Analysis of foetal DNA in the woman's blood: non-invasive prenatal testing (NIPT) for trisomy 13, 18 and 21

SFOG Guidelines 2016, developed by Ultra ARG interdisciplinary

This SFOG guideline effort describes the non-invasive prenatal testing (NIPT) for detecting trisomy 13 (Pataus syndrome), 18 (Edward's syndrome) and 21 (Downs syndrome). The clinical recommendations from this guideline effort were prepared following GRADE (1) with a foundation from SBU Alert 2015-03 (2), where in-depth information with explanations and references are found. The guidelines were prepared in accordance with the current state of knowledge and are valid until the publication of a re-evaluation by SFOG.

Recommendation for the use of NIPT in Sweden

Information about and access to prenatal testing shall be offered to all pregnant women regardless of age. An ultrasound examination at the end of the first trimester (weeks 12-13), to verify the viability, gestational age, multiple pregnancy and identification of foetal abnormalities, should be a first step for further prenatal testing. The ultrasound examination should preferably be performed as CUB (Combined Ultrasound and Biochemistry). The results of CUB then constitute the basis for further investigation with NIPT as indicated below:

With a CUB probability of ≥1 / 50, invasive prenatal testing is offered. Analysis of the full karyotype or microarray should be considered. The majority of trisomies are identified in this group, but also chromosomal abnormalities of clinical significance that are not detected with NIPT.

CUB probability of 1/51 – 1/1000, NIPT is offered.

CUB probability of <1/1000, no further action besides the basic MHV programme.

If NIPT indicates a chromosomal abnormality, invasive sampling shall be offered.

NIPT should be offered to women who had previous pregnancies with trisomies and to women for whom foetal testing is desired but invasive testing should be avoided, for example, chronic infection (HIV, hepatitis).

In multiple pregnancies, the scientific evidence for NIPT is still insufficient. The offer of NIPT in multiple pregnancies should therefore be made after careful consideration.

For ethical reasons, it is important that NIPT is used for the analysis of a few clearly defined abnormalities. The introduction of the method shall not result in offering the test for other abnormalities and disease conditions than specified, without renewed medical and ethical evaluation. If an analysis of sex chromosome abnormalities is performed with NIPT, the woman shall be informed that the current scientific basis is insufficient and that the analysis has a significantly lower accuracy than for trisomy 21.

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Issue

Can NIPT (cffDNA), as a non-invasive method, identify with high accuracy trisomy 13, 18 and 21 in an unselected population and a population with increased probability of chromosomal abnormalities?

Conclusions based on the scientific evidence

Grading of evidence according to GRADE (1)

Among women with **increased likelihood** (high-risk population) for chromosomal abnormalities in the foetus, the following applies:

- for **trisomy 21**: There is moderately strong scientific evidence that NIPT almost always gives correct diagnosis of the existence of a chromosomal abnormality in the foetus or that such can be excluded. (GRADE ⊕⊕⊕◯)
- for **trisomy 18**: There is moderately strong scientific evidence that NIPT almost always gives correct diagnosis of the existence of a chromosomal abnormality in the foetus or that such can be excluded. (GRADE ⊕⊕⊕◯)
- for **trisomy 13**: There is limited scientific evidence that NIPT often gives correct diagnosis that a deviation exists or that such can be excluded. (GRADE ⊕⊕◯◯)

Among **other** women, i.e. women who **do not have an increased probability** (general population) for chromosomal abnormalities in the foetus, the following applies:

- for **trisomy 21**: there is moderately strong scientific evidence that NIPT almost always gives correct diagnosis that a deviation exists or that such can be excluded. (GRADE ⊕⊕⊕◯)
- The method's performance in the case of trisomy 18 and trisomy 13 in this group cannot be assessed because there are too few and too small studies. (GRADE ⊕◯◯◯).

Clinical recommendations below are based on current scientific evidence in relation to the test method's costs and assessed benefit to patients, as evaluated using GRADE (1).

1. To reduce the number of invasive tests with associated risk of miscarriage, NIPT is the recommended method for assessing the likelihood of trisomy 13, 18 and 21.
STRONG recommendation

2. NIPT as well as other prenatal testing should be offered to pregnant women, regardless of age (3).
STRONG recommendation

3. With the current state of knowledge, NIPT should be preceded by an ultrasound examination (e.g. CUB) in the first trimester.
STRONG recommendation

4. The caregiver who offers prenatal testing is responsible for making sure that staff have training and competence, as well as can convey the survey results in a non-biased and professional manner.
STRONG recommendation

5. NIPT should only be offered after detailed information is provided, and you can ensure that the woman has had the opportunity and time to think about it in order to make an informed choice.
STRONG recommendation

6. NIPT and other prenatal testing may not be offered in order to determine the sex of the foetus, unless there is a genetic predisposition for a hereditary sex-linked disorder in one of the biological parents. If the foetus' sex is apparent on examination, information about the sex is to be provided only if the pregnant woman so requests (3).
STRONG recommendation

7. If NIPT indicates trisomy 13, 18 or 21, it shall be verified by invasive test.
STRONG recommendation

8. The caregiver is responsible for making sure that the NIPT and other prenatal testing is reported and quality assured with the national registry.
STRONG recommendation

