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Cell-free DNA screening in singleton and twin pregnancies: three years of clinical experience

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Objective

Fast adoption of a non-invasive prenatal testing (NIPT) in clinical practice is a global tendency last years. Here, we describe our experience with the implementation of genome-wide non-invasive prenatal testing (gw-NIPT) in pregnancies at increased risk for common aneuploidies in Valencian Region over the past three years. We analyzed the uptake of basic NIPT vs gw-NIPT, redraw/failure rate, fetal fraction, the PPV of cfDNA screening test in the detection of fetal chromosomal anomalies.

Methods

Pregnant women with intermediate risk based on first trimester combined prenatal screening (cFTS) or second trimester biochemical screening were offered NIPT as contingent screening test between 1st March 2020–31st December 2022. Patient samples from both singleton and twin pregnancies were included in our study. The assay provides an Anomaly Detected (high-risk) or No Anomaly Detected (low-risk) result for common trisomies, RAAs, and CNVs, with an option to request the reporting of SCAs. Patients consented to either basic screening (common trisomies) or genome-wide screening (inclusion of RAAs and CNVs). A fetal fraction (FF) estimate is also provided during the sample analysis. Cases with positive screening results by NIPT detection were validated where possible using prenatal diagnosis.

Results

In total, 6,098 maternal blood samples were collected. Basic cfDNA screening was chosen in 218 cases (3.6 %) and gw-cfDNA screening in 5880 cases (96.4 %). Of these, 5950 samples (97.60 %) were from singleton pregnancies, 122 (2%) samples were from twin pregnancies and 26 (0.40 %) were from vanishing twin (VT) pregnancies. There were 76 samples (1.2%) that had no-call result after first sampling with a final no-call rate of 0.33 % after a second sampling. Fetal fraction ranged from 2%-34%, with an average of 10.09 % (±4.32%). In total, 204 (3.3%) had a high-risk NIPT result. Of the these, there were 76 cases of T21, 21 cases of T18, 7 cases of T13, 29 cases of SCAs, 31 cases of rare autosomal aneuploidies (RAAs), 31 cases of copy number variants (CNVs) and 9 cases with multiple chromosomal anomalies (MCA). NIPT positive rate for T21, T18, T13, SCA, RAA, CNV and MCA were 1.25% (76/6,098), 0.34% (21/6,098), 0.11% (7/6,098), 0.47% (29/6,098), 0.51% (31/6,098), 0.51% (31/6,098) and 0.15% (9/6,098), respectively. The uptake of invasive prenatal diagnosis for NIPT positive cases were as follows: 89.5 % (68/76) of T21 cases, 81% (17/21) of T18 cases, 85.7% (6/7) of T13 cases, 75.9% (22/29) of SCA cases, 90.3% (28/31) of RAAs cases, 90.3% (28/31) of CNVs cases and 88.9% (8/9) of complex anomalies cases. The positive predictive value (PPV) in cases of T21, T18, T13, SCA, RAA, CNV were calculated: 95.6% (95% CI: 87.6–99.1%) (65/76), 76.58% (95% CI: 50.1–93.2%), 66.7% (95% CI: 22.3–95.7%), 68.2% (95% CI: 45.1–86.1), 10.7% (95% CI: 2.3–28.2%) and 10.7% (95% CI: 2.3–28.2%), respectively. Confirmed SCA concordant cases included five cases of monosomy X (3 of which were mosaics), 2 cases of XXY, 2 cases of XYY and 5 cases of Triple X; and one discordant result for triple X (positive call for monosomy X, where confirmatory prenatal test finally showed triple X). The most common RAAs in this cohort were trisomy 7 (5 cases) and trisomy 16 (5 cases). Only three cases were confirmed to be concordant with the cfDNA screening result: two cases of trisomy 16 (both mosaic) and one case of trisomy 22 (mosaic). There were 31 CNV cases in our study cohort, which were found on 13 different chromosomes. Of the 28 patients with diagnostic testing, three cases were confirmed to be concordant: one case with del(18)(p11.32p11.22), one case with del(13)(q13.3q14.3) and one case with del(13)(q31.1q31.3). In one case an amniocentesis was carried out and the karyotype was normal. However, array showed LOH chr 5, arr[GRCh38] 5p15.33p13.3(113462_30725947)x2 hmz. There were also nine patients with MCA. Six of these patients had a normal result by amniocentesis, although one of these patients had suspected a maternal malignancy that finally was confirmed. For the other three cases, an anomaly was noted on either amniocentesis or POC tissue, but not multiple anomalies. Finally, three of the twin pregnancies (3/122) and six of the VT (6/26) pregnancies had a high-risk cfDNA screening call. Eight cases underwent diagnostic testing with two twin cases (one case positive call for T21 and one case positive for del(10)(p15.3p12.31) and five of the 6 VT (positive NIPT call for T13, T18 (2 cases), T12, T12 and T15, dup 21q21.1q22.3) cases showing a false positive result. One twin pregnancy was confirmed (true positive), with T21 in one of the fetuses.

Conclusion

In our population, almost all women chose gw-NIPT fDNA testing rather than basic NIPT. RAAs and CNVs represented one third of all positive NIPT cases, each being more frequent than T18 and T13 together. Screening for fetal trisomies by cfDNA analysis of maternal blood, contingent on the results of the combined test, showed higher PPV for common aneuploidies compared to classic screening test, even in cases of RAAs and CNVs. cfDNA test is very accurate but does not give a definite answer.

Table 1. Patient demographics (n=6098)

Variable	Value
Maternal Age, yrs	
Mean	35.49
Median	36.00
Range	18–53
SD	5.17
Gestational Age, wks	
Mean	13.51
Median	13.00
Range	10–37
SD	2.36
BMI	
Mean	25.32
Median	24.17
Range	14.69–54.20
SD	5.12
Type of Pregnancy, n (%)	
Singleton	5950 (97.60%)
Twin	122 (2%)
Vanishing Twin	26 (0.40%)

Table 2. NIPT positive rate (n=6098)

cfDNA Result	Number of cases	NIPT+ve rate (%)
T21	76	1.25
T18	21	0.34
T13	7	0.11
SCAs	29	0.47
RAA	31	0.51
CNV	31	0.51

Table 3. Cell-free DNA Screening Results and Concordance with Clinical Truth for Common Trisomies, Sex Chromosomal Aneuploidies, RAA and CNVs.

cfDNA Result	Number of cases	No diagnostic testing	Confirmed (TP)	Not confirmed (FP)	PPV, % (95% CI)
T21	76	8	65	3	95.6 (87.6–99.1)
T18	21	4	13	4	76.5 (50.1–93.2)
T13	7	1	4	2	66.7 (22.3–95.7)
SCAs	29	7	15	7	68.2 (45.1–86.1)
RAA	31	3	3	25	10.7 (2.3-28.2)
CNV	31	3	3	25	10.7 (2.3-28.2%)

Figure 2. Overview of RAA calls by prenatal cfDNA screening.