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Implementation of non-invasive prenatal genetic screening for trisomy 21 (Down Syndrome) in the practice of health care in Belgium

In this scientific advisory report, the Superior Health Council of Belgium assesses the test performance of non-invasive prenatal genetic testing (NIPT) for Down Syndrome.

The aim is to provide policy makers, the medical sector, pregnant women and future parents with specific recommendations regarding the implementation of NIPT and its position in the Belgian healthcare system.

May, 7, 2014

SUMMARY

In recent decades, many prenatal tests have become available. They are mainly used to screen for anomalies during pregnancy as a part of genetic counselling, but not exclusively so. Several types of invasive prenatal diagnoses have been performed so far, which include screening for foetal trisomy 21 (T21 or Down syndrome) based on the results of a combined test (CT), namely an ultrasound examination combined with maternal serum markers. Invasive sampling techniques (using chorionic villus sampling or an amniocentesis) are a very effective tool to diagnose a genetic disorder in the foetus. On the downside, they have an attendant risk of miscarriage (0,5 – 1%).

At present, it is possible to isolate free circulating foetal DNA (cell-free foetal DNA, cffDNA) in the mother's blood and identify a foetus carrying trisomy (21, 18, 13) by means of a non-invasive genetic test. The Belgian genetics centres are or soon will be able to perform this test in their own laboratories.

Introducing these non-invasive prenatal tests (NIPT) into the healthcare system should provide a solution to the drawbacks of current prenatal screening for T21. NIPT will prevent many women from undergoing amniocentesis after a false positive CT. Ideally, the non-invasive procedure should improve prenatal screening and make it safer, with less invasive tests performed. In addition, implementing NIPT is expected to result in a reduction of the large number of foetuses carrying T21 that are missed by current CT-based screening.

This advisory report aims to evaluate the advantages and disadvantages of NIPT in prenatal screening and to formulate the basic requirements for using it. It addresses this issue only in the context of screening for T21.

NIPT has two advantages over existing techniques. First, it reduces the number of invasive diagnostic tests performed. This is expected to result in an increase in the number of foetuses carrying T21 identified compared to the number of miscarriages induced by invasive procedures. in addition, introducing NIPT into the healthcare system should reduce the number of false-negative cases that are the result of the variable sensitivity of the CT used in our country.

NIPT can be included in the procedure of prenatal screening for T21 for all pregnant women. In case of pathological findings, this non-invasive and risk-free method must be validated by means of invasive prenatal diagnosis after having provided genetic counselling to the mother and expectant parents.

Drawing a comparison between current prenatal screening for T21 and the use of NIPT reveals that recommending the latter is fully justified. Ideally, NIPT should be implemented as a first-tier test for prenatal screening. Compared to the method currently used for prenatal screening, the number of pregnant women wrongly referred for an invasive test based on a false positive CT-result drops significantly. This decrease goes hand in hand with a concomitant drop in the number of foetuses carrying T21 that are missed, which will become rare. The use of NIPT as a second-tier test is another good option, but of second choice. Practised as a second-tier test following the CT, NIPT significantly reduces the number of invasive tests, but its added value is limited by the relatively poorer performance of the CT. Consequently, the number of unidentified foetuses carrying T21 during prenatal screening is even likely to increase.

NIPT cannot replace the current techniques to monitor pregnancies, such as ultrasound scans, since NIPT only detects a few chromosomal anomalies. Besides these, prenatal care, including ultrasound screening, can detect many other anomalies as well (including non-genetic anomalies).

It is advisable to set up a pilot phase for the introduction of NIPT into the healthcare system in order to make a thorough assessment of all aspects of its implementation.

a number of fundamental conditions are required when implementing NIPT in prenatal screening in Belgium. They concern the cascade of information and genetic counselling, formulating prescription procedures and guidelines, imposing quality standards, setting up a monitoring system, providing the expertise and capacity that are needed to perform tests, taking into account ethical and legal aspects.

Keywords

Keywords	**Mesh terms***	**Sleutelwoorden**	**Mots clés**	**Stichwörter**
Non-invasive prenatal diagnosis		Niet-invasieve prenatale diagnostiek	Diagnostic prénatal non invasif	nicht-invasive Pränataldiagnostik
Non-invasive prenatal testing		Niet-invasieve prenatale test	Test prénatal non invasif	nicht-invasiver pränataler Test
		Invasieve prenatale test	Test prénatal invasif	invasiver pränataler Test
Trisomy 21		Trisomie 21	Trisomie 21	Trisomie 21
Down Syndrome	Down Syndrome	Syndroom van Down	Syndrome de Down	Down-Syndrom
Test performance		Testperformantie	Performance du test	Testleistung
Delivery of Health Care	Delivery of Health Care			
Genetic Testing	Genetic Testing	Genetische test	Dépistage génétique	genetische Testung
Pregnant Women	Pregnant Women	Zwangere vrouwen	Femmes enceintes	Schwangere

** MeSH (Medical Subject Headings) is the NLM controlled vocabulary thesaurus used for indexing items for PubMed.*

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ABBREVIATIONS AND SYMBOLS

BELAC	the Belgian Accreditation Body
CBN	Advisory Committee on Bioethics of Belgium
cffDNA	cell-free foetal DNA
CGH	comparative genomic hybridization
NT	nuchal translucency
CT	combined test
DNA	deoxyribonucleic acid
GGOLFB	Gynecologists Obstetricians group of French Language in Belgium (Groupement des Gynécologues Obstétriciens de Langue Française de Belgique)
hCG	human chorionic gonadotropin
HON	Health On the Net Foundation
MTP	Medical Termination of Pregnancy
ISP	Scientific Institute of Public Health
IVT	invasive test
KCE	Federal Centre of Expertise Health Care
CRL	Crown-rump length
MLPA	Multiple Ligation dependent Probe Amplification
MoM	multiple of the median
MPS	massively parallel sequencing
NGS	next generation sequencing
NIPD	non-invasive prenatal diagnosis
NIPT	non-invasive prenatal testing
NPV	negative predictive value
PAPP-A	pregnancy associated plasma protein A
PCR	polymerase chain reaction
PPV	positive predictive value
SE	sensitivity
SHC	Superior Health Council
SP	specificity
T21	trisomy 21
VTP	Voluntary Termination of Pregnancy
VVOG	Flemish Association Gynecology and obstetrics (Vlaamse Vereniging voor Obstetrie en Gynaecologie)

1 INTRODUCTION AND ISSUES

In recent decades, many prenatal tests have become available. They are used for research purposes, but their main purpose is to screen for anomalies during pregnancy (screening) as a part of genetic counselling, though not exclusively so.

Several types of invasive prenatal diagnostics have been practised so far: cytogenetic and/or molecular prenatal diagnostics planned during pregnancies that are at a high risk of genetic disorders, prenatal diagnostics carried out in the light of the ultrasound scan revealing a malformation in the foetus and finally as the most common prenatal test, i.e. screening for foetal trisomy 21 (T21 or Down syndrome) based on the results of a combined test (CT), the latter being a combination of an ultrasound and an analysis of maternal serum markers. Invasive sampling techniques to diagnose a genetic disorder in the foetus are very effective but they have the disadvantage of being accompanied by attendant risks. Although these risks are exceptional in the mother, the foetus is more likely to be affected. The main risk concerns foetal loss (0.5 to 1%), which often concerns foetuses free of chromosomal anomalies.

Therefore, until very recently, analyzing foetal DNA to detect genetic anomalies required performing a transabdominal puncture on the mother to collect chorionic villi or to draw amniotic fluid. These past few years, however, it has become possible to determine the sex and Rhesus factor of the foetus based on an analysis of the mother's blood (Lo *et al.,* 1989; Lo *et al.,* 1997).

