1/301-1/1000 and both had a NT in 97.5 percentile (p97.5th).

NT and unbalanced karyotypes

There were a total amount of 38 cases with NT higher than p97.5th and low risk for T21 at FTCS (21 cases, Group A + 17 cases, Group B, Table 5), and 33 of these pregnant women were under 35 years old. There were 35 cases with a NT between p97.5th-p99th and only T21 cases have been detected. The two false negatives of the FTCS for T21 were in this group (Group A, Table 5). Of the 142 fetuses with a NT ≥ p99th (Group B, Table 5), 17 cases presented low risk for T21 and normal outcome.

cf-DNA as contingent screening model

The introduction of the cf-DNA test for T21, T18 and T13 will be recommended in our hospital as first choice in pregnant women with an intermediate risk at FTCS (1/51-1/300) and in those with a NT ≥ p97.5th and low risk at FTCS (<1/300). An invasive test will be recommended in pregnant women with a risk at combined test ≥1/50.

Discussion

In our series, 74% (60/81) of chromosomal unbalanced abnormalities with serious effect on fetus phenotype, including uncommon aneuploidies, had a high risk (R ≥ 1/50) at the FTCS. At this risk cut-off we can detect the highest percentage of T21 (32/42) and T18 (18/19) cases as well as 50% (10/20) of others unbalanced karyotypes: monosomy X, T13, triploidy and the uncommon chromosomal abnormalities associated with adverse outcome. Our results are according with previous reports [11,14]. Also, it seems that the ratio between unbalanced karyotype and pregnancies on each risk groups support the recommendation of an invasive test (between 1/1 and 1/4.5, Table 2).

We have considered as intermediate risk the pregnancies which have a FTCS between 1/51-1/300 because unbalanced karyotypes represent only 2.3% (9/386) of the cases. In this intermediate risk group the ratio between chromosomal abnormalities and pregnancies on each risk groups suggests a cf-DNA test (between 1/28 and 1/58, Table 2). Other authors raise the possibility of offering cf-DNA test at a risk cut-off of 1/11 to 1/500 or 1/1000 [15]. In our series there are 11.9% of T21 and 26.3% of T18 between the risk cut-off 1/11-1/50 which makes us consider an invasive test in this group.

There are reports which analyze the distribution of risk from FTCS according to T21, T18, T13 and others chromosomal abnormalities outcome. The authors propose different policies for the introduction of cf-DNA test. With a cut-off risk at 1/1000, their detection rate for T21 was 98% [15,16]. In our series the detection rate at this cut-off would be 88% but between 1/301–1000 there were 1048 pregnant women and only two cases of T21. So this option would increase the cost of cf-DNA test implementation. Besides we could detect this two T21 cases through the NT

Table 4
False negatives cases at FTCS for T21.

Case number	Trisomy 21 risk at [¶]FTCS	Maternal age at term	[†]Nuchal Translucency mm (Percentile)	Indication for prenatal counselling
1	1/445	28	3.1 (p97.5th)	NT>3 mm
2	1/1167	39	1.5 (p5th)	[§]FHD
3	1/1273	28	1.8 (p75th)	[§]FHD
4	1/1626	38	1 (p10th)	[‡]AMA
5	1/882	30	2.9 (p97.5th)	
6	1/2687	24	1.9 (p75th)	
7	1/5116	30	1 (p10th)	

[¶] **FTCS:** First trimester combined screening.
[†] **NT** millimeters (percentile) depending on CRL [13].
[§] **FHD:** Fetal heart defect.
[‡] **AMA:** Advanced maternal age.

Table 5
Karyotype and Nuchal Traslucency ≥97.5th percentile [13].

	Group A: p97.5th≤ †NT < p99th (35 cases)						Group B: NT ≥ p99th (142 cases)						
‡FTCS	§HR-T21		¶IR-T21		††LR-T21		HR-T21		IR-T21		LR-T21		
Age (years)	< 35	≥ 35	< 35	≥ 35	< 35	≥ 35	< 35	≥ 35	< 35	≥ 35	< 35	≥ 35	Total
Trisomy 21	–	1	–	–	[a]2	–	8	21	1	–	–	–	33
Other Unbalanced Fetal Karyotype	–	–	–	–	–	–	4	17	–	–	–	–	21
Normal Karyotype	–	–	5	8	14	5	27	25	12	10	[b]17	–	123
Total	1		13		21		102		23		17		177

† **NT:** Nuchal translucency.
‡ **FTCS:** Combined First Trimester Screening test.
§ **HR-T21:** High Risk for T21 (1/2-1/50).
¶ **IR-T21:** Intermediate Risk for T21 (1/51-1/300).
†† **LR-T21:** Low Risk for T21 (<1/300).
[a] false negatives cases for T21.
[b] 14 cases with NT < 3,5 mm.

percentile because these two cases had a NT percentile between p97.5th–p99th and low risk at FTCS in a <35 years old women. The scope of maternal age on the calculation of risks in FTCS is probably modifying the risk despite the NT measurement. We could detect more T21 cases if we offered a cf-DNA test to all pregnancies with NT ≥ p97.5th when FTCS risk is low and it suppose a small increase in cost because there are 21 pregnant women that meet these criteria (Group A, Table 5).

