Non-invasive prenatal cell-free fetal DNA testing for down syndrome and other chromosomal abnormalities

Neinvazivno predrojstveno testiranje proste plodove DNA za downov sindrom in ostale kromosomske nepravilnosti

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Izvleček

Izhodišča: Amniocenteza in biopsija horionskih resic kot dokončni diagnostični metodi sta zlati standard predrojstvene diagnostike za ugotavljanje kromosomskih nepravilnosti ploda. Metodi sta invazivni in v približno 0,5–1 % povzročita prekinitev nosečnosti zaradi spontanega splava po posegu. Neinvazivno predrojstveno DNA testiranje (NIPT) temelji na analizi proste plodove DNA iz krvi nosečnice. Je visoko zanesljiv presejalni test za odkrivanje najpogostejših kromosomskih nepravilnosti ploda. V naši raziskavi predstavljamo rezultate testiranja NIPT v Diagnostičnem centru Strah v zadnjih 3 letih.

Metode: V raziskavo je bilo vključenih 123 nosečnic med 11. in 18. tednom nosečnosti. Vsaka nosečnica je opravila priporočeni ultrazvočni pregled zgodnje morfologije ploda z merjenjem nuhalne svetline. Določeno je bilo bodisi nizko bodisi visoko tveganje za kromosomske nepravilnosti.

Rezultati: 5 od skupno 6 primerov, pri katerih je NIPT določil visoko tveganje (3 primeri Downovega sindroma in 2 primera Klinefelterjevega sindroma) je bilo potrjenih s kariotipizacijo. 1 primer – Edwardsov sindrom – je bil lažno pozitiven. Kromosomske nepravilnosti sindrom Patau, trojni X-sindrom ali Turnerjev sindrom niso bile zaznane pri nobeni nosečnici. O lažno negativnih primerih ni bilo poročano. Glede na podatke, pridobljene v raziskavi, je testiranje NIPT za vse omenjene kromosomske nepravilnosti pokazalo 100-odstotno občutljivost (95-odstotni interval zaupanja: 46,29 % – 100,00 %) in 98,95-odstotno specifičnost (95-odstotni interval zaupanja: 93,44 % – 99,95 %). Pri določanju le Downovega sindroma sta tako občutljivost (95 % interval zaupanja: 31,00 % – 100,00 %) kot specifičnost (95-odstotni interval zaupanja: 95,25 % – 100,00 %) enaki 100 %. V letu 2015 je bilo povprečno trajanje analize vzorca 8,3 dni od dneva odvzema krvi. V 2 primerih (1,6 %) je bil zaradi neuspešne analize potreben ponoven odvzem vzorca.

Zaključki: Naši rezultati potrjujejo, da je NIPT hiter, varen in visoko zanesljiv napreden presejalni test za določanje najpogostejših kromosomskih nepravilnosti pri plodu. NIPT bi v do sedaj uveljavljeni klinični praksi lahko značilno znižal število nepotrebnih invazivnih preiskav in s tem tudi število spontanih splavov, ki jih invazivna diagnostika lahko povzroči.

Abstract

Background: Chorionic villus sampling and amniocentesis as definitive diagnostic procedures represent a gold standard for prenatal diagnosis of chromosomal abnormalities. The methods are invasive and lead to a miscarriage and fetal loss in approximately 0.5–1 %. Non-invasive prenatal DNA testing (NIPT) is based on the analysis of cell-free fetal DNA from maternal blood. It represents a highly accurate screening test for detecting the most common fetal chromosomal abnormalities. In our study we present the results of NIPT testing in the Diagnostic Center Strah, Slovenia, over the last 3 years.

Methods: In our study, 123 pregnant women from 11th to 18th week of pregnancy were included. All of them had First trimester assessment of risk for trisomy 21, done before NIPT testing.

