Positive testing for trisomy 13 and 18 using non invasive prenatal testing: how to improve the high false positive rate

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OBJECTIVE : Non Invasive Prenatal Testing (NIPT) has proven high sensibility and specificity for trisomy 21 detection. Detection of trisomy 13 and 18 has also high sensitivity, but lower specificity and hence low positive predictive value, due to a high false positive rate. A variety of potential causes of false positives has been identified including vanishing twin, maternal tumours, maternal mosaicism, confined placental mosaicism. The actual z-score "cut-off" is +3.95 for both trisomy 13 and 18. The aim of our study was to identify biological and statistical factors which could improve false positive rate of trisomy 13 and 18.

METHODS : Our study included all cases of positive NIPT results for trisomy 13 and 18 performed by massive parallel sequencing by the Cerba laboratory from march 2013 to November 2016. Over this period, 9325 NIPT were negative for both trisomy 13 and 18. 24 were positive for trisomy 13 and 26 were positive for trisomy 18. Respectively 11 and 16 were confirmed trisomy 13 and 18 on fetal karyotype, whereas 13 and 9 were false positives of trisomy 13 and 18. Hence, the calculated positive predictive value (PPV) for trisomy 13 and 18 was respectively 41 and 67% using the actual z-score cut-off of 3.95. We used ROC-curve analysis including all positive NIPT for trisomy 13 or 18 respectively and 200 truth negative NIPT of euploïd singleton fetuses to determine the best z-score "cut-off". We also analysed the CADET profile for all positive and negative cases. We calculated performance of the test using the new "cut-off" and using both the new "cut-off" and CADET profile analysis. At last, we tested performance of the new test (using both techniques : new "cut-off" alone or combined new "cut-off" with CADET profile analysis) on an anonymous sample of 30 positive NIPT for trisomy 13 and 18 and 200 negative NIPT performed from March to December 2016 .

RESULTS : ROC curve analysis suggested a z-score cut-off at 10.0 for trisomy 13 and 18. Using this cut-off, sensitivity and specificity of the test were 88.9% and 97.7% for trisomy 13; and 88.9% and 98.6% for trisomy 18 respectively. PPV for trisomy 13 and 18 were 61.5% and 84% respectively. Negative predictive value (NPV) were 99.5 % and 99 % respectively. Using the combined method of the modified cut-off and CADET profile analysis, sensibility and specificity became 88.9% and 98.1% for trisomy 13 and 88.2% and 99.0% for trisomy 18 (see table 1). When testing the two new methods on the anonymous sample of positive NIPT performed from March to December 2016, characteristics of the test were the same as on the initial sample for trisomy 18. No statistical analysis was possible to evaluate the new technique's performance for detection of trisomy 13 in the control sample because we had no true positive for trisomy 13 during the study period.

Table 1. Characteristics of NIPT for trisomy 13 and 18 using the different tested methods

Trisomy 18	**Se (%)**	**Sp (%)**	**PPV (%)**	**NPV (%)**
NIPT result (Z-score 3.95)	100.0	95.0	65.0	100.0
Z-score 10	88.8	98.6	84.2	99.0
combined test Z-score and CADET profile	88.2	99.0	88.2	99.0
Trisomy 13				
NIPT result (Z-score 3.95)	100.0	93.8	40.9	100.0
Z-score 10	88.8	97.6	61.5	99.5
combined test Z-score and CADET profile	88.8	98.1	66.7	99.5

Se : Sensibility, Sp: Specificity, PPV : Positive Predictive Value, NPV : Negative Predictive Value.

CONCLUSION : **Our results suggest actual z-score cut-offs of NIPT for trisomy 13 and 18 are one of the causes of a high false-positive rate. Using a higher z-score cut-off could improve performance of the test without lowering its sensibility. For z-scores close to the chosen cut-off (3.95 – 16.05), analysing the CADET profile can help improving positive predictive value of the test and avoid false negative results.**

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