Prenatal Cell-free DNA testing: Analysis of "No Call" and Low Fetal Fraction Results

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Objective

1. Identify any significant adverse outcome on the "no call" group. 2. Evaluate the performance rates in different groups of FF. Identify any significant outcome adverse on the FF 4 to 5,9% group.

Methods

NIPT Screening analysis was carried out from January 2013 to March 2022. 25705 women were screened for trisomy 21, 18 and 13 by cfDNA testing from 10 weeks of gestation. Without any further processing, maternal blood samples were sent via courier to the USA for analysis using a direct DNA analysis (Harmony Prenatal Test®). Risk scores for trisomy 21, 18 and 13 were provided in the test report and were presented to each patient, with a mean turnaround time of 7,64 days. The risk scores were represented as a percentage, with ranges capped at >99% and <0.01%. The Harmony Prenatal Test® analyses the relative number of chromosomes in maternal blood to provide an objective individualized and dependable result with high sensitivity and specificity. Descriptive statistics were used for data analysis; the Student's t test, the Fisher exact test and Mood's Median Test was employed. P-values (2-sided test) less than 0.05 were considered significant.

Results

In this period (from January 2013 to March 2022), CML-GS has performed 25705 cfDNA tests. Maternal age mean was 36.17 years, and gestational age mean was 14.06 weeks. In our population the failure rate is 0,7%. In the "no call" group, there was a statistically significant increase (p-value 0.007) in the miscarriage rate, compared to the control group (with results) of 11.63% and 2.29%, respectively. There are no statistically significant changes in Normal and Abnormal outcomes in the two groups analysed. To analyse the performance rates in different groups of FF, we grouped FF into 4 categories [4-5.9; 6-9.9; 10-14.9; >=15]. Test performances, and outcome adverse in each group were determined. We observed a statistically significant increase (p-value <0.001) of miscarriage rate in the group with the lowest FF, when compared to the other groups (1.27; 0.12; 0.11; 0.05), respectively. We also verified a statistically significant increase (p-value 0.006) in the FPR% in the group with lower FF, when compared to the other groups (0.80; 0.13; 0.04; 0.05), respectively. Remaining test performance parameters without statistically significant changes. High risk result test for Trisomy 13 and 18 shown a lower FF (p-value <0.001) when compared to pregnancies with high risk for trisomy 21. When comparing the BMI of the different FF groups, an inverse correlation was observed between the BMI and the FF, with the group with the lowest FF having the highest BMI (median 26.42, p-value <0.001). We also identified an increase in BMI associated with miscarriage; the main adverse outcome (median 24.56 p-value 0.049).

Conclusion

One of the limitations of the cfDNA testing as method of screening for aneuploidies is the failure to provide a result. The reasons for a "no call" result could be related do technical issues such as difficulties with blood collection or inadequate transportation of the samples, to incorrect labelling, for example or assay problems, as failed DNA extraction, amplification, or sequencing. However, the major reason for failure appears to be associated to low FF (below 4%) (1,3,9). The most significant influence in low FF is recognized to be the maternal weight; the higher the weigh, the lower is expected to be the FF (12). However, other biologicals influences had been identified to decrease the levels of FF, such as mosaicism, triploidy, maternal autoimmune diseases, IVF reproduction, parity, multiple gestation (decreases the level per fetus) and maternal age (1). The association between a failed result due to low FF generates clinical concern to an increased risk of aneuploidy, as low FF has been associated with a higher risk of aneuploidy (1, 2, 3). Miscarriage was the most common outcome adverse identified in both groups of "no call" and low fetal fraction results, in this analysis. The performance rates across the groups were consistent as expected. However, in the group with lower levels of FF (between 4-5,9%) a FPR increased and a higher number of miscarriages, were identified. As the information regarding the causes of the miscarriages are not available, one possible explanation for the data found could be the association of cfDNA contribution from a non-identified vanishing twin gestation, as the demised fetus could continue released cfDNA into maternal blood by the time of blood collection. Vanishing twin cases had been associated with discrepant results and are not recommended to perform the test (1, 15, 16, 17). Trisomies 13 and 18 are associated with low levels of FF and higher rates of "no call" results (8) and could eventually explain the increased miscarriage rates in the "no call" group, as those trisomies and other chromosomal abnormalities are also commonly associated with miscarriages. (13, 14). Another possible explanation could be confined placental mosaicism, also correlated with low FF. Confined placental mosaicism, is an abnormal cell line that occurs almost solely in the placenta. The aneuploid placental cell lineages can affect the placental function and 16% to 21% of pregnancies with CPM show prenatal complications, which include miscarriages. The presence of mosaicism in the placenta can also influence risk estimation of a possible miscarriage. It's interesting to mention that chromosome 18 and 13, are frequently associated with mosaicism (11). Regarding the increased BMI identified in the group of miscarriage, also associated with low FF and "no call" group, this association may be explained by the effect of BMI directly on the total cfDNA levels. As mentioned before, the correlation between maternal weight and FF levels is well described. The presence of increased total cfDNA levels in obese pregnant women suggests its association with complications that may affect both maternal and fetal health. The raw DNA values need to be adjusted for maternal BMI for adequate interpretation of clinical tests that involve assessment of the FF (5).