VALIDATION STUDY OF UTILIZATION OF TRISOMY TEST FOR NONINVASIVE PRENATAL TESTING OF COMMON TRISOMIES

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INTRODUCTION AND AIM OF THE STUDY

Identification of fetal DNA in maternal circulation led to redefinition of possibilities in the field of noninvasive prenatal testing (NIPT) [1]. From detection of qualitative fetal genetic markers (e. g. fetal gender) with advances in sequencing technologies also quantitative analysis of fetal DNA became possible (e. g. detection of chromosomal aneuploidies) [2, 3]. With increasing availability of genomic sequencing NIPT based on analysis of circulating cell-free fetal DNA tests focused on detection of most common chromosomal aneuploidies (trisomy 13 – T13, trisomy 18 – T18 and trisomy 21 – T21) have became integral part of prenatal genetics and in last few years different laboratories made such NIPT available worldwide.

Aim of the study was prospective validation of Trisomy test, home-made NIPT test that is using paired-end low coverage whole genome sequencing approach combined with proprietary bioinformatical algorithm for z-score calculation.

RESULTS

In analysed cohort containing 1938 samples 1827 samples were reported as euploid, 35 as trisomic and 76 as uninformative (Figure 1). All samples with z-score lower than 2.5 were concluded as negative and higher than 4 as positive for trisomy (Figure 1, 2). Based on these criteria 22, 8 and 5 samples were reported as T21, T18 and T13, respectively (Figure 1, 2). Samples with z-score between 2.5 – 4, falling to so called grey zone (23 samples), and samples with fetal fraction below 5% after first blood draw (53 samples) were clasified as uninformative (Figure 1). Mean fetal fraction in informative samples was 14.51% with maximum at 44.56% (Figure 3).

Out of 35 samples reported as trisomy positive, 33 were true positive and 2 false positive (1x T21 and 1x T13). From 1827 samples reported as trisomy negative 2 were false negative (1x T21 and 1x T18) (Figure 2).

The overall sensitivity of the test for detection of selected trisomies was 94.29% and specificity was 99.98% (Table 1).

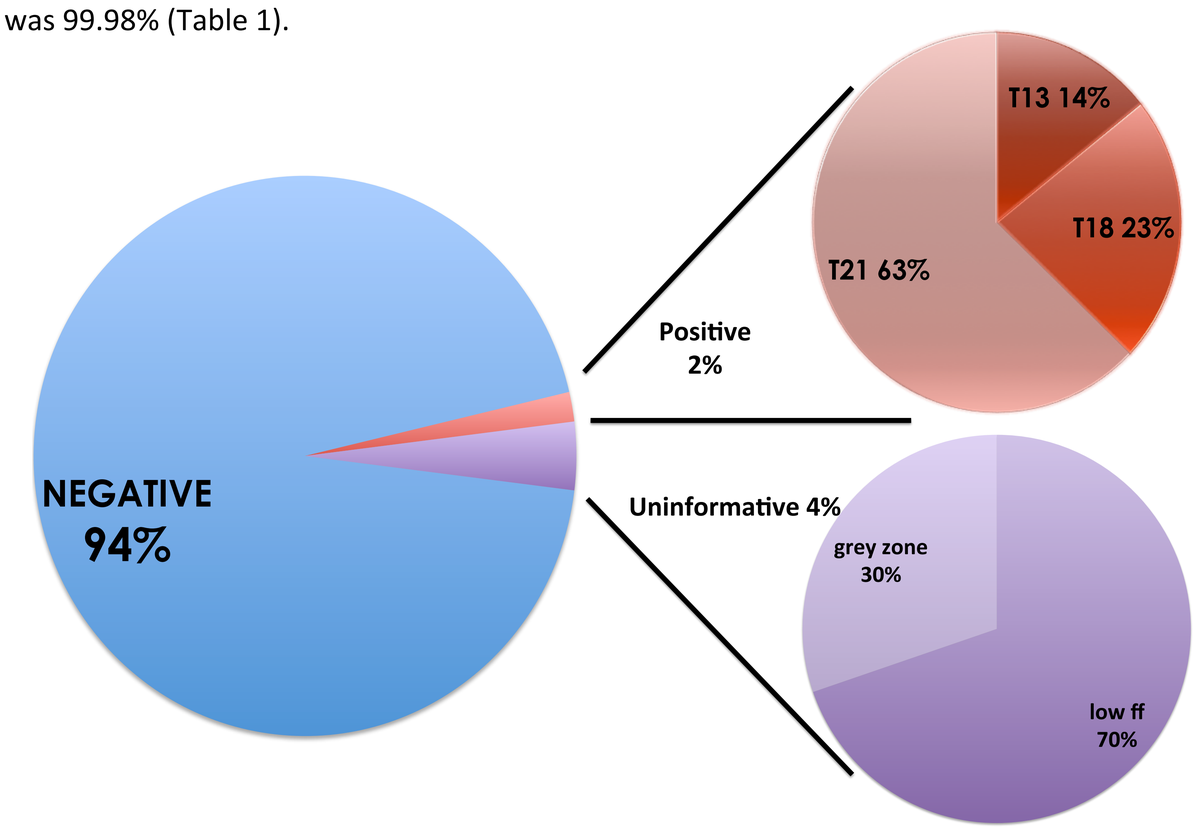

Figure 1: Reported results proportions
grey zone – z-score between 2,5 and 4, low ff – fetal fraction <5%