NIPT requires training efforts

The simplicity of a blood sample from the pregnant woman for prenatal testing places new demands on maternity healthcare's capacity to, in a non-controlling way, guide the parents-to-be to make informed choices regarding prenatal testing and its consequences. Specific demands are placed on how the actual offer of prenatal testing is formulated and communicated so that the voluntary nature is clearly ensured. The information shall be formulated in accordance with Chapter 4 of the National Board of Health and Welfare's regulations and general advice on prenatal testing and pre-implantation genetic diagnosis (4). A need for improved information is emphasised in the SBUs report "Methods for early prenatal testing, a systematic literature review" (3). Special resources are necessary to ensure that parents-to-be are guided to informed choice regarding NIPT.

Background to the work on the guidelines

Prenatal testing is aimed, based on the woman's informed choice, at identifying foetal malformations, genetic abnormalities, and pregnancy complications that are of importance for the decision to continue the pregnancy as well as to optimise the treatment during pregnancy and upon the child's birth.

Current available methods are ultrasound diagnostics, biochemical methods including CUB and invasive procedures (amniocentesis and chorionic villus sampling). Today, there is a wide variation in the use and availability of these methods in Sweden.

In recent years, a genetic analysis method for trisomy 13 (Pataus syndrome), 18 (Edward's syndrome) and 21 (Downs syndrome) has been developed. The method, called non-invasive prenatal testing (NIPT), involves analysis of cell-free foetal DNA (cffDNA) in the maternal blood. NIPT can be used for diagnosis of various conditions in the foetus and is already used in Sweden for analysis of the foetal blood type. NIPT for these trisomies can be performed from 9-10 weeks of pregnancy, but the earliest accepted sampling day varies between laboratories. The method's high accuracy means that the need for invasive procedures may decrease compared to the current procedures, but abnormal findings shall be verified by an invasive test.

Legislation, National Board of Health and Welfare's regulations and general advice 2012: 20, reports from the Swedish National Council on Medical Ethics (SMER) and the Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU) (2,3,4,5) lay the foundation for this work on the guidelines. The scientific evidence is therefore reported only briefly in this document and is found with detailed explanations, via a link to SBUs website (http://sbu.se/sv/Publicerat/Alert/Analys-av-foster-DNA-i-kvinnans-blod-icke-invasiv-fosterdiagnostik-NIPT-for-trisomi-13-18-och-21/) and from SFOGs website under Guidelines. The proposed guidelines are developed by a multidisciplinary group representing SFOGs working group for ultrasound, ethics, maternity healthcare and perinatal medicine as well as the Swedish Society of Medical Genetics (SFMG) and SBU. A representative from the Swedish National Council on Medical Ethics (SMER) has participated in the working group meetings. The proposal has been revised following feedback from the Swedish National Down Syndrome Association and the Swedish National Association for Persons with Intellectual Disability (FUB).

Rationale and discussion for the clinical recommendation proposal

The proposal is based on the current state of knowledge and cost of NIPT analysis (cffDNA). For anyone (regardless of age), who, after adequate information, wishes to have prenatal testing in the first trimester, NIPT is considered to be the best choice for chromosomal abnormalities and ultrasound examination for abnormalities. Ultrasound examination also includes the diagnosis of miscarriage, multiple pregnancies and determination of gestational age. As long as the cost for NIPT is high, it is not considered economically feasible to offer NIPT as the first screening method to all who request prenatal testing. To offer NIPT after a probability assessment in the form of CUB, which is a well-established method and routinely

used in most of the 21 regional councils, can be essentially cost neutral. Such an implementation method has so far been the most common internationally.

Rationale for the clinical recommendation:

- Invasive sampling at a CUB probability of ≥1 / 50

Reduced number of invasive tests compared with today's routine.

Invasive testing offered when CUB shows a probability of ≥1 / 50 results in a clear reduction in the number of invasive tests compared with today's routine (invasive test at a probability of > 1 / 200-300).

The majority of trisomies are in this group, and a positive NIPT shall be verified with an invasive test.

According to the Pregnancy Registry, 79% of all trisomy 21 is found in the group probability according to CUB ≥1 / 50. If NIPT is offered instead of an invasive test, a positive result shall still be verified with an invasive test even if the positive predictive value of NIPT is high for trisomies (50–80%). If the analysis is done with full karyotype, other rare but clinically significant chromosomal abnormalities can be detected, which today's NIPT cannot detect. Two studies indicated that 23% and 17%, respectively, of clinically significant chromosomal abnormalities, were not trisomies in a prenatally screened high-risk group, and those who underwent invasive sampling with karyotype (6.7). These abnormalities are therefore not detected with NIPT. The percentage of abnormalities that are not trisomies is estimated at about just under 2% in the group with a probability according to CUB ≥1 / 50. When using micro-array analysis, it is expected that a number of additional chromosomal abnormalities can be identified.

