Single Nucleotide Polimorphism-based non-invasive prenatal screening for fetal aneuploidies: clinical utility and follow up, experience of one centre.

Authors: Anamarija Brezigar MD, Anja Plot MS
Medgen Centre for Medical Genetics, Ljubljana, Slovenia

Objective

We reviewed the results of 766 consecutive blood samples analysed with SNP based non-invasive prenatal screening from May 2014 to March 2017, taken from patients who attended our clinic for genetic counseling for non-invasive prenatal screening.

Methods

756 women with singleton pregnancy were counseled and, after providing informed consent, enrolled for cell-free NIPS screening. Patients selected among three different NIPS test extension variants. Blood samples were sent out for cell-free DNA analysis based on single nucleotide polymorphisms to Natera's San Carlos laboratory without prior knowledge of karyotype or outcome. Patients gave birth in local obstetric departments. Invasive karyotyping was recommended to all patients with high risk NIPS and performed in 3 Slovenian diagnostic centres. Blood samples were redrawn when first analysis did not generate a result. Newborns were examined by neonatologists. Mothers were interviewed about pregnancy outcome and the medical data was reviewed by the first author in cases of adverse pregnancy outcome, miscarriage, intrauterine death, or fetal structural anomalies. The screening performance was evaluated.

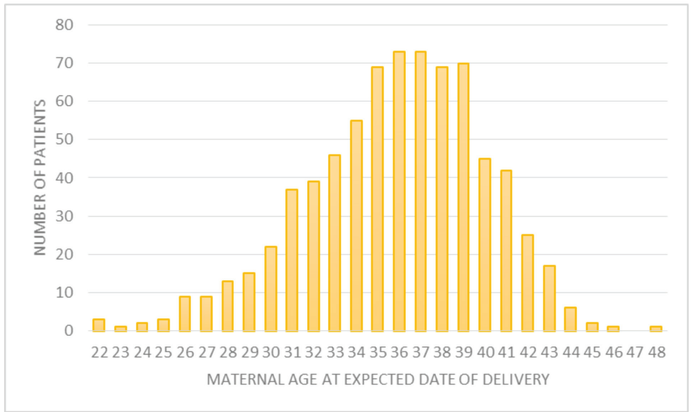

Figure 1: Maternal age at expected date of delivery. Median maternal age was 36 years (from 22 to 48 years).

NIPS TEST EXTENSION VARIANTS	SCREENING OPTIONS	No. SAMPLES
BASIC	tri 13, tri 18, tri 21, monosomy X, sex chromosome trisomies, triploidy/ vanishing twin, fetal sex (optional)	331
BASIC PLUS	tri 13, tri 18, tri 21, monosomy X, sex chromosome trisomies, triploidy/ vanishing twin, fetal sex (optional), DiGeorge syndrome del 22q11.2	63
EXTENDED	tri 13, tri 18, tri 21, monosomy X, sex chromosome trisomies, triploidy/vanishing twin, fetal sex (optional), DiGeorge syndrome del 22q11.2, Prader-Willi syndrome, Angelman syndrome, del 1p36, Cri-du-chat syndrome	362

Table 1: Test extension selected after counseling. 331 (44%) women selected the basic aneuploidy test, 63 (8%) the aneuploidy test with del 22q11.2 and 362 (48%) the aneuploidy test with five microdeletions.

Medgen
Medicinska genetika
Družba za zdravstvene dejavnosti d.o.o.
Ulica Bratov Babnik 10, 1000 Ljubljana

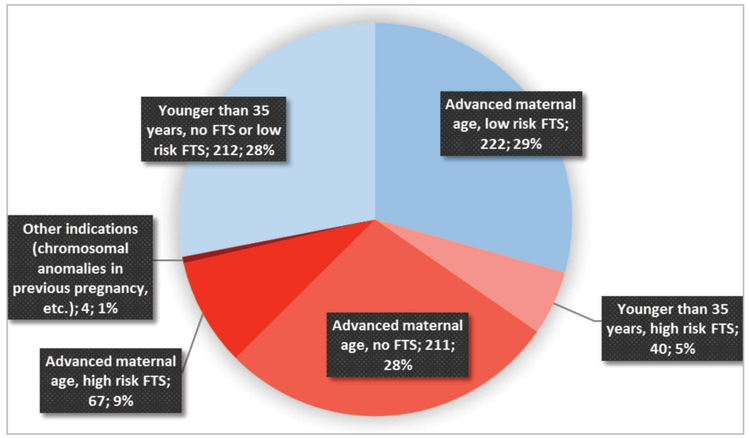

*Figure 2: 322 (42,6 %) high risk for trisomies and 434 (57,4%) low risk pregnant women with singleton pregnancy were enrolled. Average length of gestation at sampling was 13 weeks 1/7 (from 9 2/7 to 30 4/7). *FTS- first trimester screening. **Blue shades represent low risk cases while red shades represent high risk cases.*

Results

322 (42,6 %) pregnant women with high risk for trisomies and 434 (57,4%) low risk pregnant women with singleton pregnancy were enrolled. Average length of gestation at sampling was 13 weeks 1/7 (from 9weeks 2/7 to 30 weeks 4/7), average maternal age at expected date of delivery was 36 years (from 22 to 48 years), average BMI was 22,9 (from 16,8 to 48,8).

