



HAUTE AUTORITÉ DE SANTÉ

RECOMMENDING
PUBLIC HEALTH STRATEGIES

REPORT

Tests based on cell-free DNA in the context of screening for trisomy 21

Appropriateness of detecting other chromosomal abnormalities

Validated by the HAS Board on 26 September 2024

Summary

In France, standard pregnancy monitoring schedules the option of prenatal screening for trisomy 21 (Down syndrome) at the first-trimester medical consultation, based on an assessment of a combination of factors (primarily maternal serum markers (MSM), maternal age and ultrasound measurement of foetal nuchal translucency). Since 2018, when the risk identified by combined screening is between 1/1,000 and 1/51, noninvasive prenatal testing (NIPT) based on a circulating cell-free DNA test has been offered. In this context, or in the event of multiple pregnancies, a history of pregnancy with T21, or for parents carrying a Robertsonian translocation involving chromosome 21 (conditions stipulated by the Order of 14 December 2018), the cost of the test is fully covered by the French National Health Insurance system. Invasive tests (amniocentesis or chorionic villus sampling) performed for diagnostic purposes (by karyotyping in particular) are then performed only if first-trimester combined screening demonstrates a risk of 1/50 or more or if the result of the cfDNA test is positive for T21.

The first objective of introducing cfDNA testing into the prenatal screening strategy for T21 was to reduce the number of invasive procedures performed in pregnant women in the context of karyotyping, which was immediately offered to women with an estimated risk of more than 1/250 following combined screening up until 2017. However, it had been pointed out that the introduction of cfDNA testing may result in the lower detection of chromosomal abnormalities other than T21 (due to the decreased number of karyotypes).

In this context, the French Ministry of Health consulted the HAS to have it assess the benefit and impact of detecting other chromosomal abnormalities by circulating cell-free DNA testing in maternal blood, performed in the context of screening for T21. The French Biomedicine Agency was consulted in parallel to define the methods for informing women and professionals, the consent process and the informed consent form.

Taking into consideration the data in the literature relating to the frequency and clinical features of the different chromosomal abnormalities, the performance of cfDNA tests, current practices in France and other countries and discussions with the working group, the main conclusions are as follows:

Evolution of screening by cfDNA testing in France since 2018

In France, almost 130,000 cfDNA tests were performed in 2022. In the event of a positive result (1.36%), an invasive test for diagnostic purposes (by karyotyping, FISH or CPA) can be used (70% to 80% of positive tests), or a more rapid ultrasound scan, at 18 weeks' gestation, may also be proposed depending on the suspected abnormality. The NIPT detection rate is 1.36% (61% of positive results are for T21) with a slight increase since 2019 (1.19%) related to the increase in detection of trisomy types other than T21. Given the low frequency of rare autosomal trisomies (RATs) and segmental abnormalities (in the general population and on NIPT), the number of additional invasive tests resulting from this screening is expected to be low and, consequently, should not generate a significant increase in the iatrogenic miscarriage risk.

In addition, while positive NIPT results only account for 8.6% of karyotyping indications, 76.5% of the karyotype tests performed in 2022 due to a "positive aneuploidy screening result on cfDNA", demonstrated the presence of a foetal unbalanced chromosomal abnormality. In comparison, 23.3% of ultrasound signs (excluding NT \geq 3.5 mm) and 16.1% of serum markers alone with a risk $>$ 1/50 lead to the identification of an unbalanced chromosomal abnormality, demonstrating the good predictive value of screening by cfDNA testing compared to the other karyotyping indications.

The different types of chromosomal abnormalities

The scope of the assessment considered the chromosomal abnormalities liable to be identified by the cfDNA tests currently available in France and identifiable by karyotyping (i.e. with a minimum size of 5-7 Mb), compatible with a progressing pregnancy (monosomies and polyploidies are therefore excluded) and likely to have particularly serious foetal or obstetric consequences.