At present, it is also possible to isolate the "cell-free foetal DNA" (cffDNA) from maternal blood and identify foetuses carrying a trisomy (21, 18, 13) by means of a non-invasive genetic test (see section 3.2.4). Since October 2012, this screening method for trisomies, and more specifically for Down syndrome, has been offered in the larger centres in the United States and in other countries like China (completed by an invasive diagnostic test). Initially, all the analyses were performed by a single company. Since then, other companies, including some in Europe and Asia, have offered kits to perform a non-invasive prenatal test, which certainly results in a clinical benefit when applied correctly. Belgian genetics centres are or will soon be able to perform this test in their own laboratories.

This advisory report addresses this issue only in the context of the detection of T21, given:
(1) the higher frequency of T21 compared to T13 and T18, which are more rare;
(2) the fact that current prenatal screening does not specifically test for T13 and T18 and
(3) the information currently available regarding the performance parameters of the test.

Introducing these non-invasive prenatal tests (NIPT) into the healthcare system should provide a solution to the drawbacks of current prenatal screening for T21. NIPT prevents many women from undergoing amniocentesis after a false positive CT. Currently, about 7,600 invasive prenatal tests are performed in Belgium, of which an estimated 76% are carried out within the framework of T21 diagnostics. Consequently, introducing NIPT into the healthcare system will lead to a reduction in the absolute number of miscarriages induced by the invasive method. Ideally, the non-invasive procedure should improve the overall performance of prenatal screening and make it safer with less invasive tests performed.

In addition, NIPT should reduce the large number of carriers of T21 missed by the current CT-based screening procedure.

The advantage of non-invasive methods (drawing a simple blood sample from the mother) is that they provide the opportunity to perform a genetic test at virtually no risk to the foetus and the mother. As a result, they could be implemented to improve large-scale population screening. Whilst this genetic test offers a safer means of providing information to medical professionals, expectant mothers and parents, it does not affect the latter's freedom of choice regarding the

monitoring of the pregnancy. However, it does contribute to strengthening the principle of informed consent as regards the available medical options in the event of the foetus carrying T21. In accordance with the recommendations of the European Commission on the implications of genetic diagnostics and population-based screening programs, it is essential to provide information and set up regulations in this area (McNally *et al.,* 2004).

Society, legislators, physicians, paramedics, pregnant women and future parents should therefore prepare for the practical and ethical implications of prenatal screening for and diagnosis of T21 and in the near future for those of foetal genome sequencing performed on a maternal blood sample.

The purpose of prenatal screening for trisomy is to offer information to pregnant women and future parents regarding the different choices they have.

This report therefore provides an answer to the following questions:

1. What is the added value of introducing NIPT in prenatal screening for T21:
   a. with respect to the reduction in the number of prenatal invasive tests;
   b. with respect to the reduction in the number of false-negative screening tests.

2. What are the prerequisites for the introduction of NIPT in Belgium:
   a. information and counselling;
   b. prescribing procedures and guidelines;
   c. quality requirements for different procedures;
   d. system for monitoring the screening process and health outcomes;
   e. management of the capacity to perform the various procedures;
   f. ethical considerations;
   g. introduction of NIPT in the healthcare system.

The issues pertaining to the health economics of introducing and using NIPT technology for prenatal screening are discussed in a report of the KCE (Hulstaert *et al.,* 2014). Within the framework of the Belgian "Health Research System", the partner institutions coordinated their work and issued their advisory reports at the same time, whilst paying heed to the characteristics of each of the other partners.

To answer these questions, an *ad hoc* working group was set up by the SHC, which included experts on medical and clinical genetics, health economics, molecular biology, medical epidemiology, bioethics, general medicine, obstetrics, health education, public health, etc.

2 FURTHER DETAILS AND ARGUMENTATION

2.1 Methodology

After analysing the request, the Board and working group Chair identified the necessary areas of expertise. The working group experts provided a general and an *ad hoc* declaration of interests and the Committee on Professional Conduct assessed the potential risk of conflicts of interest. The SHC coordinated its views with its four partner institutions from the Belgian "Health Research System", who issued their advisory reports at the same time, whilst paying heed to the specificities of each of the other partners.

This advisory report is based on a review of the scientific literature as well as on the views of the experts.

In order to do so, the Council made an overview of literature and selected relevant studies, dating from January, 1st,1997 onwards, in the PubMed database. In addition, lists of references were reviewed to glean more studies and those suggested by colleagues and reviewers were evaluated to determine whether they too could be taken into consideration. We included those studies that mentioned the diagnostic accuracy of one or several molecular techniques for NIPT for T21, as well as the sensitivity (SE) and specificity (SP) of this test. We excluded studies that examined the use of NIPT for other disorders.

Once the draft advisory report was approved by the working group and by the standing working group, it was ultimately validated by the Board.

2.2 Elaboration

2.2.1 Scientific literature

2.2.1.1 Down Syndrome

Individuals carrying T21 (Down Syndrome - Down, 1866) have a series of characteristics caused by the presence of a 3rd copy of chromosome 21, as a whole or partially, in all or most of the cells in their body (Down, 1866; Lejeune *et al*., 1959).

The most common morphological features include e.g. a round face, up and outwards slanted palpebral fissures, a small nose with a flat root, epicanthus, a small mouth, small ears and a flat neck with excess skin. Regarding the extremities, a single palmar crease, clinodactyly of the 5th finger and increased space between the 1st and 2nd toe can be observed. Individuals carrying T21 usually have a short stature.

The degree of intellectual disability varies from one individual to another: it may be mild (IQ 50 - 70), moderate (IQ 35 - 50) or sometimes severe (IQ 20 - 35). The main observed disorders include heart malformations (50%), gastrointestinal atresia (12%), cataracts (15%) and Hirschsprung disease (< 1%). Several medical problems require medical care and monitoring, such as hearing impairment (75%), obstructive sleep apnea (50 – 79%), otitis media (50 – 70%), ocular refraction conditions (50%). The thyroid function can be altered (4 – 18%) and epilepsy occurs in 1 – 13% of individuals.

Hematologic monitoring is necessary in cases of anemia due to iron deficiency (10%), an early transient myeloproliferative disorder (10%) and later leukemia (1%). Gluten intolerance occurs in 5% of cases (Bull, 2011).

In our country, some hospitals have set up a specific multidisciplinary consultation for children carrying T21 to ensure that they receive optimal medical monitoring and thus prevent associated disorders.

The social circumstances of individuals carrying T21 vary with age and the facilities provided in each country. In Denmark, 80% of all individuals carrying T21 attend 10 years of primary school. Only 1% of the individuals carrying a “classic” form of T21 pursue secondary education and hold a full time job. However, most receive government support (Zhu *et al*., 2014). In Italy, the access to education for young people is adequate but there is still a great deal of progress to be made to ensure a satisfactory standard of living for adults (Bertoli *et al*., 2011). In Belgium, the education of children carrying T21 is usually provided within the family and sometimes in regular schools, but mostly in special educational facilities. In adulthood, caring facilities are difficult to find outside of the family circle and their funding largely depends on the communities, regions and the private sector and/or charities.

In adulthood, earlier aging and Alzheimer's disease occur more frequently (Wisniewski *et al.,* 1985). The risk of stones in the gallbladder is 3.52 times higher than in a control population (Tyler *et al.,* 2004).

The life expectancy of these individuals has increased markedly in recent decades, mainly due to the treatment of heart defects. The median age of death is now almost 60 years (Zhu *et al.,* 2013; Englund *et al.,* 2013).

There is currently no etiological treatment available for T21. *In vitro* assays to silence the extra chromosome have been performed recently. They open the door to the potential development of “chromosomal therapy” *(Jiang et al.,* 2013).

Animal experiments in mice suggest that prenatal treatment of Down syndrome might be possible (Guedj and Bianchi, 2013). Moreover, early prenatal diagnosis could make it possible to provide brain treatment and thus improve the postnatal cognitive abilities of the individuals concerned (Guedj *et al.,* 2014).