Results: 5 of total 6 high-risk NIPT cases (including 3 cases of Down syndrome and 2 cases of Klinefelter's syndrome) were confirmed by fetal karyotyping. One case–Edwards syndrome was false positive. Patau syndrome, triple X syndrome or Turner syndrome were not observed in any of the cases. Furthermore, there were no false negative cases reported. In general, NIPT testing had 100 % sensitivity (95 % confidence interval: 46.29 %–100.00 %) and 98.95 % specificity

(95 % confidence interval: 93.44 %–99.95 %). In determining Down syndrome alone, specificity (95 % confidence interval: 95.25 %- 100.00 %) and sensitivity (95 % confidence interval: 31.00 %–100.00 %) turned out to be 100 %. In 2015, the average turnaround time for analysis was 8.3 days from the day when the sample was taken. Repeated blood sampling was required in 2 cases (redraw rate = 1.6 %).

Conclusions: Our results confirm that NIPT represents a fast, safe and highly accurate advanced screening test for most common chromosomal abnormalities. In current clinical practice, NIPT would significantly decrease the number of unnecessary invasive procedures and the rate of fetal loss caused by invasive diagnostics.

Introduction

Aneuploidies represent a major cause of perinatal death and childhood handicap. Consequently, the detection of chromosomal abnormalities constitutes the most frequent indication for invasive prenatal diagnosis. However, invasive procedures, such as amniocentesis or chorionic villus sampling (CVS), are associated with a 0.5–1 % risk of fetal loss due to miscarriage. It is indicated only in pregnancies considered to be at high risk for aneuploidies. Screening by a combination of fetal nuchal translucency, maternal serum free-β-human chorionic gonadotropin and pregnancy–associated plasma protein-A, can identify about 90 % of fetuses with trisomy 21 and other major aneuploidies, with a false-positive rate of 5 %.[1] Similar results were found in Slovenia where detection rate is about 85 %, with a false-positive rate of less than 3 %.[2] Unfortunately, high rate of false positive screening results remains a major problem.

In recent years, advances in molecular biology have enabled the development of highly accurate non-invasive prenatal tests (NIPT), based on cell-free fetal DNA (cffDNA).[3,4] The discovery of cffDNA in maternal plasma in 1997 represents a key breakthrough to further progress that has been made in the field of non–invasive prenatal testing.[5,6] On average, 10–20 % of cffDNA is present in the maternal plasma. The proportion varies strongly and it depends on different factors, such as gestational period and BMI of the pregnant woman.[7] In the last decade, several methods of NIPT for detecting chromosomal aneuploidies in early pregnancy have been developed. The massively parallel sequencing (MPS) method has been indicated as the most accurate, appropriate and therefore most commonly used. Clinical studies have shown that the method of MPS together with its variations is suitable as a highly reliable screening for most common chromosomal aneuploidies in the first trimester of pregnancy[4,8] and later in gestation.

The experts agree that the NIPT method represents a highly accurate advanced screening test. Therefore, in the case of high-risk results, patients should still undergo one of

Table 1: Epidemiological data for study population (123 pregnant women).

Characteristic	Average	St. dev*	Median	Min	Max
Age	36.8	4.1	38	27	47
Weight	65.2	11.7	62	45	120
Height	168.1	5.2	168	155	180
Gravida	2	0.9	2	1	5
Twin pregnancy:	3 (2.4 %)				
Singleton pregnancy:	120 (97.6 %)				

** st. dev = standard deviation*

Table 2: Type of prior screening test.

Prior test:	$n_{pregnancies}$ (%)
1st trimester assessment of risk (other clinics)	2 (1.6 %)
1st trimester assessment of risk (age + NT*)	90 (73.2 %)
1st trimester assessment of risk (age + NT + bioch**)	31 (25.2 %)

** NT = nuchal translucency*
***bioch = biochemistry markers*

the conventional invasive diagnostic procedures.

First commercial NIPT tests were offered in the USA at the end of 2011. In our institution, we have performed the first NIPT test one year later. In this study, we present the implementation of NIPT testing and the results obtained in the Diagnostic Center Strah, Slovenia, over the last 3 years.

Methods

One hundred and twenty-three participants were included in a retrospective observational study between 5 February 2013 and 20 May 2015. Samples were taken at the gestational age from 11th to 18th week of pregnancy. One hundred and twenty women were pregnant with a single child, 3 of them were carrying twins. Epidemiological data are summarized in Table 1.