Three types of abnormalities were retained in the context of this assessment:

- chromosomal abnormalities with a risk of serious clinical consequences on the foetus: these are **trisomies 2, 8, 13, 14, 15, 18 and 22**;
- chromosomal abnormalities with a risk of serious obstetric consequences: in view of the data currently available, only **trisomy 16** has been retained currently, given the significant placental impact;
- **noncryptic unbalanced segmental abnormalities.**

Trisomies 1, 3, 4, 5, 6, 7, 10, 11, 12, 17, 19 and 20 were not retained given their low frequency in the general population and on NIPT, the low rate of diagnostic confirmation, the low foetal mosaic risk and/or the low risk of impact on the foetus or placenta.

Except in the case of trisomies 13,16 and 18, published data on autosomal chromosomal abnormalities are rare, primarily due to their lower frequency in the general population and on NIPT. The studies are heterogeneous. This heterogeneity is related to the country, the year of the study, the very diverse definitions of at-risk populations and the inclusion of women with no increased risk of aneuploidy, the variable rates of follow-up of women for diagnostic confirmation, the thresholds selected for segmental abnormalities and the techniques used (platform used, sequencing type and depth).

“Common” trisomies 13 and 18

Trisomies 13 and 18 are the most common types after trisomy 21. The foetal impact is very significant, since 95% of cases result in *in utero* death. For live births, the consequences are very serious, leading to death during the first year in 90% of infants. However, depending on the mosaicism, some may live to adulthood.

First-trimester combined testing intended to screen for T21 appears to be just as effective for detecting T13 and T18 (although with different serum marker thresholds).

In 2022, in France, 159 and 305 cfDNA tests were positive for T13 and T18 respectively, i.e. 0.1 and 0.2% of cfDNA tests performed (versus 0.8% for T21).

In the population at increased risk of aneuploidy (variable definition between studies), irrespective of gestational age, the false-positive rate is less than 0.50% and the positive predictive value (PPV) is greater than 80% for T13 and T18.

Around 30 European countries, as well as the USA, Canada, Australia and South Korea offer screening for trisomies 13, 18 and 21 using cfDNA testing, sometimes with the option of including testing for sex chromosomal aneuploidy or certain microdeletions for at-risk populations. In France, since 2022, almost all cfDNA tests have also screened for T13 and T18 in addition to T21, generally at no additional cost to the pregnant woman despite not being on the standard list of funded procedures.

Rare autosomal trisomies (2, 8, 9, 14, 15, 16 and 22)

These are generally fatal *in utero* when they are homogeneous. In the event of foetal mosaic trisomy, the clinical signs and their severity can vary greatly depending on the chromosome involved, the rate of mosaicism and the type of cells affected. The value of detecting placental mosaicism primarily concerns trisomy 16, which increases the risk of intrauterine growth retardation (IUGR), preterm delivery or pre-eclampsia.

The detection of rare autosomal trisomies (RATs) is also complex, with serum markers of low specificity and highly variable ultrasound signs. If a RAT is suspected on the first-trimester ultrasound scan, the situation does not fall within the scope of NIPT since a diagnostic test will be offered from the outset. However, ultrasound signs are usually detected during the second-trimester morphology ultrasound scan. Here, NIPT would be useful for obtaining an earlier diagnosis and limiting the psychological impact of a late termination of pregnancy. IUGR can be a sign of T16 but it is usually noticed in the third trimester of pregnancy, whereas adapting pregnancy follow-up could limit the risks of serious consequences. Of the 129,804 cfDNA tests examined in 2022, 106 results (0.08%, i.e. 6% of the 1,767 positive tests) indicated a suspected rare aneuploidy.

The PPVs of cfDNA tests for RATs are highly variable from one study to another, ranging from 0 to 57% (mostly < 20%) and the highest PPVs are observed on low sample sizes or incomplete follow-ups of pregnant women (lack of confirmatory diagnostic tests).

Outside France, Belgium allows the reporting of incidental results for RATs, and in the USA, although it is possible to test for RAT, learned societies do not recommend it due to limited performance data. In France, in 55% of NIPTs performed due to an increased risk of T21, other abnormalities are also tested for (RAT, segmental abnormalities) with variable costs depending on the laboratories and the care pathway.

Noncryptic segmental abnormalities

The phenotype expression of noncryptic segmental abnormalities is variable and depends on factors such as the chromosome involved, the breakpoints and mosaicism. The *de novo* and unbalanced nature of a segmental abnormality, along with databases, nonetheless enable a prognosis to be made in most cases.