2.2.1.2 Screening for and prenatal diagnosis of T21

Prenatal screening and diagnosis are performed to enable pregnant women and expectant parents to take decisions or act with the full knowledge of the choices available to them by providing them with correct information about trisomy, the various successive procedures and related health implications. Therefore, multi-phase counselling is an essential part of T21 screening and diagnosis.

In the current Belgian context, an invasive prenatal diagnosis can be performed in the light of the result of a combined screening test (probability algorithm based on age, a set of combined serological tests and ultrasound examination (see Appendix 1). An invasive prenatal diagnostic test is rarely carried out directly.

2.2.1.2.1 *The prenatal chromosomal diagnosis of T21*

T21 is the most common chromosomal anomaly in newborns. The risk of foetal T21 increases with maternal age (Table 1, cf. Thompson and Thompson, 2007).

Table 1 Incidence of Down Syndrome depending on maternal age (cf. Thompson and Thompson, 2007).

Maternal age (Years)	At birth	At amniocentesis (16 weeks)	At biopsy of chorionic villi (9-11 weeks)
15-19	1/1250	-	-
20-24	1/1400	-	-
25-29	1/1100	-	-
30	1/900	-	-
31	1/900	-	-
32	1/750	-	-
33	1/625	1/420	1/370
34	1/500	1/333	1/250
35	1/385	1/250	1/250
36	1/300	1/200	1/175
37	1/225	1/150	1/175
38	1/175	1/115	1/115
39	1/140	1/90	1/90
40	1/100	1/70	1/80
41	1/80	1/50	1/50
42	1/65	1/40	1/30
43	1/50	1/30	1/25
44	1/40	1/25	1/25
45 or more	1/25	1/20	1/15

The difference between the incidence of T21 at the time of prenatal diagnosis and at birth is the result of the spontaneous mortality of these foetuses during pregnancy.

The first prenatal chromosomal diagnosis of T21 was made in 1968 (Valenti *et al.,* 1968). For nearly 50 years, setting up a standard karyotype by performing a microscope examination of chromosomes from cultured amniotic fluid cells or chorionic villus sampling was the preferred invasive technique for a prenatal diagnosis. A commonly accepted criterion to perform an invasive prenatal diagnostic test is that the risk of foetal anomaly must be at least as great as the risk of miscarriage or other complications linked to the procedure. The risk of miscarriage associated with chorionic villus sampling and the risk related to amniocentesis is assessed differently by different authors. It obviously depends on the expertise and experience of the person carrying out the puncture, as well as on the mode of presentation of the foetus. The risk is estimated to be approximately 1/300, 1/200 or even 1% (Tabor *et al.,* 1986; Evans and Andriole, 2008).

Conventional chromosomal analysis is very accurate, but the time needed to obtain the result is relatively long, 10 to 15 days, as it requires cultivating amniotic cells or villi. Therefore, faster methods for detecting aneuploidy of the main chromosomes involved have been developed through different techniques, such as fluorescence *in situ* hybridization, quantitative fluorescent PCR (polymerase chain reaction) and MLPA (multiple ligand dependent probe amplification) (Shaffer and Bui, 2007; Cirigliano *et al.,* 2009; Boormans *et al.,* 2008). With these techniques, a diagnosis of T21 can be made rapidly, viz. within 48 to 72 hours. They are usually completed by a conventional karyotype. Currently, invasive prenatal diagnosis by means of rapid and conventional molecular analyses of chromosomes tends to be supplanted by molecular karyotyping obtained through DNA microarrays. With these microarrays, chromosomes can be analysed at high resolution, thus allowing the detection of minute alterations (duplication - deletion), which, though invisible under the microscope, are responsible for congenital malformations and developmental disorders (Hillman *et al.,* 2011; Wapner *et al.,* 2012; Vetro *et al.,* 2012; Vanakker *et al.,* 2014).

2.2.1.2.2 *Ultrasound and serum screening for T21 (the "combined test")*

Prenatal screening for chromosomal anomalies in the foetus is one of the main indications for prenatal diagnostics.

Currently, this search is carried out by means of an invasive approach, such as chorionic villus sampling (at 11 weeks into the pregnancy) or amniotic puncture (around 15 weeks), which entails a small yet real risk of miscarriage (0.5 – 1%). Most children with Down syndrome are born to mothers under the age of 35, who are at a lower risk of having a child carrying T21. This can be accounted for by the fact that the absolute number of pregnancies among these women is much higher. It follows that invasive diagnostic tests cannot be offered to these women on a routine basis. Instead, various non-invasive screening tests have been performed so far.

To avoid this risk to the foetus, non-invasive tests have been developed, such as maternal serum screening combined with an ultrasound scan to measure the thickness of the nuchal skin fold or nuchal translucency (NT). These tests search for **indirect** epiphenomena (not the chromosomal anomalies themselves), with reduced sensitivity and specificity as a corollary.

Chronologically, the first screening test is offered during the second trimester of the pregnancy; 3 substances are measured in the maternal serum: alpha-fetoprotein, β-hCG *(*human chorionic gonadotropin*)* and unconjugated estriol, hence the common name of "triple test". Since recently, some add a 4th substance, viz. inhibin A.

Currently, this screening is performed as early as the first trimester. It quantifies different serum markers in the mother's blood between 11 and 13 weeks into the pregnancy and takes into account the ultrasound measurement of the subcutaneous edema in the foetal neck, also called NT, to calculate the risk of T21 (Snijders *et al.,* 1998). The measured maternal serum markers are "Pregnancy Associated Plasma Protein A" (PAPP-A) and the free β-subunit of hCG. This combined screening can detect 85 – 90% of foetuses with T21 at a false positive detection rate of 5% (Wald *et al.,* 2003; Malone *et al.,* 2005). If the screening is performed **sequentially** in the first and the second trimester in centres with a quality system, its sensitivity for T21 is estimated to be 95% with a false positive rate of 5% (Reddy and Menuti, 2006).

Most women with a positive screening result for T21 undergo an invasive prenatal test. In most cases, the latter reveals that their foetus has normal chromosomes. Consequently, many invasive diagnostic procedures prove to have been unnecessary.

CT-based prenatal screening is widely used in Belgium (about 60% of all pregnancies in the first trimester and nearly 20% in the second trimester). The outcome of the prenatal screening test is determined by estimating the probability of T21 based on a mathematical algorithm (see Appendix 1), which uses the results of serum markers, the ultrasound measurement of the nuchal translucency and maternal age. The overall result of the CT is deemed positive if the probability of T21 exceeds a given cut-off point. The cut-off point used in Belgium is in the order of magnitude of 1/300 (0.0033), or 1/250 (0.0040). The impact of these different cut-off points is such that a higher value (for example 1/250 vs. 1/300) increases the specificity of the CT but reduces its sensitivity, which means that, on the one hand, fewer pregnant women receive a false positive result (for which they would have been offered an invasive test), but on the other hand, fewer foetuses carrying T21 will be identified during the screening procedure.

There are no data available concerning the performance of the CT in Belgium. Based on results obtained in Flanders, which concern about 40% of the combined tests, the sensitivity of the CT can be estimated between 0.70 and 0.85, whilst its specificity is around 0.95. The screening performance parameters and particularly its sensitivity are deemed to be low (Chitayat *et al*., 2011). The poor performance can be attributed to the fact that there is no quality assurance system for the ultrasound measurement of the nuchal translucency. This advisory report takes

into account estimated SE and SP values for different cut-off points as reported by Hulstaert *et al.* (2014) (e.g. for the cut-off point 1/300, the SE is 0.7254 and the SP 0.9503), as well as better test performance settings (SE = 0.9100, SP = 0.9750) due to better-quality data obtained through ultrasound measurements (De Catte, oral communication).