Conventional prior screening tests were performed in all participants. Ninety pregnant women had First trimester assessment risk, based on maternal age and fetal nuchal translucency. Thirty-one women had a combined screening test, based on maternal age, fetal nuchal translucency and biochemistry in the 1st trimester as well. Two participants came for the NIPT with the First trimester risk assessment results, done in other clinics (Table 2).

Indications for NIPT were advanced maternal age (37 years or older) or high-risk result (cut-off 1/300), based on First trimester assessment of risk for trisomy 21, 18 and 13. In addition, some pregnant women with none of the high risk factors opted for NIPT on demand.

Pre-test counseling was provided to all the participants. Written informed consent was obtained before blood sampling. 10 mL of venous blood sample from each woman was used for NIPT molecular testing for T21, T18, T13 and sex chromosome aneuploidies. Samples were analyzed at BGI Diagnostic Laboratories. Pregnant women with high-risk NIPT results were sent to genetic counseling and were advised to undergo the invasive diagnostic procedure. Pregnant women with low-risk results underwent routine antenatal care, provided by their obstetricians. Telephone interviews were performed in women with high-risk results and in women with low-risk result who had given birth until 30 May 2015 in order to find out the outcome of pregnancy.

Statistical analyses were performed. Sensitivity, specificity and positive predictive value were calculated based on following formulas:

$$SEN = TP / (TP + FN);$$

$$SPC = TN / (TN + FP);$$

$$PPV = TP / (TP + FP);$$

SEN = sensitivity; SPC = specificity; PPV = positive predictive value; TP = true positive; FN = false negative; TN = true negative; FP = false positive.

Study was approved by the Slovenian Ethic Committee.

Results

NIPT was performed in 123 pregnant women. Seventy-six women (61.8 %) were 37 years of age or older (advanced maternal age). Twenty-one women (17.1 %) had high-risk results for T21, T18 or T13, based on prior screening testing. Thirty-nine women (31.7 %) with no high-risk factors decided to

Table 3: Indications for NIPT.

Indication	$n_{pregnancies}$ (%)
High-risk result at prior testing (> 1:300)	21 (17.1 %)
Age (37 and more)	76 (61.8 %)
High-risk result at prior testing + age (37 and more)	13 (10.6 %)
No indication	39 (31.7 %)

undergo NIPT (low-risk population). Indications for NIPT are summarized in Table 3.

High-risk NIPT results were found in 6 cases including 3 cases of T21, 1 case of T18 and 2 cases of XXY. Five of them were confirmed by subsequent fetal karyotyping, while case T18 was found to be false positive. T13, XXX or Xo were not observed in any of the cases. Furthermore, there were no false negative cases reported.

