INFORMATION NOTE

NON-INVASIVE PRENATAL TESTING (NIPT)

TO DETERMINE THE RISK OF TRISOMY 21, 18 AND 13 BY ANALYSING THE DNA IN THE MATERNAL PLASMA

Dear Madam,

since the Emilia-Romagna Region has complemented its programme of prenatal diagnosis by introducing testing to determine the risk of aneuploidy of chromosomes 13, 18 and 21 by analysis of the free-circulating foetal DNA in the maternal bloodstream, we are writing to inform you of the characteristics and limitations of the proposed test.

The risk of conceiving a child with trisomy 21 (Down's syndrome), trisomy 18 (Edwards' syndrome) or trisomy 13 (Patau's syndrome), which represent the majority of chromosomal abnormalities found at the prenatal stage by means of amniocentesis or chorionic villus sampling, varies with maternal age.

Maternal plasma contains a proportion of free DNA from the mother and a proportion of free DNA from the foetal placenta. This has made it possible to develop Non-Invasive Prenatal Tests (NIPT) based on molecular genetics techniques capable of detecting chromosomal abnormalities as early as the tenth week of gestation.

The sensitivity (ability to identify affected foetuses) of NIPT is very high (99% for trisomies 21 and 13, 98% for trisomy 18), with extremely low percentages of false positives (less than 0.1%) (1).

The Emilia-Romagna Region has therefore decided to offer pregnant women residing in the region, or under the care of the Regional Health Service (and therefore registered in the regional register of service users, including women registered as temporarily present foreigners), or under the charge of regional health service units, NIPT testing to assess the risk of trisomy of chromosomes 21, 18 and 13 in order to ensure the lowest possible number of false positives (thus avoiding recourse to invasive prenatal testing) and false negatives (with failure to diagnose trisomy 21, trisomy 18 or trisomy 13).

NIPT is a **risk assessment screening test, not a substitute for diagnostic tests** (foetal karyotype in chorionic villi and amniotic fluid), and **is not intended to make a conclusive diagnosis**. **As such, a low-risk result does not guarantee the absence of pathology, and conversely an increased-risk result does not provide certainty of the presence of trisomy.**

WHO IS IT AIMED AT?

NIPT testing forms part of the prenatal risk assessment programme offered to all eligible pregnant women residing in the Emilia-Romagna Region, or under the care of the Regional Health Service (and therefore registered in the regional register of service users, including women registered as temporarily present foreigners), or under the charge of Regional Health Service units.

The test can be performed from the tenth week of gestation, both in the case of pregnancies deriving from natural conception and by means of homologous or heterologous medically assisted reproduction techniques. The test can be performed in the case of both single and twin pregnancies. In the latter case, however, since the NIPT result relates to the foetal DNA of both foetuses, it is not possible to distinguish the condition of the individual foetus. **In the event of a positive result**, therefore, **the test does not indicate which foetus is affected**.

PROCEDURE

To perform the analysis, it is necessary to take two samples of the maternal blood. This is done at the centre to which the women concerned have been referred by their obstetrician or gynaecologist, who

inform the pregnant women about the test, obtain their informed consent and make the necessary referral. The blood samples are then sent to the relevant regional laboratory at Azienda USL di Bologna (Laboratorio Unico Metropolitano - LUM), where analyses are centralized.

RESULTS

The NIPT test report will be interpreted and explained by the referring professional within the context of the overall clinical picture of the pregnancy.
Where there is evidence of increased risk, it is advisable to arrange a specialist consultation with a medical geneticist or gynaecologist with expertise in prenatal diagnosis. During this consultation, you will be given the opportunity to arrange further diagnostic investigations based on invasive techniques (chorionic villus sampling, amniocentesis), about which you will be given specific information, for the purpose of consent to the health treatment.
In the event of a positive test result, contact your prenatal diagnostic centre if you have not already been contacted.

In some cases, the specialist may recommend further blood tests, evaluation of the mother's and/or father's chromosome structure, or additional specialist examinations.

RISKS AND LIMITATIONS OF THE TEST

The test **carries no risk** for the foetus or mother.
NIPT is only a risk assessment screening test. It **does not replace invasive diagnosis** and does not obviate the need to carry out the other clinical, laboratory and instrumental investigations that form an integral part of pregnancy monitoring. Only diagnostic investigations such as chorionic villus sampling or amniocentesis currently make it possible to definitively confirm or rule out a chromosomal abnormality of the foetus at the antenatal stage.
Where the foetus is the result of consanguinity (i.e. if the partners are related to some degree), the test may not provide a risk probability due to the presence of less informative alleles. For this reason, you will need to communicate this information before the sample is taken and you may be advised to have a pre-test genetic consultation to assess the most appropriate clinical diagnostic pathway.
You may be contacted and asked to give another sample, if it was not possible to assess the risk on the basis of the first sample for reasons that might include non-conformity of the sample taken, an instrumental malfunction or an inconclusive or indeterminable result. If the second result is also inconclusive, you may be advised to have a genetic and/or prenatal consultation to assess the clinical diagnostic pathway to be taken.

NIPT testing is not validated for multiple pregnancies with more than two foetuses.
Furthermore, it cannot detect an increased risk of chromosome abnormalities such as microdeletions, microduplications, balanced chromosomal rearrangements, foetal and/or placental chromosome mosaicisms, point mutations, methylation defects, or the presence of any foetal genetic diseases other than trisomies 21, 18 and 13, which may be associated with malformations and/or disabilities in the unborn child.

It is important to be aware of the sensitivity of the test, which, as indicated above, is 99% for trisomies 21 and 13, and 98% for trisomy 18. Therefore, in rare cases, some pregnancies with a trisomal foetus may yield a "low risk" result and thus not be identified (false negative). Rarely, some pregnancies involving a foetus without trisomy may yield an "increased risk" result (false positive). In these cases, the NIPT result can only be verified by means of invasive diagnosis (chorionic villus sampling or amniocentesis).

Lastly, we would like to remind you that if you have any further questions or needs, you can contact your clinic, the centre that referred you for the test, or your gynaecologist/obstetrician, who will provide you with all the necessary information.

Bibliography

1. Gil MM et al. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2015 Mar;45(3):249-66. doi: 10.1002/uog.14791. Epub 2015 Feb 1. Update in: Ultrasound Obstet Gynecol. 2017 Sep;50(3):302-314. PMID: 25639627
2. Norton ME et al. Cell-Free DNA Analysis for Noninvasive Examination of Trisomy. New England Journal of Medicine, vol. 372, fasc. 17, Apr 2015, pp. 1589–97. DOI.org (Crossref) https://doi.org/10.1056/NEJMoa1407349.

Reference guidelines

Ministry of Health, Higher Health Council Section I, Non-invasive screening of foetal DNA (NIPT) in public health, 2021.