2.2.1.2.3 *Non-invasive prenatal diagnosis (NIPD) / non-invasive prenatal testing (NIPT) for foetal chromosomal aneuploidy*

With a view to detecting foetal chromosomal anomalies **directly** in the maternal serum, research initially focused on the (difficult) isolation of (rare) foetal cells circulating in maternal blood. After many years of intensive research, less than half of the male pregnancies could be identified by detecting male foetal cells in maternal blood (Bianchi *et al*., 2002).

In 1997, the discovery of cffDNA in maternal serum offered new perspectives (Lo *et al*., 1997). This cell-free foetal DNA originates in the trophoblast and is detectable in maternal blood as early as 5 weeks into the pregnancy. It disappears within the first hour after birth, unlike foetal cells, which remain in the mother's blood for a longer period of time (Lo *et al.*, 1998; Ingargiola *et al.,* 2003).

Techniques to determine the sex of the foetus have been developed by studying the free foetal DNA in the context of sex-linked disorders, identifying the RhD factor of the foetus in Rhesus-negative mothers and in the search for paternal mutations for some autosomal dominant disorders. However, the small amount of foetal DNA in maternal blood, which essentially contains maternal DNA, complicated the screening for foetal alleles that were not inherited from the father. Indeed, foetal DNA represents only a small percentage of the total DNA in maternal blood (about 10% in the first trimester) (Lo *et al*., 1998). This requires sensitive detection techniques to differentiate trisomic foetuses (carrying 47 chromosomes) from a normal foetus if only the DNA in maternal blood is analysed, as the foetal supernumerary chromosome will have only a small effect on the total DNA in maternal blood.

To distinguish between the maternal and foetal DNA, the first tests focused on allelic variations between mother and child (Lo *et al.*, 2007b; Tong *et al.*, 2006; Dhallan *et al.*, 2007). This approach has the disadvantage of being dependent on the presence of genetic polymorphisms in some *loci* of the genome and consequently, is only applicable to well defined populations. The development of universal tests that do not depend on polymorphisms (based on digital PCR) nevertheless encountered technical difficulties that were mainly due to the small percentage of foetal DNA in maternal blood (Fan and Quake, 2007; Lo *et al.,* 2007b). These difficulties have been overcome by the development of "next-generation sequencing" (NGS) technology, also called "high-throughput sequencing". The advantage of NGS compared to the first generation sequencing technology is that it makes it possible to sequence millions of DNA fragments simultaneously, after a step of clonal amplification, (therefore also referred to as "Massively Parallel Sequencing" - MPS), based on very small amounts of DNA and using a minimum of reactants. Thus, with NGS, millions of fragments ("reads") can be generated and analysed quickly and at reasonable cost, multiplying by over 100 ("coverage") the determination of each nucleotide in the genome. This was the ideal method to achieve a quantitative and accurate measurement of small differences in the concentration of foetal DNA in maternal blood.

In 2008, NGS was used for the first time for NIPD/NIPT by two independent research groups (Chiu *et al.*,; Fan *et al.*, 2008). The DNA in maternal plasma was sequenced and the origin of the fragments obtained ("reads") was determined ("mapped") by comparison with the human reference genome. Then, the (relative) number of fragments per chromosome was counted. In case of a trisomic foetus, the number of fragments of the supernumerary chromosome was (statistically significantly) higher compared to a normal (diploid) foetus. Given the fact that the chromosome 21 represents less than 1.5% of the sequenced genome in a normal individual, the

establishment of a statistically significant difference between normal foetuses and foetuses carrying T21 requires the analysis of several million of DNA fragments. Chiu *et al*. showed that it was possible to diagnose 100% of foetuses carrying T21 based on a sample with an average of 2.3 million sequenced fragments ("reads"). On the other hand, if the sample contained only 0.3 million, the detection rate dropped to 79% (Chiu *et al.*, 2011). However, this approach requires extensive DNA sequencing, whereas only one chromosome is of interest (*in casu* chromosome 21). This explains why targeted analysis methods have been developed to replace methods that cover the whole genome ("whole genome approach"), the latter being more time consuming and expensive. With this targeted approach, it is not the genome as a whole that is analysed. Instead, it targets specific chromosomes or chromosome regions (e.g. chromosomes 18 and 21). Several studies have shown that this approach significantly reduced (from 5 to 10 times) the number of sequenced fragments ('reads') needed to make an accurate diagnosis of trisomy 18 or 21 (Sparks et *al.*, 2012; Ashoor *et al.*, 2012a; Norton *et al.*, 2012). This "targeted approach" offers the advantage of being faster, feasible on simpler NGS devices and therefore of being superior in terms of cost-effectiveness (Boon and Faas, 2013).

- NIPD or NIPT?

The issue whether the non-invasive prenatal method has to be considered as a screening test (NIPT) or as a diagnostic test (NIPD) deserves our attention.

A non-invasive prenatal screening **test** refers to the non-invasive assessment of foetal health and in particular, the analysis of cffDNA in maternal blood. A screening test allows to identify individuals likely to be affected. A positive screening test has to be confirmed by a diagnostic test. This is particularly the case for the assessment of foetal aneuploidy: an invasive test needs to be performed to confirm any positive NIPT. Currently it is the accepted term to refer to the screening for foetal aneuploidy by analysing the cffDNA in maternal plasma because this test has not yet acquired diagnostic accuracy. The main argument in favour of using cffDNA as a diagnostic tool is the fact that the number of false positives should be lower or at least equivalent to that of the invasive test.

The non-invasive **diagnosis** of a condition in the foetus is defined as the diagnosis made without direct access to foetal tissue (chorionic villus sampling or amniocentesis). Consequently, there is no risk of miscarriage associated with this procedure. This term is now generally used for diagnostics analysing cffDNA in maternal plasma. The result of a non-invasive diagnostic test (e.g. foetal sex determination) can immediately be used for clinical purposes and generally does not require confirmation by means of an invasive test. The current test for foetal aneuploidy has not yet reached the same diagnostic accuracy as the invasive test.

Benn *et al*. (2013) reviewed a number of clinical series studying the cffDNA in maternal blood and conclude that NIPT, whilst it is a highly effective screening test, cannot replace invasive prenatal diagnosis as a means of diagnosing T21 (Chiu *et al.*, 2011; Ehrich *et al.*, 2011; Palomaki *et al.*, 2011; Bianchi *et al.*, 2012; Ashoor *et al.*, 2012a; Sparks *et al.*, 2012; Norton *et al*., 2012; Palomaki *et al.*, 2012; Lau *et al.*, 2012; Nicolaides *et al.*, 2012).

- NIPT for high-risk pregnancies or all pregnancies?

Most existing studies have focused on the study of cffDNA in pregnancies at a high risk of aneuploidy. The detection rate for trisomy 21 was found to be 99.3% (CI 95%, 98,2 to 99,8%) with a false positive rate of 0.16% (CI 95%, 0.08 to 0.31%). This detection rate is far superior to all previous screening protocols for T21 by means of the combined test. According to Benn *et al*. (2013), analysing cffDNA in women at a high risk of T21 followed by a confirmatory invasive diagnostic test – in the event of the NIPT result being positive - is not likely to cause any notable change in the detection rate, but it will reduce the rate of false positives drastically (about 300 times). In their conclusion, Mersy *et al*. (2013) state that NIPT will probably replace the

current risk assessment of T21 by means of serum markers but that larger prospective studies in a population of low-risk women are needed before its introduction into the public health system.