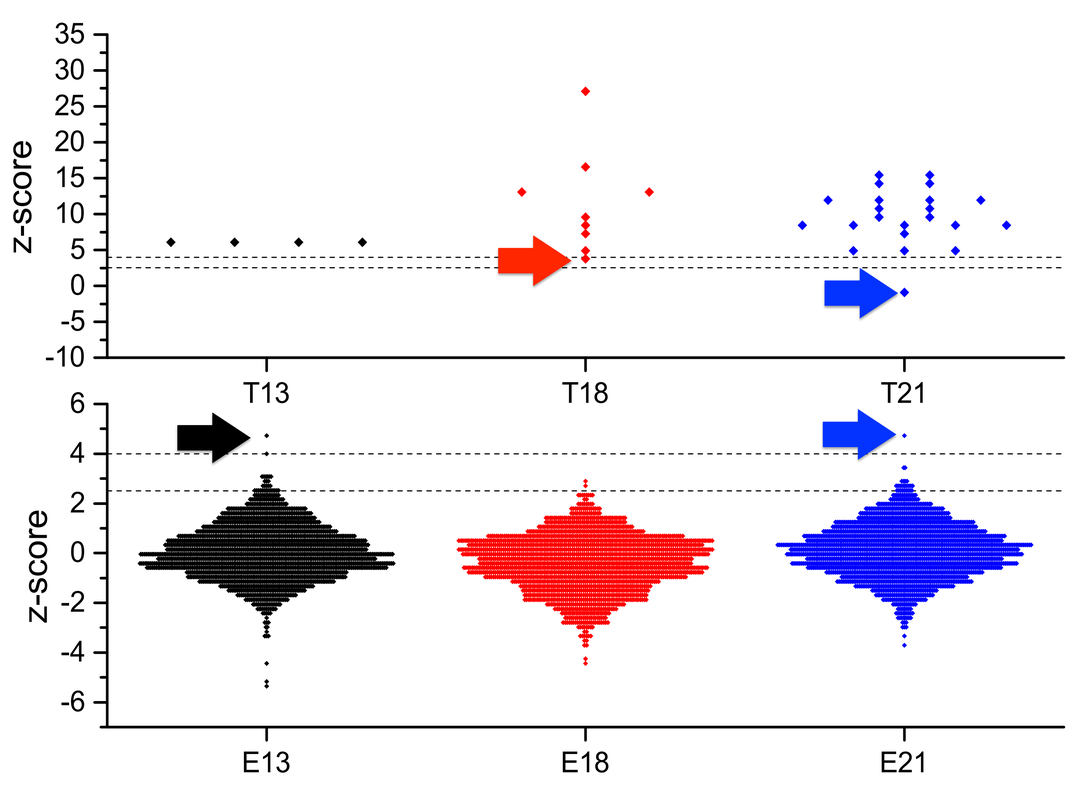

Figure 2: Z-score in analyzed samples reported as informative. Upper section is showing z-scores of samples reported as positive (trisomic - T), lower as negative (euploid – E). Samples identified as false positives and false negatives are highlighted by arrows in corresponding regions.

Table 1: Summary of reported results and calculations of sensitivity and specificity of TRISOMY test (only samples with informative results are included).

	Trisomy 21	Trisomy 18	Trisomy 13	All
True positives	21	8	4	**33**
False positives	1	0	1	**2**
True negatives	1839	1853	1857	**1825**
False negatives	1	1	0	**2**
Sensitivity	95.45%	88.89%	100%	**94.29%**
Specificity	99.95%	100%	99.95%	**99.98%**