For 620 women there is known outcome. 604 patients already gave birth in local obstetric departments. All of those who received low risk results reported no clinical trisomy in babies born or in follow up diagnostic karyotyping in adverse pregnancy outcome. 11 mothers (1,5%) received high risk report for aneuploidy result, 10 of them further pursued the diagnosis by karyotyping. All the diagnosis were confirmed. One of them with high risk result for tri 21 refused further diagnostic testing, ultrasound of fetus at examination at 22nd week of pregnancy showed large ventricular septum defect, pregnancy is ongoing. Correct diagnosis seems likely. 5 pregnancies didn't come to term; of those, 3 low risk pregnancy results were confirmed normal by aCGH, 2 low risk were terminated because of anencephaly - spina bifida defects. Overall redraw rate was 1,2%.

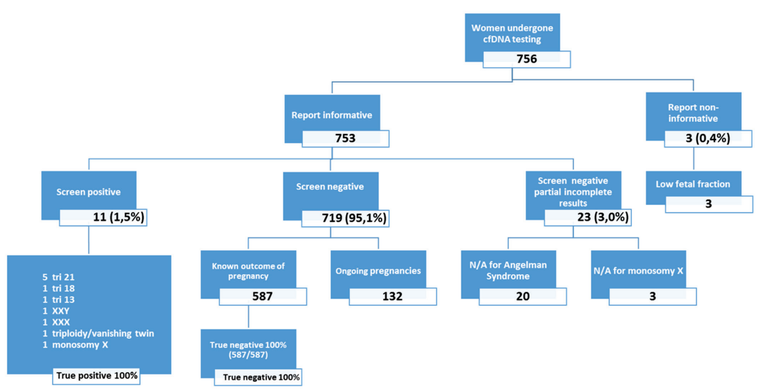

Figure 3: Flowchart summarizing test results.

3 (0.4%) samples returned with no call result, all because of low fetal fraction. All microdeletion analysis were reported as low risk. No baby showed clinical signs of the 5 microdeletion syndromes studied and none were reported as such later.

No call results	Age (year)	Gestation age (week)	Fetal fraction (%)	BMI	Year of test performed
Patient A1	39	12 3/7	3,6	37,8	2014
Patient A2	44	10	1,1	18,6	2016
Patient A3	36	15 2/7	2,0	25,2	2016

Table 2: No call result details. First patient who had BMI 37,8, diagnostic karyotype was normal, the second declined all further testing and delivered a healthy child, the third miscarried.

Non-informative results for monosomy X	Fetal sex	Gestation age (week)	Fetal fraction (%)	US chek-up	Prenatal kariotyping	Year of test performed
Patient B1	non-informative	15 6/7	14,9	Yes	46 XY	2014
Patient B2	female	15 3/7	7,4	Yes	No	2016
Patient B3	female	16 2/7	13,6	Yes	No	2016

Table 3: Details of the non-informative results for monosomy X. All of them delivered healthy babies.

Low risk reports with no result for monosomy X were received for 3 patients (0,5 %). Redraw was not recommended. For that reason we recommended expert ultrasound check- ups with emphasis to exclude cystic hygroma, hydrops, subcutaneous edema, narrowed aortic arch, renal anomalies or short femur and sex of the fetus. We also proposed patients to do invasive prenatal karyotyping which was elected by one patient. All of them delivered healthy babies.

Non-informative results for Angelman syndrome	Gestation age (week)	Fetal fraction (%)	US chek-up	Year of test performed
Patient C1	12 4/7	5,9	Yes	2015
Patient C2	9 3/7	6,1	Yes	2015
Patient C3	14	6,2	Yes	2015
Patient C4	12 1/7	6,0	Yes	2015
Patient C5	16 4/7	5,1	Yes	2016
Patient C6	12 1/7	6,4	Yes	2016
Patient C7	12 3/7	4,2	Yes	2016
Patient C8	12 2/7	6,0	Yes	2016
Patient C9	21	5,6	Yes	2016
Patient C10	12 2/7	6,0	Yes	2016
Patient C11	10 1/7	4,7	Yes	2016
Patient C12	16 1/7	5,3	Yes	2016
Patient C13	14 2/7	5,4	Yes	2016
Patient C14	12 4/7	8,3	Yes	2016
Patient C15	10 1/7	6,8	Yes	2016
Patient C16	12 5/7	5,7	Yes	2016
Patient C17	10 4/7	4,9	Yes	2016
Patient C18	12 4/7	6,7	Yes	2017
Patient C19	13 4/7	6,7	Yes	2017
Patient C20	9 5/7	5,8	Yes	2017

Table 4: Relatively low median fetal fraction 5,95 % was detected in non-informative results for Angelman syndrome.

All 362 patients who selected microdeletions screening received low risk report. 20 reports were risk unchanged for Angelman syndrome. Relatively low median fetal fraction 5,95% was observed in these samples. Apart from regular ultrasound check-ups no additional diagnostic testing was performed in these pregnancies. 16 patients delivered healthy babies with no clinical signs of Angelman syndrome. 4 pregnancies are ongoing. All babies born to these 362 patients showed no clinical signs of any of the 5 microdeletion syndromes studied at birth and none were reported later.

Conclusion

No false positive or false negative cases were found in this cohort of 620 patients with known outcome. Skilled post-test counseling proved to be essential for best pregnancy outcome. The results of our study confirm that the non-invasive prenatal cell- free fetal DNA SNP-based screening test has a very high detection rate.