Several studies have been conducted in a population of women who were not at an increased risk of aneuploidy (Fairbrother *et al*., 2013). These limited results suggest that the detection rate is 99%, with a false positive rate of about 0.2%. More recent studies show the feasibility of using NIPT for prenatal screening for T21 within the general population, with the latter showing a better test performance compared to standard screening (Bianchi *et al*., 2014). This study involving 1,914 women at a low risk of T21 showed a significantly lower rate of false positivity for NIPT compared to screening based on serum markers, both with and without the measurement of nuchal translucency (0.3% vs 3.6% for T21). In addition, the positive predictive value for T21 was superior to that of standard screening (45.5% vs 4.2%). The negative predictive value was 100% (CI 95%, 99.8 to 100%) (Bianchi *et al.*, 2014).

There are a number of cases in which the analysis of foetal DNA failed because no foetal DNA could be amplified, e.g. when the foetal fraction (the proportion of foetal DNA relative to the total circulating DNA) was insufficient. Ideally, the foetal fraction should be greater than or equal to 10% and currently it should always exceed 4%. These failed NIPTs should be viewed in the light of the gestational age and maternal weight (Wang *et al.*, 2013; Ashoor *et al.*, 2012b; Haghiac *et al.*, 2012).

Few data are currently available regarding the diagnosis of T21 in twins. Further research is needed to assess the zygosity of twins and there is a risk of false positivity when one of the twins is normal and the other one dies with an aneuploidy (Canick *et al.*, 2012; Lau *et al.*, 2013; Futch *et al*., 2013). Recently, a study involving 189 twin pregnancies showed the feasibility and accuracy of detectingT21 with NIPT (Huang *et al*., 2014).

NIPT does not detect foetuses carrying mosaic T21 and it may lead to false positive results if the foetus has normal chromosomes whilst the placental cells are trisomic (Pan *et al.*, 2013; Wang *et al*., 2013).

Finally, a mismatch between a pathological NIPT and a normal foetus has been described in a case of maternal cancer (Osborne *et al*., 2013).

- <u>Failures and contra-indications for NIPT</u>

Currently, a distinction can be drawn between maternal or foetal contra-indications for using NIPT.

As regards the mother, these contra-indications are linked to the presence of cells that do not match the initial maternal genome, which can be due to:

- a stem cell therapy, immunotherapy, an organ transplant in the mother;
- a blood transfusion in the mother right before the test;
- a cancerous tumour in the mother that may cause cells with chromosomal rearrangements to reach the bloodstream, thus disturbing the analysis.

For the foetus:

- twin and/or multiple pregnancy (Conick *et al.*, 2012; Lau *et al.,* 2013; Futch *et al.*, 2013; Huang *et al.*, 2014);
- a nuchal translucency greater than 3.5 mm during the first trimester of the pregnancy;
- malformations in the foetus visible on the ultrasound at any time during the pregnancy.

Indeed, these last two sonographic signs may be indicative of another chromosomal anomaly that requires genetic counselling and possibly the carrying out of an invasive diagnostic analysis (Devers *et al.*, 2013; Vanakker *et al.*, 2014).

According to the literature, NIPT fails in 0.7 to 6% of all cases. The most frequently reported technical reasons are insufficient blood sampling, too long a delay between the time of blood collection and its receipt at the laboratory, problems with the extraction of DNA or a problem with the sequencing. Concerning the pregnant woman, the foetal DNA fraction should be greater than or equal to 10%, with the lower limit currently around 4%. It follows that the blood of the mother should not be taken too early in pregnancy: ideally at 11 weeks into the pregnancy. Maternal obesity is another factor that reduces the recovered fraction of foetal DNA.

2.2.2 The context of the implementation of NIPT as part of prenatal screening for T21 in Belgium

2.2.2.1 Selecting the parameters to carry out the NIPT and the consequences of these different scenarios

Given the lack of observable parameters to assess the combined test used in this country, it is not possible to draw a comparison with the introduction of NIPT. Therefore, the SHC had to consider scenarios based on different theoretical assumptions about the parameters of the combined test. This advisory report takes into account estimated SE and SP values for different cut-off points, as reported by Hulstaert *et al*. (2014) (e.g. for the cut-off point 1/300, SE = 0.7254 and SP = 0.9503), as well as better performance parameters for the test (SE = 0.9100, SP = 0.9750) due to better-quality data from the ultrasound (De Catte, oral communication). Regarding the results of NIPT, most pertain to high-risk populations. More recent data from the literature concern pregnant women among the general population. In both cases, the SE (ranging from 0.9930 to 1) and SP (0.9970 to 0.9984) are significantly higher than those for CT. In the scenarios described below, the NIPT SE and SP used are set at 0.9930 and 0.9970, respectively.

In order to simulate the introduction of NIPT in screening for and/or prenatal diagnosis of T21 in Belgium, several theoretical scenarios and their impact on health outcomes are outlined below.

The details of all scenarios are given in Appendix 2; a comparative overview of the described scenarios is provided in Table 2. For all scenarios, the following data were used:

- 100,000 pregnancies in the first trimester;
- prevalence of T21 (1st trimester): 0.0024 (1/416);
- rate of miscarriages linked to invasive testing: 0.01.

Scenario 1 outlines the current practice with the combined test (cut-off 1/300, SE 0.7254 and SP 0.9503); scenario 1B (see Appendix 2) with a better SE (0.9100) and SP (0.9750). Simply improving the SE results in a greater number of patients being referred for invasive testing (174 vs. 218), all of which actually turn out to be positive. A higher SP reduces the number of false positives (4,958 vs. 2,494). In the more efficient CT, the number of invasive tests is lower (5,132 vs. 2,712) with a higher detection/miscarriage rate (3.4:1 against 8.0:1).

In scenarios 2, 3, 4 and 5, NIPT is offered as the second-tier test, i.e. after a positive combined test, with different cut-off points (1/300, 1/600 and 1/1,200 for the scenarios 2, 3 and 4, respectively), whilst in scenario 5, the test quality is considerably improved for the cut-off value 1/300 (see scenario 1B). Changing the cut-off from 1/300 to 1/1,200 increases SE and reduces SP without affecting the intrinsic quality of the test. The introduction of NIPT as a second-tier test reduces the overall SE and increases the overall SP compared to the parameters of the first-tier tests.

- In scenario 2, the number of detected foetuses carrying T21 goes down rather than up, given the lower SE - but the number of invasive tests drops dramatically.
- Scenarios 3 and 4 make it possible to detect a higher number of foetuses carrying T21 compared to current practice.
- In scenario 5, the quality of the CT, which is the first-tier test, is improved. More foetuses carrying T21 are identified and fewer women are wrongly referred for NIPT. The net result of the combination of CT and NIPT is the higher number of detected foetuses carrying T21 and the lower number of unidentified foetuses carrying T21. Under these conditions, the detection/miscarriage ratio is high (108:1).
- In scenario 6, NIPT is offered as a first-tier screening test. In this case, the number of invasive tests is slightly higher compared to scenarios 2, 3, 4 and 5, but the number of detected foetuses carrying T21 is far greater than is the case in current practice (scenario 1A) and better compared to the other scenarios, viz. 2-5.