As with RATs, detection by ultrasound (including in the second trimester of pregnancy) or serum markers is difficult.

Their frequency on NIPT is also equivalent to that of RATs (115 positive results in 2022).

The PPV of cfDNA tests ranges from 3 to 74.2% for segmental abnormalities, primarily depending on the size threshold chosen and the pregnancy follow-up rate.

Outside France, the Netherlands and Belgium report the results of certain segmental abnormalities discovered by chance. In France, some laboratories offer testing for abnormalities of more than 7 Mb and the French-language cytogeneticists association (ACLF) recommends reporting structural abnormalities only if they concern a rearrangement compatible with a conventional structural cytogenetic abnormality (translocation derivative, inversion recombinant, etc.).

Women's preferences for cfDNA tests

In France, women are increasingly having cfDNA tests to detect chromosomal abnormalities other than T21, reflecting a clarification of the care pathway but also their desire to have access to as much information as possible on the health of their foetus (with an increase in cfDNA tests performed for personal reasons). This desire needs to be weighed up against the anxiety generated by a positive cfDNA test result, which may persist even following a normal diagnosis, and the risks incurred by the diagnostic tests offered following a positive screening test. The treatable nature of a condition (with an impact on the foetus, infant or mother) is also an important factor for women in terms of having cfDNA tests.

The challenges involved in detecting other chromosomal abnormalities

Extending the detection of chromosomal abnormalities beyond trisomy 21 raises a number of issues:

- Public health challenges: the aim is to improve the effectiveness of prenatal screening for chromosomal abnormalities without significantly increasing the number of invasive tests in order to limit the risk of iatrogenic foetal loss. An established screening programme will enable harmonisation of practices and the care pathway offered to pregnant women, as well as the provision of reliable and consistent information;
- Economic challenges: extending the abnormalities screened for should incur limited costs for society thanks to the very predominant use in France of whole genome sequencing methods (no additional reagent costs), at the same time ensuring equal access to screening for all women (no additional costs for certain pregnant women). In addition, the early management of certain conditions screened for prenatally, such as T16, could limit morbidity thanks to the implementation of appropriate follow-up.
- Organisational challenges, mainly based on the time required to provide pregnant women with accurate and easy-to-understand information so that they can give their informed consent;
- And ethical challenges, which underpin all the others and are based on the principles of respect for autonomy, non-maleficence, justice and fairness, and are very closely linked to information and communication challenges. Documented, objective information that is understood by the pregnant woman and delivered outside of an emergency situation (e.g. first-trimester combined screening with increased risk) is essential if an informed decision is to be made. This requires the provision of regularly updated information documents (based on the latest available data on the consequences of abnormalities, technological developments and test performance) and appropriate training for prescribers. The right not to know must also be respected. The screening strategy should be based on a principle of equal access to screening services, with no individual or regional disparities.

It would be useful to assess couples' understanding of cfDNA tests.

Recommendations

Considering the following factors in favour of extending detection of other chromosomal abnormalities by cfDNA tests performed in the context of screening for trisomy 21:

- The risk of serious foetal consequences for infants born with partial or mosaic trisomy of chromosomes 2, 8, 9, 13, 14, 15, 18 and 22;
- The specific case of placental trisomy 16, in which it is particularly important for healthcare professionals to be informed given its relatively high frequency and the significant consequences for the mother, the child and the organisation of the end of pregnancy.
- The poor prognosis associated with noncryptic unbalanced segmental abnormalities with a *de novo* character;
- The current difficulties in identifying these abnormalities by first-trimester combined screening, maternal serum markers or ultrasound signs in the first trimester of pregnancy, given the diversity of possible defects;
- The low expected increase in invasive diagnostic test rate and the low risk of induced foetal loss;
- Recognition that this technique has been employed for several years, leading to better mastery of its use, as emphasised by the WG experts, and a reduction in the failure rate;
- The absence of an additional cost for screening for other chromosomal abnormalities given the very predominant use of whole genome sequencing methods;
- Current inequalities in terms of access to screening tests and the costs borne by pregnant women;

However, considering limitations related to:

- Uncertainties with respect to the clinical consequences associated with certain RATs highly dependent on the chromosome involved, the region concerned, its size and mosaicism;
- The rare data available for trisomies 1, 3, 4, 5, 6, 7, 10, 11, 12, 17, 19 and 20, which currently demonstrate a low frequency in the general population and on NIPT, a low rate of diagnostic confirmation, and a high probability of being confined to the placenta (and hence a low probability of being present in the foetus);
- The lack of studies with a good methodological quality and sufficient sample size currently available in the literature to assess the performance of tests for rare chromosomal abnormalities;
- The heterogeneity of the studies in terms of the technology employed, the sequencing method, the characteristics of the patients included and the definitions of the populations at increased risk of chromosomal abnormalities;
- The availability in France of only one validated cfDNA test to detect RATs and noncryptic segmental abnormalities;

The HAS recommends:

- **Offering women who meet the conditions of the order of 14 December 2018 testing for chromosomal abnormalities compatible with an ongoing pregnancy and liable to have particularly serious foetal or obstetric consequences.** As knowledge currently stands and in view of the prevalence of foetal involvement and the known consequences of placental involvement, the abnormalities meeting these criteria are trisomies **2, 8, 9, 13, 14, 15, 16, 18, 21 and 22 and noncryptic segmental abnormalities.**

- **Extending the indications for cfDNA testing to the following situations:**
 - **In the event of a history of pregnancy with aneuploidy,**
 - **If one of the parents is a carrier of a Robertsonian translocation involving chromosome 13,**
 - **In the event of a first-trimester maternal serum marker profile suggestive of trisomy 13 or 18.** This recommendation implies that a probability of T13 or T18 be indicated by laboratories following first-trimester combined screening;
- That **pregnant women¹ be provided with information that they can easily understand so that they can make an informed decision with respect to the performance of screening and diagnostic tests;**
- That prescriber training be scheduled in order to guarantee the quality of the information provided and women's autonomy in terms of decision-making, particularly in the context of an increase in the number of abnormalities screened for;
- That a **dedicated time slot for providing screening information** be scheduled in the care pathway for pregnant women, prior to prescription of the test, with fair remuneration of practitioners. The methods for informing pregnant women will be defined by the French Biomedicine Agency (ABM). This implies appropriate human and financial resources dedicated to implementing and monitoring the extension of screening for chromosomal abnormalities.

Before implementing these recommendations, it will be necessary to use data collected for the French Biomedicine Agency's annual assessments to ensure the feasibility of monitoring:

- The impact of extending the indications of cfDNA tests and chromosomal abnormalities on the number of invasive diagnostic tests performed;
- Compliance of the rate of detected chromosomal abnormalities with the expected rate;
- The stability or improvement of the rate of abnormalities diagnosed after a positive cfDNA test result in the years following implementation of the guideline.

¹ Women are central to the mechanism and make all decisions relating to their pregnancy. Their autonomy must be respected. However, it is recommended that the couple be involved where possible, while respecting the woman's wishes.

[\(Order of 14 December 2018 amending the decree of 23 June 2009 - JORF No. 0294 dated 20/12/2018\)](#)

The HAS encourages the implementation and publication of good-quality studies, with recent data and in a population comparable to the target population in France for cfDNA tests, in order to assess the detection rate and performance of cfDNA tests for the different chromosomal abnormalities screened for.

The list of RATs for which screening is recommended will need to be reviewed on the basis of the data that becomes available relating to the consequences of the various chromosomal abnormalities and the performance of the cfDNA tests.

The consent methods will be defined by the French Biomedicine Agency, which was consulted in parallel by the French Ministry of Health to define the methods for informing women and professionals, the consent process and the informed consent form. Uncertainties related to the results of cfDNA tests will have to be included in this information.

The HAS specifies that:

- The question of prenatal screening for certain sex chromosomal aneuploidies with a later impact (early childhood, adolescence, or even adulthood) should be raised in future studies, as should the question of certain micro-rearrangements, if the technology evolves and good-quality data are made available;
- In the context of whole genome sequencing, since identification of maternal disorders - such as the presence of a maternal tumour process or pre-eclampsia - is possible using cfDNA testing and the measures to be taken have not yet been codified, consideration needs to be given to these aspects.