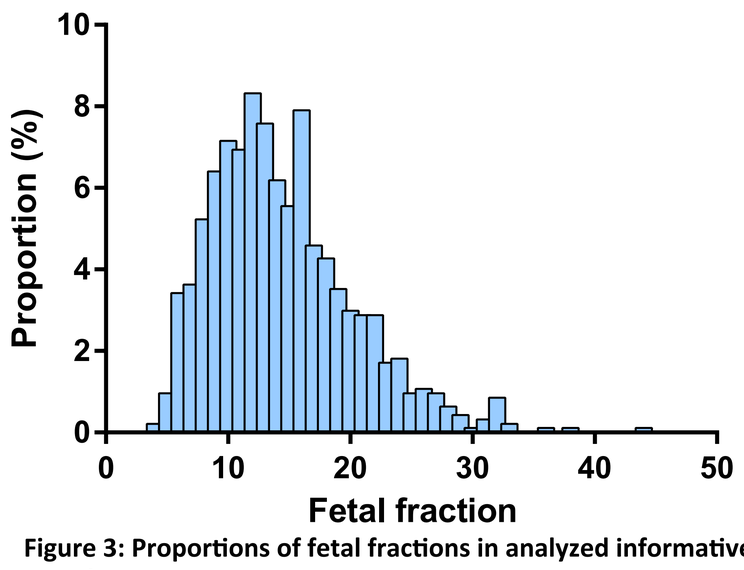

Figure 3: Proportions of fetal fractions in analyzed informative samples

NONINFORMATIVE SAMPLES

In case of 76 samples reported after 1st blood draw as uninformative, repeated blood draw was asked from relevant patients (blood redraw was asked two weeks - 2nd blood draw, and four weeks - 3rd blood draw, after the original one). The 3rd blood draw was asked if the fetal fraction increased between 1st and 2nd analysis but stayed below 5% and the week of pregnancy was not too high for performing confirmatory amniocentesis in the case of positive result. Out of the 76 samples 56 were reported as informative after 2nd and 3 samples after 3rd analysis (Figure 4). The most frequent reason of uninformative results after repeated blood draw and analysis were low fetal fraction in 11 samples, of which 3 samples were associated with increased body weight (> 90 kg) of pregnant women. In 1 case the combination of increased body weight and treatment by LMWH was observed (Figure 4). 6 pregnant women decided not to repeat the test after noninformative result of sample from 1st blood draw (Figure 4).

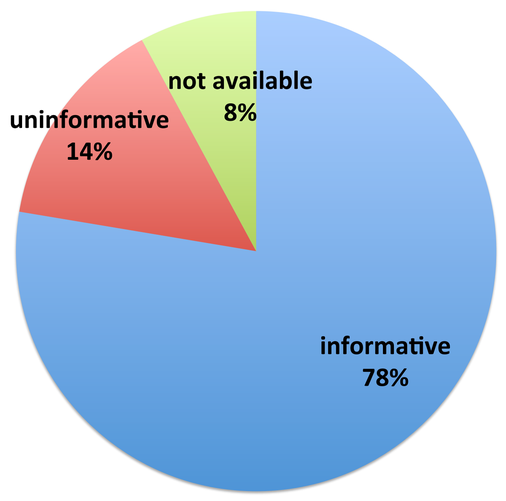

Figure 4: Trisomy test results of uninformative samples after 2nd and 3rd analysis.

CONCLUSION

Trisomy test performed in our laboratories and based on paired-end low coverage whole genome sequencing showed similar rates of sensitivity and specificity as presented in recently published metaanalyses [4, 5].

MATERIAL AND METHODS

From September 2015 till August 2016 altogether 1938 samples of pregnant women were analyzed. The mean age of women in our cohort was 34.8 years with age distribution showed on Figure 5. Periferal blood was taken using EDTA or Streck cfDNA tubes. Blood was centrifuged twice and DNA was isolated by QIAamp DNA Blood Mini Kit from Qiagen. Isolated DNA was quantified with Qubit dsDNA HS Assay Kit from Thermo Fisher Scientific. DNA libraries were prepared using TruSeq Nano DNA Library Prep Kit from Illumina with few modifications [6]. MiSeq Reagent Kit v3 (150 cycles) and MiSeq platform were used until end of June 2016, from July 2016 till August 2016 NextSeq 500/550 High Output Kit v2 (75 cycles) and NextSeq 500 platform were used for sequencing of libraries. Gained genomic data were analyzed by home-made bioinformatic pipeline, which calculated fetal fraction and z-scores by proprietary algorithm, that is currently patent pending.

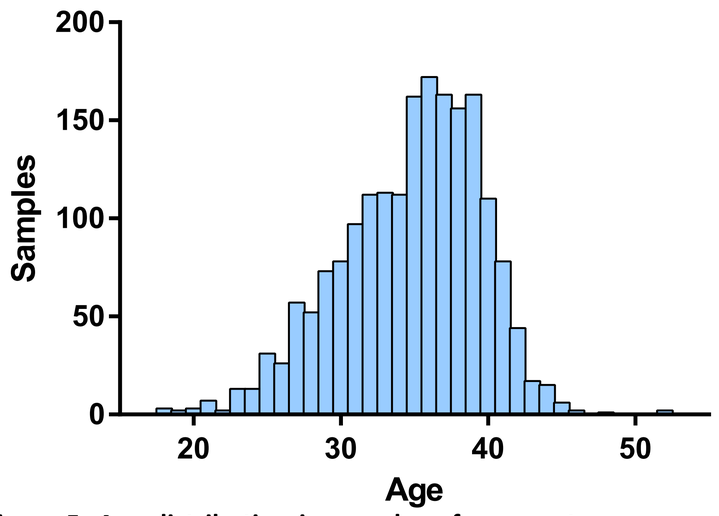

Figure 5: Age distribution in samples of pregnant women received for analysis.

References
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Authors disclose no conflicts of interest.