Table 2 Comparative table with different theoretical scenarios

	SCEN1	SCEN 2	SCEN3	SCEN4	SCEN5	SCEN6
cut-off point	1/300	1/300	1/600	1/1,200	1/300	no
SE CT	0.7254	0.7254	0.8099	0.8521	0.9100	n.a.
SP CT	0.9503	0.9503	0.9088	0.8449	0.9750	n.a.
SE NIPT	n.a.	0.9930	0.9930	0.9930	0.9930	0.9930
SP NIPT	n.a.	0.9984	0.9984	0.9984	0.9984	0.9984
PPV	0.0339	0.9558	0.9324	0.8904	0.9818	0.5980
Combined test						
number of T21	174	174	194	205	218	-
number of false positives	4,958	4,958	9,098	15,473	2,494	-
number of false negatives	66	66	46	35	22	-
NIPT after positive CT	-	5,132	9,292	15,677	2,712	100,000
number of T21	-	173	193	203	216	238
number of false positives	-	8	14	25	4	160
number of false negatives	-	67	47	37	24	2
Invasive test	5,132	181	207	228	220	398
number of iatrogenic miscarriages	51	2	2	2	2	4
number of avoidable miscarriages	50	0	0	0	0	2
detection/miscarriage rate	3.4:1	86:1	96:1	102:1	108:1	60:1
Difference compared to SCEN 1						
number of invasive tests	**-**	**- 4,951**	**- 4,925**	**- 4,904**	**-4,912**	**- 4,734**
number of T21	**-**	**- 1**	**19**	**29**	**42**	**64**

1st trimester: 100,000 pregnancies; T21 prevalence: p = 0.0024 (1/416); invasive tests/miscarriages ratio: 0.01;
CT = combined test; NIPT = *non-invasive prenatal test*; SE = sensitivity; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

These scenarios make it possible to validate the introduction of NIPT for pregnant women and to discuss different procedures.

NIPT may be introduced either as a second-tier test to be performed in the light of the results of the CT or as a first-tier test without CT. Both approaches result in a substantial reduction in the number of invasive tests performed (based on a theoretical population of 100,000 pregnancies with an T21 incidence of 0.0024) and, depending on the scenario used, the number of tests performed drops from over 5,132 to around 181 to 220 IVTs when NIPT is offered as a second-tier test and to 398 IVTs when it is offered as the first-tier test.

The introduction of NIPT also reduces the number of false negatives, depending on the scenario under consideration. Thus, it drops from 67 to 24 false negatives when NIPT is performed as the second-tier test and to 2 false negatives when it is the first-tier test.

Scenario 5 clearly shows the importance of a better performing CT.

2.2.2.2 Benefits and limitations of NIPT as a first-tier or second-tier test

2.2.2.2.1 *NIPT as a second-tier test after CT*

Benefits:

- greater reduction in the number of IVTs (although the difference is not substantial);
- considerably greater positive predictive value (0.89 to 0.98 vs. 0.60 for the first-tier NIPT): this is due to the fact that NIPT is practised only among women with a positive CT, which increases the net specificity of the two consecutive tests substantially;
- few NIPTs (between 2,700 and 16,000 tests depending on the scenario).

Limitations:

- lack of standardization of the CT (as the first-tier test) with greater variability expected in the quality of the results: improving the standardization and quality of the ultrasound examination would provide a solution to this limitation (see scenario 5);
- larger number of false negatives: this number decreases as the SE of the CT goes up, but never reaches the same level as a first-tier NIPT test.

2.2.2.2.2 *NIPT as the first-tier test*

Benefits:

- does not depend on a prior test with unknown and variable quality in Belgium;
- number of false negatives near 0.

Limitations:

- extremely high number of NIPTs (close to the total population of pregnant women);
- compared to the use of NIPT as the second-tier test, the decrease in the number of invasive tests performed is slightly lower;
- higher number of false positives, resulting in a slightly lower positive predictive value.

2.2.2.2.3 *Conclusion*

- Both approaches for the introduction of NIPT, namely as a first-tier or as a second-tier test, substantially improve prenatal screening for T21. This results in:
    - a substantial drop (over 90%) in the number of IVTs performed;
    - a rise in the number of foetuses identified that potentially carry T21 due to a considerably greater positive predictive value.
- The use of NIPT as the only diagnostic test for T21 cannot be recommended because of the number of false positives obtained for the SP value used in the scenarios.
- The use of NIPT as the first-tier test in prenatal screening:
    - generates more false positives for the SP value used in the scenarios. Referring these false positives for invasive testing results in a potentially greater number of preventable miscarriages among these pregnancies compared to scenarios in which NIPT is used for secondary screening. However, the absolute difference is very small and is not clinically relevant, even in the conservative scenario used, with a high miscarriage rate, viz. 0.01.
    - leads to the lowest number of foetuses carrying T21 missed by prenatal screening.
- The use of NIPT as a second-tier test after a positive CT results in:
    - a substantial drop in the number of invasive tests performed. The artificial adaptation of the performance parameters of the CT, changing the cut-off point for referral for NIPT from 1/300 to 1/1,200, results in an overall increase of the SE without any great loss in SP for the combined approach (NIPT after the current

test). The detection/miscarriage ratio can be used to identify the optimal cut-off point.

- The added value of CT as the first-tier test remains questionable given the actual intrinsic performance level of the CT. However, it is necessary to improve the quality of the current CT, and particularly the intrinsic test performance of the ultrasound examination, to keep the CT as a first-tier test in the battery of prenatal screening tests for T21 (CT followed by NIPT) (see scenario 5).

Prenatal testing/screening for T21 can be set up by introducing NIPT as the first-tier test followed by an invasive test after a positive result. However, NIPT cannot replace the current monitoring of pregnancies, as it only looks for certain chromosomal anomalies, whilst there may be many other prenatal anomalies that can be found by means of an ultrasound examination. With the ultrasound examination required for other indications in actual practice and given the time required to evaluate the results of NIPT (1 to 2 weeks), it is unlikely that NIPT will be performed without a preceding or simultaneous ultrasound examination.

2.2.2.3 Prerequisites for the introduction of NIPT

2.2.2.3.1 *The cascade of information and genetic counselling*

Counselling pregnant women and expectant parents is a procedure that takes place at different stages of the diagnosis and prior to initiating each step of the process (Devers *et al*., 2013). They should be provided with information prior to any search for T21. This can be done by first-line medical and paramedical staff by means of information sheets and HON- certified web sites ("Health on the Net"-label attesting the quality of the scientific information)[1].

The content of the information has to be clear and precise to avoid misunderstandings (Gregg *et al*., 2013). The objectives and limitations of the test, the reasons for the different stages, the options after each test, the advantages and disadvantages of each procedure, the contra-indications and possible failures should be clearly presented (Benn *et al*., 2013). This information should enable pregnant women and expectant parents to take a decision with the full knowledge of the choices available to them and without any pressure before and after each step of the screening process. An example of how this cascade of information and counselling may be provided is shown in Figure 1.

