FIRST LABORATORY FOR NON-INVASIVE PRENATAL TESTING IN CROATIA: INSIGHTS BASED ON 3.5 YEARS OF CONTINUOUS OPERATION

Poliklinika Breyer

Laboratoriji za medicinsku biokemiju, mikrobiologiju i citologiju

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INTRODUCTION AND GOALS

Analysis of cfDNA in maternal plasma to determine fetal risk for chromosomal aneuploidies is an example of rapid and efficient clinical integration of NGS methodology in healthcare. By May 2022, more than 50 000 samples had been analyzed in the NIPT laboratory at Breyer.

The main goal was to establish continuous (24/7) laboratory operations while ensuring the highest quality results. Other objectives were to improve test parameters, to systematically follow up cases with rare findings and to assess preferences for prenatal testing in Breyer.

METHODS

Mother peripheral blood was collected in cfDNA preservation blood tubes (1) (Figure 1a), plasma was separated and cell-free DNA isolated using an automated liquid handler.

Prior to PCR amplification, DNA fragments underwent sticky ends fill-in and DNA barcoding. The amplified DNA was manually purified and quantified before pooling the same mass of all samples over > 2ng/µl. DNA was denatured and ligase was used to prepare DNA circles used as templates to create DNBs by RCA.

DNBs were placed on the patterned chip and WGS with CPAS was performed in conjunction with on-site bioinformatics and report validation (2) (Figure 1b).

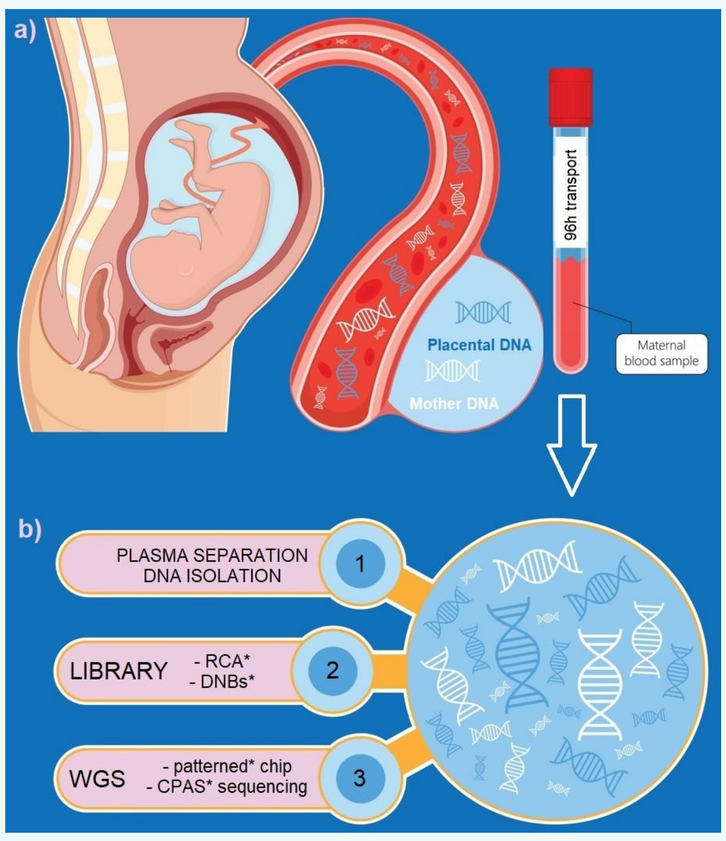

Figure 1. a) Maternal peripheral blood contains small percent of cell-free DNA fragments originating from placenta mixed with mother DNA fragments. Plasma is separated from whole blood to avoid white and red blood cells and to concentrate placental DNA for laboratory process. **b)** Laboratory process includes DNA isolation, preparation and amplification in order to prepare sufficient amount od DNBs for sequencing. Steps that reduce errors and improve results are marked with „*".

RESULTS

Patient basic clinical background - *Age*

One of the main indications for NIPT is the age of the patient, since women > 35 years are at higher risk for some chromosomal aneuploidies. However, our study shows that as many as 52% of patients under the age of 35 underwent NIPT.

Patient basic clinical background – *Gestational age*

90% of Breyer patients predominantly tested early in pregnancy: before 14 weeks and 0 days of pregnancy (14+0).

Laboratory accepts samples at least 8 weeks after VTS was confirmed by ultrasound if VTS occured before 8th week of pregnancy. 10% of patients that tested >14+0 were egligible to come for testing after 8 week waiting period following VTS.

NIPT test options distribution

In the period from January 2019 till May 2022, 2006 patients opted for NIPT testing in Breyer.

For 67% of patients (1342 patients) the whole laboratory analysis was performed in house (Figure 2a).

Breyer offers four testing options for patients based on different range of chromosomal abnormalities being reported as described on Figure 2b.
Most of the patients (59%) chose the widest testing option (Figure 2b).