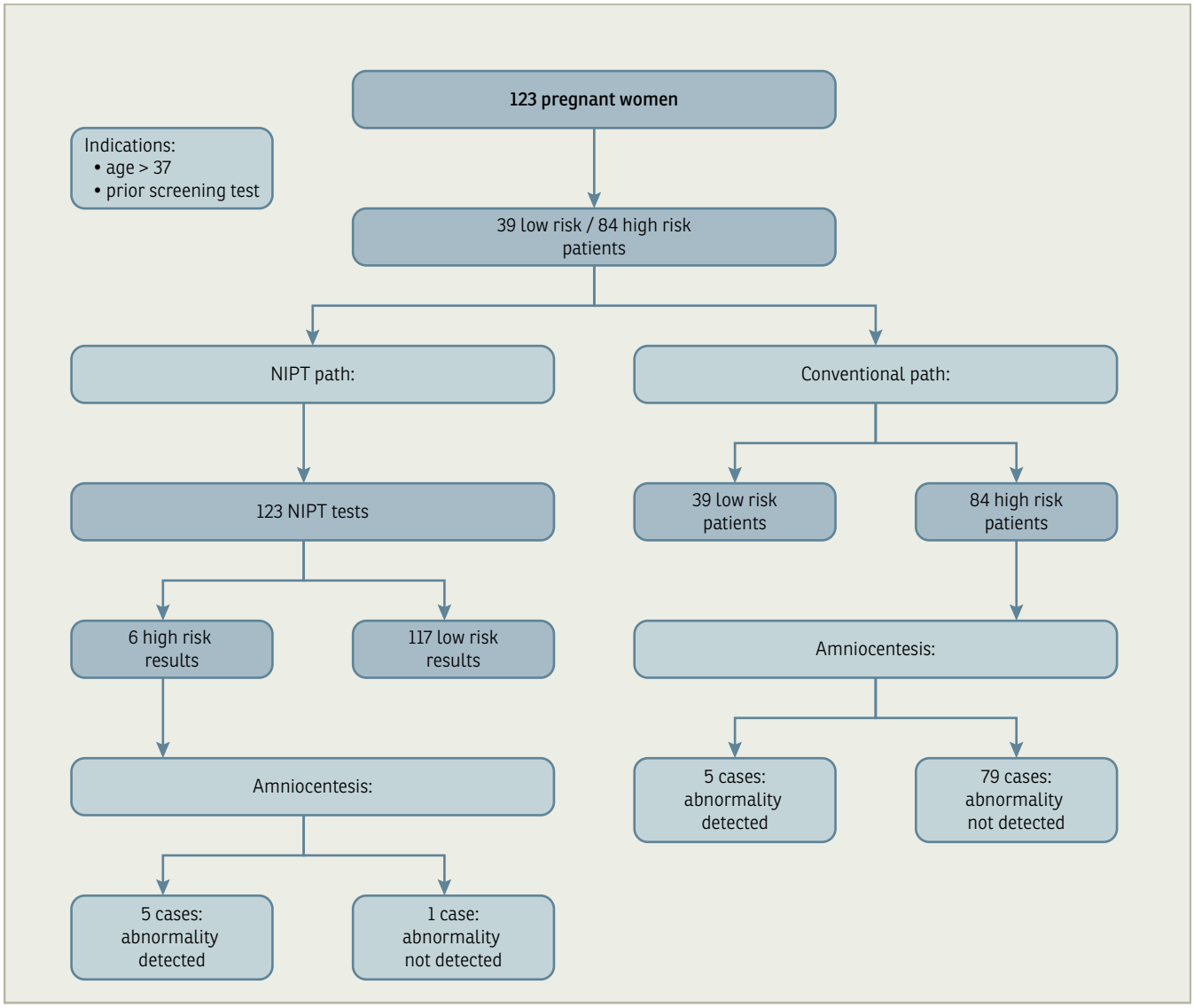
Specificity, sensitivity and positive predictive value were calculated based on 94 born children and 6 cases that were confirmed by diagnostic procedure (Table 4). NIPT test shows 100 % sensitivity (95 % confidence interval – 95 % CI: 46.29 %–100.00 %) and 98.95 % specificity (95 % CI: 93.44 %–99.95 %). According to a separate analysis for T21 alone, NIPT was 100 % sensitive (95 % CI: 31.00 %–100.00 %) and 100 % specific (95 % CI: 95.25 %–100.00 %). In 2015, the average turnaround time was 8.3 days from the day when the sample was taken. Repeated blood sampling was required in 2 cases (redraw rate = 1.6 %).

Out of 123 women, six with high-risk NIPT results underwent invasive diagnostic procedure (amniocentesis). Their characteristics (age and prior risks) are summarized in Table 5. In 5 of 6 cases, the high-risk NIPT results were confirmed. One case was false positive. Without NIPT, 84 high-risk pregnant women would undergo invasive diagnostic procedures (amniocentesis), which would result in 79 unnecessary amniocenteses, as shown in Figure 1.

Table 4: NIPT test statistics.

Average turnaround time in 2014	10.6 days			
Average turnaround time in 2015	8.3 days			
Redraw rate	2 (1.6 %)			
True positive	5 (83.3 % of all high risk results)			
False positive	1 (16.7 % of all high risk results)			
True negative	94 (100 % of all low risk results and born yet)			
False negative	0 (0 % of all low risk results and born yet)			
Specificity (95 % CI)	98.95 % (93.44 %–99.95 %)			
Sensitivity(95 % CI)	100.00 % (46.29 %–100.00 %)			
Positive predictive value (95 % CI)	83.33 % (36.48 %–99.12 %)			
Comparison between NIPT outcome in high-risk (advanced age or prior testing) and low-risk population				
Population	TP	FP	TN	FN
Low-risk (no indication):	0	0	30	0
High-risk (indication):	5	1	64	0

* CI = confidence interval



Picture 1: Comparison between NIPT screening and diagnostic path which include NIPT and hypothetic conventional path without NIPT, assuming that amniocentesis would be performed to all high-risk pregnant women.