An increased NT (p99th or ≥3.5 mm) is associated with a high risk of chromosomal abnormalities [11], being monosomy X, T21 and T18 cases the more frequent unbalanced karyotypes found in these cases [14,17,18]. NT in p99th is an indication for invasive test but we think that it is necessary to get the result of FTCS in these pregnancies because it provides information that can help women to make a decision about all their options, mainly when the NT is <3.5 mm. In our series, there was only one abnormal karyotype (T21) with intermediate risk for T21 and none with low risk for T21 despite of the NT in p99th. Although other authors report different percentages of uncommon chromosomal abnormalities in pregnancies with a NT in p99th depending on the technique used for its detection [19,20] in our series all the complementary molecular studies were normal and had a normal outcome. So we could offer cf-DNA test to all pregnancies with NT in ≥ p99th and low risk for T21.

Evidence data about the performance of cf-DNA test in a general population show a positive predictive value range for T21 from 45.5% [21] to 94.4% [22] and show high sensitivity and specificity in low risk population [23]. Because the positive predictive value of FTCS is less than in cf-DNA test and the FTCS false negative cases for T21 are more frequent in women under the age of 35, it would be necessary to perform the cf-DNA test into general obstetric population [24]. Currently this is not possible in our public health service due to the cost of the test.

There are several studies that have shown that contingent model can be cost-effective because it implies a reduction in the rate of the invasive tests and a lower procedure-related loss rate [25,26]. Although the cost effectiveness studies should be done with caution due to the large number of topics that must be evaluated, in our cohort we have 7 false negatives cases for T21 that would mean an additional cost between 594,000–790,000 € for the health and social care per case [27,28], and therefore that would imply an additional cost between 337–448 € for each pregnant woman. With this theoretical saving we could introduce the cf-DNA to all pregnant women, but maintaining the FTCS that allows us to have additional information on PAPP-A levels to assess the risk of preeclampsia or preterm delivery. Besides, both FTCS and TN allow us to identify other chromosomal abnormalities other than T21 which are not estimated by the cf-DNA. In addition, cf-DNA also has false negative and positive results, although there are very few. Detecting pathological cases does not always imply cost reduction since we ought always to respect the autonomy of the pregnant women. In the near future a more accurate study about economic feasibility should be done.

Available data indicate that cf-DNA test has less accuracy for SCAs than for T21 and T18, mainly due to a confined placental or maternal mosaicism that can increase the false positive rates [29–31]. We assume that we will not diagnose SCAs different than non mosaic monosomy X if we do not analyze the fetal sex by cf-DNA test. These SCAs have a mild phenotype and their diagnosis is usually fortuitous when invasive test are performed by AMA or FTCS at risk to rule out a T21 fetus [32]. In addition, X chromosome aneuploidies have been associated to AMA and these cases probably remain undiagnosed at this moment because in our cohort, 88% (3,022/3,423) of pregnant women with AMA and low risk at FTCS declined an invasive test.

It is necessary to remind the important role of ultrasound scan screening that could detect fetus at risk for any chromosomal abnormalities, including uncommon abnormalities, triploidies and false negative cases of cf-DNA test or FTCS. In our series, three of the FTCS false negatives, one triploidy and a partial deletion of 13q chromosome mosaicism were detected by an abnormal ultrasound scan and they could be missed by cf-DNA test or FTCS.

Regardless of our recommendations, all pregnant women must be informed about the chromosomal abnormalities that couldnt be detected by cf-DNA test or by second trimester ultrasound as well as about all prenatal diagnosis options.

One of the limitations of this study is that the cohort belongs only to our center so this proposed policy cannot be applied to another population. Another limitation could be the lack of MLPA or array-CGH results that detect unusual cryptic anomalies in all invasive tests of pregnant women at intermediate risk and that could have a future clinical impact on newborn, although the data at birth were normal. The low prevalence of uncommon chromosome abnormalities could affect the validation of our new policy.

Here we present a contingent screening model for the introduction of cf-DNA test in our hospital in two pregnancies group: i) those with an intermediate risk at FTCS and ii) those with a low risk at FTCS and NT ≥ p97.5th. We consider that those two assumptions could allow us to detect the most common aneuploidies, and to decrease the number of invasive test and consequently, the fetal losses. Our data suggest that the others uncommon chromosomal abnormalities with severe effect in the fetal phenotype would not be underdiagnosed since all of them

had a high risk at FTCS or an abnormal ultrasound scan in which it was recommended to perform an invasive test. The new proposed policy has been implemented in our hospital since October 2015 and future analyses of those data with the previous policy will allow us to verify and to improve our prenatal protocol.

Contribution to authorship

Carmen Cotarelo-Pérez, Raluca Oancea-Ionescu and María Fenollar-Cortés contributed equally to this work.

Disclosure statement

The authors declare no conflict of interest.

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