- NIPT (cffDNA) at a probability according to CUB 1 / 51 – 1 / 1000

NIPT offered with the probability according to CUB 1 / 51 – 1 / 1000 increases the detection rate of trisomies (about 16% of trisomy 21 according to the pregnancy Registry). The cost increase is estimated at about 1 million / 10,000 pregnant women who undergo prenatal testing. To increase the group that is offered prenatal testing from 1/200 to 1/1000, it increases the identification of trisomy 21 by approximately 6%, but stigmatises a greater number of women than with today's CUB-routine (about 11% of those who do CUB). At the same time, there is probably a relatively large demand for prenatal testing in this group where NIPT, to some extent, can reduce anxiety and thus reduce the number of invasive tests. In Stockholm, in 2013, the proportion of invasive tests because of age / anxiety was 44% and after CUB only 31%. The group that dropped out tested positive in a screened population with NIPT which is extremely low (0.1%), and about 80% of those who dropped out were positive as mentioned above, true positive. Most analytical methods nowadays have fewer failed tests where a result is not received (0.5–3%).

Today, there are some counties that do not offer CUB. It is unclear how many women would accept an offer of prenatal testing according to the above proposal. In those counties where CUB is offered today, the proportion who accept varies between 15% to over 90%, and there is a greater proportion of women 35 years or older compared to the entire pregnant population (general population) who accept.

The outcome of the screening is influenced by the composition of the studied population (unselected normal population, selected low-risk and selected high-risk). The following table is designed to provide a picture of the possible effects in different populations upon offer of prenatal testing with NIPT according to the guideline model. The comparative groups are based on the Swedish Pregnancy Registry and the Foetal Medicine Foundation (FMF) records for region Östergötland. In order not to make the reporting too complex, at this stage only the detection of trisomy 21 is used according to the CUB calculation programme that is used in Sweden today. Assuming that all cases with high probability and positive NIPT undergo confirmatory invasive sampling, sensitivity for NIPT is 100%, and percentage of false positives is 0.1%, so the model provides the following effect based on:

1. Pregnancy Register, high-risk group (42% ≥ 35 years). For the years 2006–2013, n = 106,577; 463 cases with trisomy 21 (1/230).
2. Pregnancy Register, selected low-risk group (only women under 35 years). For the years 2006–2013, n = 57,670; 94 cases of trisomy 21 (1/613).
3. Region of Östergötland, close to the normal population (high proportion that undergoes CUB and non-age-controlled). For the years 2008–2014, n = 24,101; 59 cases of trisomy 21 (1/408).
4. National view with the Pregnancy Registry and FMF together as it is used today for CUB (not NIPT).

	1	2	3	4 (CUB)
Detection rate trisomy 21 (%)	95.7	90.4	96.0	89–94%
Number of invasive tests (%)	2.0	1.0	1.6	3–5 *
Number of NIPT (%)	14.0	7.5	10.2	0

* Nationally estimated information based on the Pregnancy Registry and SFOG annual reports. Stockholm about 7% in 2013.

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Abbreviations

GRADE	Grading of recommendations assessment, development and evaluation
CUB	Combined Ultrasound and Biochemistry
MHV	Maternity Healthcare
NIPT	Non invasive prenatal test
cffDNA	Cell free foetal DNA
SBU	Swedish Agency for Health Technology Assessment and Assessment of Social Services
SFMG	Swedish Society of Medical Genetics
SFOG	Swedish Society of Obstetrics and Gynaecology
SMER	Swedish National Council on Medical Ethics
SOSFS	The National Board of Health and Welfare's regulations and general advice

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GRADE (Grading of Recommendations Assessment, Development and Evaluation)

GRADE – for classification of the strength of the scientific evidence

Grading of the strength of the evidence according to GRADE[9] is based on the total scientific evidence. Study quality refers to the scientific quality of a particular study and its ability to answer a particular question in a reliable manner. Evidence strength is a measure of how reliable the results are when the overall literature is judged. Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU) uses an international evidence grading system called GRADE. For each outcome measurement, one starts from the overall assessment from the study's design. Thereafter, the strength of the evidence is influenced by the presence of debilitating factors such as study quality, consistency, transferability, accuracy of the data and the risk for publication bias. Evidence strength is graded in four levels:

- Strong scientific evidence (⊕⊕⊕⊕) Based on studies with high or medium high quality without factors that weaken the overall assessment.
- Moderately strong scientific evidence (⊕⊕⊕◯) Based on studies with high or medium high quality with the occurrence of factors that weaken the overall assessment.
- Limited scientific evidence (⊕⊕◯◯) Based on studies with high or medium high quality with strong factors that weaken the overall assessment.
- Insufficient scientific evidence (⊕◯◯◯) When studies are lacking, available studies have low quality or where studies of similar quality show conflicting results, the scientific evidence is stated as insufficient.

The stronger the scientific evidence, the less likely that reported results will be affected by new research in the foreseeable future.

GRADE –for classification of the strength of the recommendation

The recommendation can be strong or weak, for or against an intervention.
It is based on four components:
1. The strength of the scientific evidence is graded according to GRADE
2. The benefit-risk balance of the intervention
3, Possible ethical implications and other values
4. Cost Aspects
A weak recommendation can mean that certain conditions must be met, or that there is a benefit-risk balance that may be valued differently for different patients.