Discussion and conclusions

We present the results of NIPT testing in the Diagnostic Center Strah, Slovenia, over the last 3 years. NIPT in our clinical study showed high sensitivity (100.00 %; 95 % CI: 46.29 %–100.00 %) and specificity (98.95 %, 95 % CI: 93.44 %–99.95 %) for both high- and low-risk population of pregnant women. With respect to T21–Down syndrome alone, sensitivity (95 % CI: 31.00 % – 100.00 %) and specificity (95 % CI: 95.25 % – 100.00 %) were both 100.00 %. The results are consistent with other validations and clinical studies analyzing NIPT in other population groups. [8,9] For example, Zhang et al. suggest 99.02 % sensitivity and 99.86 % sensitivity for T21, T18 and T13 detection. [8] In addition, our data show a very low redraw rate (1.6 %) and short turnaround time (8.3 days).

Without NIPT, amniocentesis or other invasive methods would be recommended in all high-risk pregnancies. According to our study population, only 6.0 % of high--risk pregnant women (5 of 84) carried the fetus with chromosomal aneuploidy. Ninety-four percent (79 of 84) of them would be exposed to the risk of fetal loss due to invasive diagnostic procedure. On the contrary, in 83.3 % (5 of 6) of total high-risk NIPT the result was confirmed. One case (16.7 %) out of 6 high-risk NIPT was found to be false positive.

Amniocentesis still represents a gold standard in prenatal care. It is a diagnostic

Table 5: High risk NIPT case characteristics

	Age	Prior T21 risk	Prior T18 risk	Prior T13 risk	NIPT result	Amniocentesis result
Patient 1	29	1 : 37	1 : 18265	1 : 18998	T21	T21
Patient 2	43	1 : 78	1 : 821	1 : 1656	T21	T21
Patient 3	38	1 : 830	1 : 1624	1 : 828	T18	normal karyotype
Patient 4	38	1 : 3049	1 : 7163	1 : 20000	XXY	XXY
Patient 5	41	1 : 874	1 : 977	1 : 4462	XXY	XXY (mosaic 90 %, normal 10 %)
Patient 6	38	1 : 784	1 : 1860	1 : 2660	T21	T21

method that directly analyzes and counts chromosomes in fetal cells and can detect any aneuploidy or other chromosomal abnormalities. Nevertheless, it is an invasive method associated with a 0.5–1 % risk of fetal loss due to miscarriage. Amniocentesis cannot be performed before the 16th week of pregnancy, and it takes another 3 weeks to get the results. In addition, the method is unpleasant and very stressful for the pregnant woman.[1,10] On the contrary, NIPT, which can be performed as early as in the 10th week of pregnancy, does not represent any risk or stress for the pregnant woman or the unborn child. The results are available in 10 days after blood sampling. There are two main disadvantages of NIPT: first, the test costs are not covered by insurance, and second, the majority of fetal DNA in maternal blood is derived from the placenta and not directly from fetus, therefore the method is indirect and there is, although very low, a possibility for false positive and also false negative results.[3,4,8]

The main limitation of this study is a low sample size; considering 95 % confidence interval, the sample size is large enough for the assessment and evaluation of specificity; however, the 95 % confidence interval is too wide for the evaluation of sensitivity. In addition, pregnant women included in the study were not representative for the population as many of them were at high risk according to their age or prior screening tests. For example, positive predictive value of NIPT may be statistically significantly lower in low-risk population than in high-risk population. Nevertheless, we can say that the results of our study have confirmed that NIPT represents a highly accurate non-invasive approach to screening for Trisomy 21 and other most common aneuploidies. In current clinical practice, NIPT would significantly decrease the number of unnecessary invasive diagnostic procedures. Fetal loss caused by invasive procedures, mostly amniocenteses, would significantly decrease as well.

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