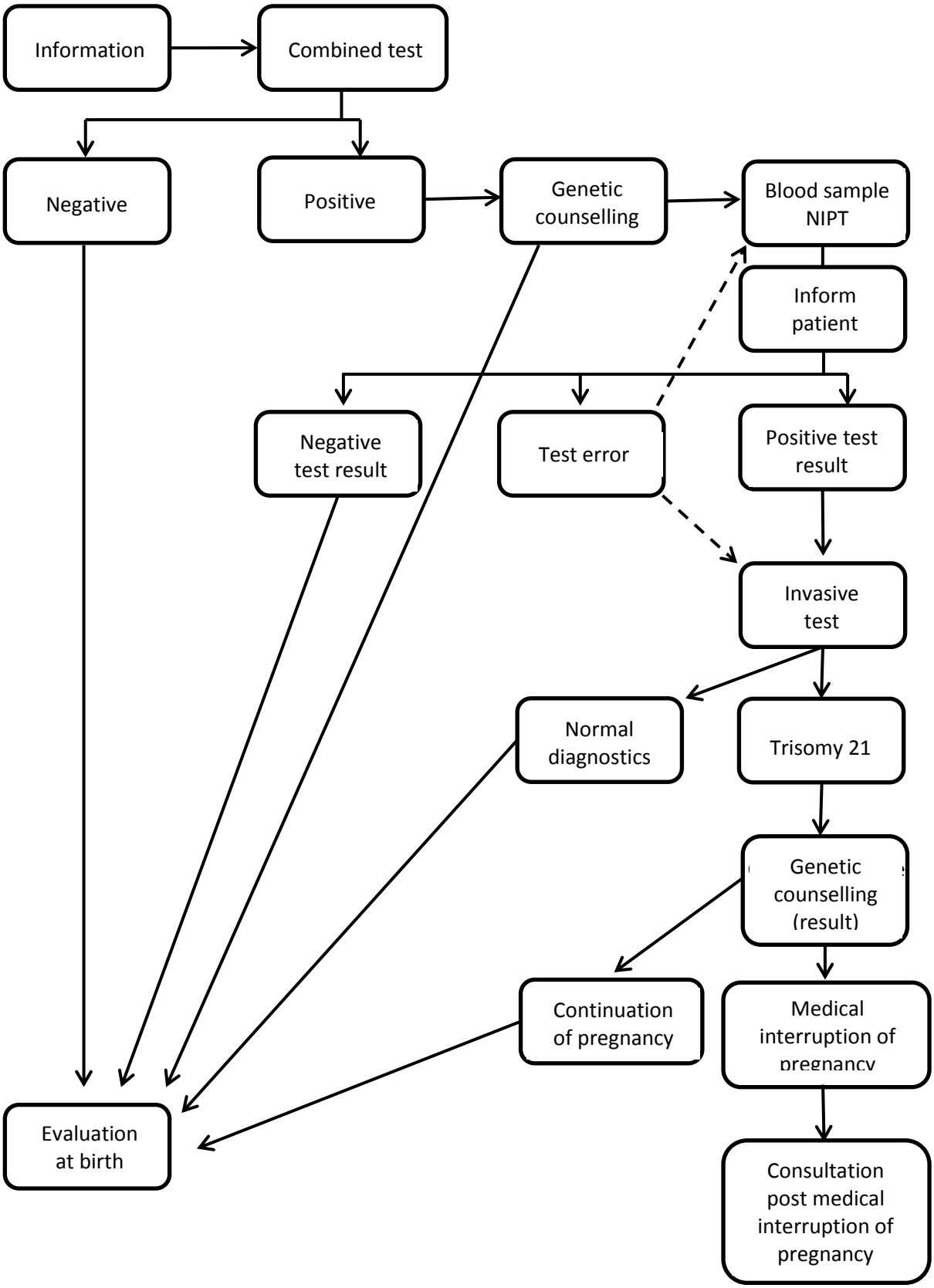
In the context of a pregnancy for which conventional medical care is provided (CT during 1st or 2nd trimester):
If the results of the CT indicate that the probability of the foetus carrying T21 exceeds the cut-off point, NIPT is offered and a blood sample drawn once the pregnant woman and expectant parents have been provided with all the necessary information .

- If NIPT is indicative of Down syndrome, an invasive prenatal diagnosis is offered after genetic counselling. In the opinion of the Council, this should be performed prior to any decision to terminate the pregnancy.

- If NIPT does not find any T21, the medical monitoring of the pregnancy continues in a conventional way, once the information has been provided to the pregnant woman and expectant parents.

[1] https://www.hon.ch/ [1] https://www.hon.ch

Figure 1: An example of the cascade of information and genetic counselling (based on scenarios 2-5).



2.2.2.3.2 *The quality criteria*

At present, we do not have any systematic information about the parameters used for the combined test algorithm (CT) in Belgium, which is based on serum markers, ultrasound imaging of the foetus and maternal age. It is very likely that there is a significant amount of variation in this respect, given the lack of standardization and quality control of the results of the ultrasound scan. The cut-off point for the result of the combined test that leads to the invasive procedure can vary between 1/250 and 1/300. For example, for p (T21 | pos CT) ≥ 0.0033 (1/300) the SE of the test and its SP in the Netherlands are known and estimated as SE (0.85 - 0.95) / SP (0.93 to 0.97) (Health Council of the Netherlands, 2013). In Belgium, however, the test performance is estimated to be much lower: SE (0.70 to 0.85) / SP (0.95). In addition, a false negative CT with a low SE leads to a greater likelihood of T21 in women with a negative test result. For example, with an SE of 0.75 and 0.85, respectively, and a 1st trimester prevalence of T21 = 0.0024, this probability (negative predictive value) is 0.0076 compared to 0.0038 (p (T21 | neg CT, SE: 0.75, SP: 0.95) = 0.00076 (1/1,587), p (T21 | neg CT, SE: 0.85, SP 0.95) = 0.00038 (1/2,632); see Appendix 2, scenarios 1 and 4).

The overall SE of consecutive tests is always lower than the SE of the first test. Introducing the NIPT as a second-tier test should not adversely affect the negative predictive value. This means that the test performance of NIPT should be such that p (T21 | neg + CT (CT NIPT | pos)) is less than or equal to 0.00076.

The quality criteria concern the entire process, including the provision of information and genetic counselling.

The performance of CT in Belgium is variable and possibly poor. Therefore, the added value of the CT as a first-tier test to be carried out before NIPT remains questionable if there is no improvement in the intrinsic test performance of the CT, especially as regards the ultrasound scan. To keep the CT as the first test in the battery of prenatal screening tests for T21 (CT followed by NIPT), it is essential to take measures to improve the overall quality of the obstetric ultrasound examination. This can be achieved by continuous training and the establishment of a certification program for sonographers in the field of prenatal screening and limiting reimbursement to the tests carried out in accredited centres.

Since the expected number of invasive tests will be considerably reduced by the introduction of NIPT, it is also recommended to take the necessary measures to ensure the quality of invasive testing and thus, keep the number of iatrogenic miscarriages minimal. This objective can be achieved by means of an accreditation program as well as by having accredited centres carrying out the invasive tests.

2.2.2.3.3 *Prescription procedures and guidelines*

Because NIPT cannot be considered without taking into account other aspects of prenatal care as well as the applied techniques, including ultrasound monitoring, the Council takes the view that the test should be prescribed by a medical specialist with the appropriate skills, i.e. a specialist working in a recognized centre of gynaecology and obstetrics or a recognized genetics centre.
Unambiguous procedures and guidelines must be set up by a joint association composed of the College of Physicians for the Centre of Human Genetics (Royal Decree of 26.11.2012 published in the Belgian Official Gazette on 12.01.2012), the Belgian Association of the French-speaking obstetrician-gynaecologists (*Groupement des Gynécologues Obstétriciens de Langue Française de Belgique* - GGOLFB) and the Flemish Association for Obstetrics and Gynaecology (*Vlaamse Vereniging voor Obstetrie en Gynaecologie* - VVOG).

2.2.2.3.4 *Monitoring system of the screening process and health outcomes*

At present, it is difficult to assess the potential impact of introducing NIPT, given the lack of systematic basic data. The introduction of new procedures requires the simultaneous setting up of a follow-up system with which the performance of various tests carried out in Belgium can be monitored individually. This also involves monitoring the health outcomes of prenatal screening.

2.2.2.3.5 *Management of the capacity to implement NIPT*

At present, a number of samples, from which the genome of the patient could be analysed, are sent abroad. It is not acceptable to send the genomic material of an entire population and generation since there is absolutely no guarantee as to the use that will be made of the maternal and foetal genome in countries that do not have the same legislation, nor the same deontological and ethical rules as our country. Therefore, the systematic offering of non-invasive prenatal screening requires this country to have the capacity to carry them out on its own soil.

Given the genetic material available in blood samples and its overall significance for population screening, NIPT is a genetic test that needs to be performed by recognized Belgian genetics centres (RD 1987; MD 1988 MD 1989) to ensure the deontological and ethical treatment of the genetic material of the Belgian population. In addition, the laboratories concerned must acquire a BELAC accreditation (the Belgian Accreditation Body).
Though a little more time is still needed to develop this test, the wait may actually be very short, with some centres already having the capacity to offer it and others setting it up for late 2014. As a first step, the number of analyses which can then be performed will make it possible to provide for a pilot phase during which the results of NIPT and CT will be assessed in parallel.