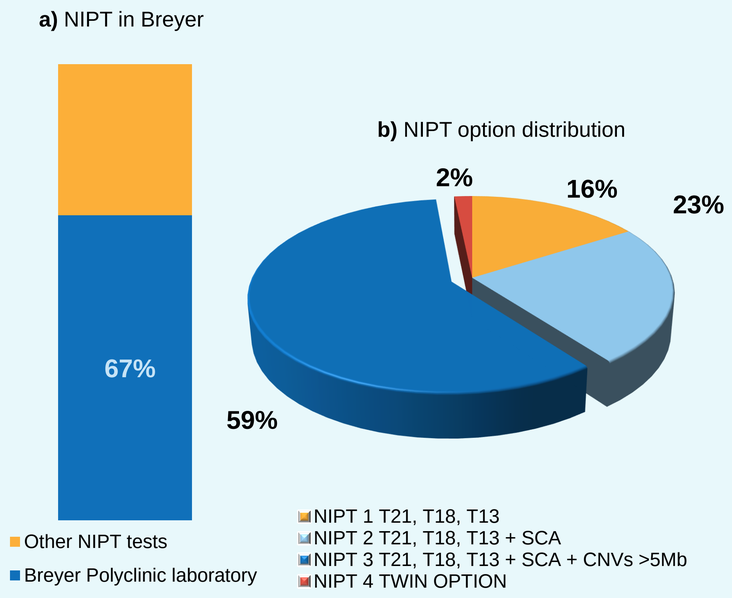

Figure 2. a) NIPT options for patients in Breyer. **b)** NIPT package distribution for Breyer patients. Statistics were generated using non-personal and non-medical data from Breyer.

Laboratory performance - TAT

Our data show following TAT for samples analyzed in Breyer: minimum waiting time was 3 days, while maximum waiting time for samples that required repeated laboratory analysis was 14 days. Average TAT is 6 days (Figure 3).

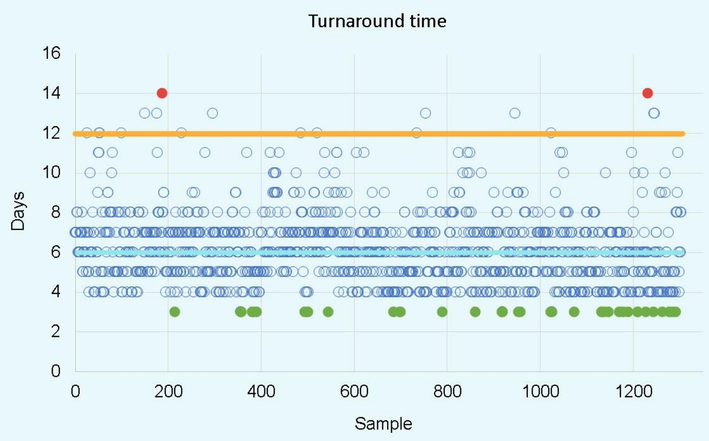

Figure 3. TAT for NIPT samples in Breyer. TAT for every NIPT sample (1342) is represented on the graph and includes non-working days. Minimum Breyer TAT is marked green, maximum red, blue line is Breyer average TAT, orange line is expected waiting time according to the informed consent. Statistics were generated using non-personal and non-medical data from Breyer.

Laboratory performance - *Rejected and redrawn samples*

Among 1342 samples, only 6 was rejected for NIPT analysis for reasons described in (Figure 4).

According to Wei Wang *et al, 2015* (3), in 2.18% of cases new sample is required in order to obtain NIPT result. From 1342 samples analyzed in Breyer, 19 had to be redrawn which equals to 1.4%.

Higher BMI (>30) is related to lower fetal fraction (4) that can cause failed analysis and repeated sampling. In Breyer laboratory new sample was needed for two patients >30 BMI following a successfull analysis.

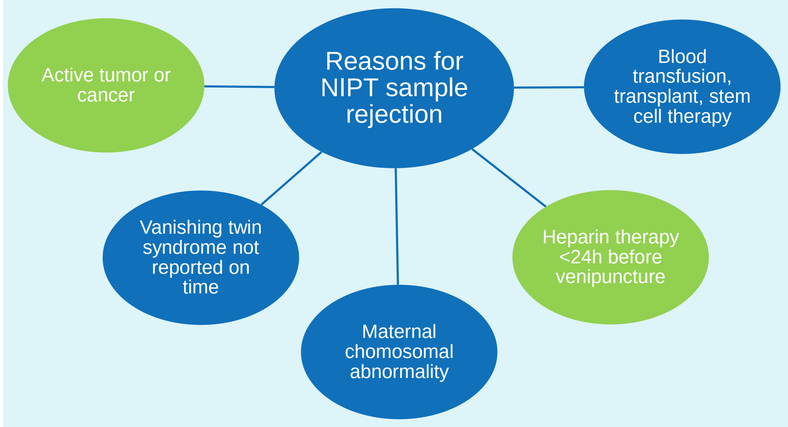

Figure 4. Overview of reasons to reject sample for NIPT analysis. Green marked reasons on image were documented in Breyer. Statistics were generated using clinical data from patients who gave informed consent.

High risk results

High risk result was reported in 27 patients (3%); 52% of were issued for patients < 35 years, while 22% for IVF pregnancies (Figure 5). High risk for common trisomies was reported for 17 patients: T18 (2) and T21 (15). Laboratory detected high risk for 2 rare trisomies (T15 and T16), sex chromosome aneuploidies: XO (1), XYY (1), XXY (3), XXX (1) and one duplication with influence of mother DNA (dup(xq24-q25.9), 74Mb).

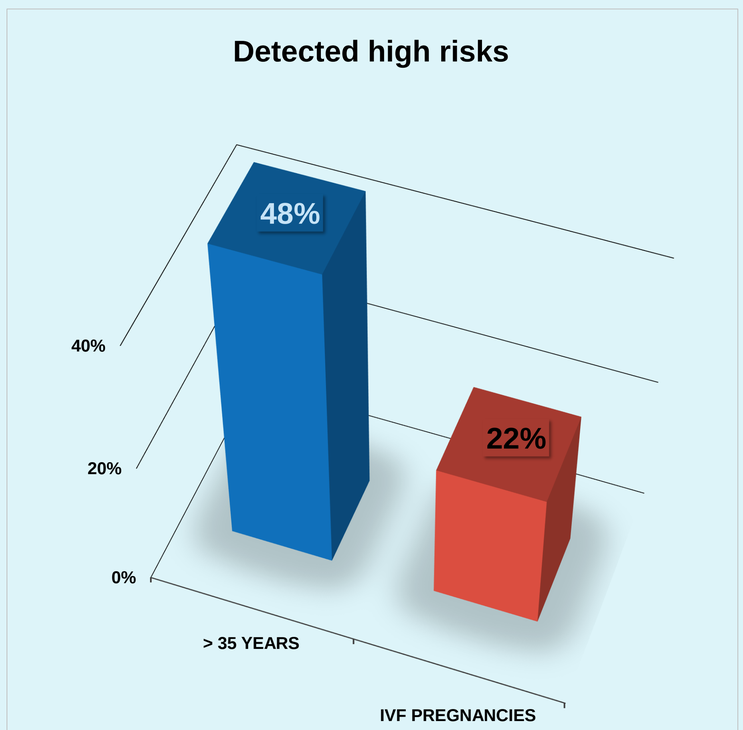

Figure 5. Distribution of samples for which high risk for chromosomal abnormalities was found. Statistics were generated using clinical data from patients who gave informed consent.

CONCLUSIONS

Real-time NIPT sample monitoring in one location is beneficial in several ways and provides initial insight into the patient testing preferences.

Our data show prompt laboratory analysis with significantly improved TAT and less rejected and redrawn samples than validation studies. Laboratory also succesfully analyzed all samples from patients with higher BMI and is currently the only laboratory that can analyze samples for some patients with VTS.

Patients decide to perform NIPT even if they do not belong to age-associated risk group; the distribution of high risks is equal among women over and under 35 years of age. Patients have preferences to perform NIPT in local laboratory rather than sending the sample abroad for analysis and they predominantly choose testing option that can provide broader spectrum of results.

LITERATURE

1 - Qin *et al*, (2014) 'Stabilization of circulating tumor cells in blood using a collection device with a preservative reagent', Cancer Cell International, 14(1):23. DOI: 10.1186/1475-2867-14-23
2 - Li *et al*, (2018) 'The application of NIPT using combinatorial probe-anchor synthesis to identify sex chromosomal aneuploidies (SCAs) in a cohort of 570 pregnancies', Molecular Cytogenetics, 11:59. DOI: 10.1016/j.cell.2018.08.016
3 - Wang *et al*, (2015) 'Non-invasive prenatal testing for trisomies 21,18 and 13:clinical experience from 146 958 pregnancies', Ultrasound Obstetrics and Gynecology, 45(5):530-8. DOI: 10.1002/uog.14792
4 – Livergood *et al*, (2017) 'Obesity and cell-free DNA "no calls": is there an optimal gestational age at time of sampling?', American Journal of Obstetrics and Gynecology, 216(4):413.e1-413.e9. DOI: 10.1016/j.ajog.2017.01.011

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ABBREVIATIONS

BMI - Body Mass Index
cfDNA - cell-free DNA
CNV- Copy Number Variation
CPAS - Combinatorial Probe-Anchor Synthesis
DNA – DeoxyribouNucleic Acid
DNBs – DNA NanoBalls
dup- duplication
IVF - In Vitro Fertilization
Mb - Megabase
NGS - Next Generation Sequencing
NIPT – Non-Invasive Prenatal Testing
PCR - Polymerase Chain Reaction
RCA - Rolling Circle Amplification
T – Trisomy
TAT - Turnaround Time
VTS - Vanishing Twin Syndrome
WGS - Whole Genome Sequencing
XO - Monosomy X (Turner Syndrome)
XXX - Trisomy X (Triple X Syndrome)
XXY - Klinefelter Syndrome
XYY - Jacobs Syndrome