In addition, sufficient attention should be paid to the necessary centralization in accredited centres of and training in the various components of this process (see section 2.2.2.3.2 Quality criteria).

- information and counselling;
- ultrasound examination;
- invasive testing procedures.

2.2.2.3.6 *Introduction into the healthcare system*

It is preferable to introduce NIPT into the healthcare system after a pilot phase, which should be organised through a joint project of the College of Physicians for the Centre for Human Genetics, the GGOLFB and the VVOG.

In pursuance of Article 8 of the RD (Royal Decree) (1999) on assessing the quality of the medical activity in hospitals, the College will do the following :

1. based on a consensus, it will develop quality indicators and assessment criteria for the correct medical practice regarding the NIPT and their results;
2. it will implement a computerized registration model and a standardised report.

This pilot phase could be conducted in collaboration with the Scientific Institute of Public Health (*Institut scientifique de Santé Publique/Wetenschappelijk Instituut voor de Volksgezondheid* – ISP/WIV).

This pilot phase should result in :

- a better definition of the guidelines, both with regard to the providing of information and counselling as well as to clinical and laboratory procedures;
- the uptake of the renewed offer by pregnant women:

    - motivation;
    - access and inequalities in healthcare;
    - health literacy;
    - impact on decision making.
- the defining of the parameters with which the processes and quality can be monitored as well as the preparation of continuous monitoring;
- the defining of performance parameters;
- the defining of the role of the serological test following the introduction of NIPT.

The Health Council of the Netherlands also advises that data be collected during the pilot phase for introducing NIPT into the healthcare system (2013).

2.2.2.3.7 *Ethical and legal aspects*

What is a genetic test?

This term often includes different concepts, yet the latter entail legal issues that make it all the more necessary to define it with great precision, viz. confidentiality and privacy, data protection, biobanks, insurance, labour laws and evaluative medicine. This is why some suggest that a distinction should be drawn between different types of clinical genetic testing, e.g. clinical examination, laboratory tests and genetic information, which includes, amongst others, the interpretation of the latter two tests (Varga *et al*., 2012).

The 'Convention on Human Rights and Biomedicine' (or Oviedo Convention) has so far been ratified by 28 countries, but Belgium has not signed it yet. The additional protocol on genetic testing for health reasons (Council of Europe, 2008) establishes standards regarding the quality of genetic service, prior information and consent, genetic counselling and population screening, and uses the concepts of 'clinical validity' and 'usefulness of genetic tests'. This is the first international instrument that governs, from a legal point of view, genetic testing for health reasons (Lwoffl, 2009). The SHC recommends that Belgium too should ratify this convention.

The implementation of innovative medical technologies can raise unprecedented ethical, legal and social dilemmas. This applies in particular to prenatal diagnosis. In some cases, the doctors, pregnant women and future parents may not share the same point of view (Williams *et al*., 2005).

The ethical issues raised by prenatal diagnosis include:

- psychosocial problems as a result of a false positive screening;
- issues related to the reporting of the results to the family;
- issues related to the discovery of unexpected results;
- ethical issues related to the informed choice and/or informed consent;
- the necessary information and counselling to patients and their families;
- issues of privacy and confidentiality;
- the right to have access to the available test technologies;
- issues in equity as regards e.g. the access to screening programs;
- issues related to the acceptability of abortion and the social stigma associated with the lower prevalence of certain conditions (Potter *et al*., 2009).

In 2013, the German Ethics Committee ruled on the introduction of genetic testing into clinical medicine (Deutscher Ethikrat, 2013). The same year, the Health Council of the Netherlands and the Comité Consultatif National d'Ethique pour les sciences de la vie et de la santé (National Consultative Ethics Committee for Life Sciences and Health) in France issued their advisory reports on foetal genetic testing on maternal blood.

These advisory reports take the view that the efficiency of screening in women at a high risk of T21 could be considerably enhanced by implementing foetal genomic testing of maternal blood, which would prevent the vast majority of invasive diagnostic tests that are required to confirm the diagnosis, i.e. invasive procedures that pose risks to the foetus and sometimes to the mother.

The French Committee argues that this test could be implemented as a first-tier screening test for all pregnant women, should its scientific relevance be confirmed. The limitations to such an implementation are of a technical, organizational and financial nature, rather than ethical. Yet on a societal dimension, there is the issue of the stigma attached to disability and its economic and social burden. The same concern is expressed by McCabe and McCabe (2011), as they describe the pressure exerted by some insurance companies or the pressure to limit the reproductive choices of parents, which could be observed in some states of the United States. This essentially boils down to eugenics.

It follows that it is of paramount importance to provide pregnant women and expectant parents with proper information and to continue to provide support and resources to those who decline prenatal testing or choose to continue a pregnancy in which the foetus is affected by a malformation or carries Down Syndrome (Hui and Bianchi, 2013).

The ethical advisory report from the Netherlands expresses concern about the potential disadvantages of “routine” NIPT. This could result in pregnant women and their spouses failing to realize that the results of these tests can lead to difficult choices. The routine test (if NIPT is used as a first-tier test) could put a heavy burden on the pregnant woman and a responsibility as a parent-to-be that may be psychologically difficult to bear .

Moreover, there is the inevitable question of the future possibilities of non-invasive testing of the foetal genome, i.e. what criteria will be used to decide whether or not screening is performed and who will take that decision.
It therefore seems really simplistic to describe the aim of non-invasive screening as the providing of informed reproductive choices.

Is there a clear line in the human world between what is normal and what is different? What are the standards that will be considered acceptable by future parents and society?

In the law on patient rights (Belgian Official Gazette 26.09.2002), the Belgian legislation provides for the right of the patients to request the professional practitioners to provide them with any information about themselves that may be necessary to understand their health status and its evolution (art. 7). In addition, the law mentioned above also provides for the right of the patients to freely consent to any intervention by the professional practitioner after having been given proper information (art. 8).

The law on abortion of 9 April 1990 (Belgian Official Gazette 05.04.1990) decriminalizes abortion beyond 12 weeks of conception when it is certain that the child will be born with a particularly serious condition that is recognized as incurable at the time of the diagnosis.

As a matter of fact, all of these questions, as well as others, will be discussed in a future advisory report by the Advisory Committee on Bioethics of Belgium (CBN).

3 CONCLUSION AND RECOMMENDATIONS

Conclusion

Prenatal screening for foetal anomalies offers individuals (pregnant women and parents-to-be) the opportunity to have relevant information on the basis of which they will be able to decide whether to continue or terminate the pregnancy. In the first case, they will have the opportunity to prepare for the birth of a sick or disabled child whilst in the second, they will be able to avoid such a birth.

The possibility of non-invasive screening for chromosomal anomalies (such as T21) should lead to an improvement of current prenatal screening.

NIPT has two advantages over existing techniques:

- Firstly, it reduces the number of invasive diagnostic tests (chorionic villus biopsy and amniocentesis are cumbersome procedures, with an attendant risk of miscarriage of 0.5 to 1%). This is believed to result in an increase in the number of cases of T21 identified in relation to the number of induced miscarriages.
- Next, introducing NIPT into the healthcare system should reduce the number of false-negative cases due to the variability of the SE of the combined tests used in our country.

NIPT can be included in the procedure of prenatal screening for T21 provided to all pregnant women. This test, which is non-invasive and therefore safe for both the foetus and the pregnant woman, can be performed as early as the end of the first trimester. In the event of pathological findings, it should be validated by means of an invasive prenatal diagnostic test after genetic counselling to the mother and future parents .

Recommendations

In the light of these data, the SHC recommends the following:

1. NIPT should be introduced into the healthcare system, both as a first and second-tier test (test performed after the combined test and based on the latter's results). Indeed, it is a major improvement in prenatal screening for T21. For identical cut-off points, it can significantly reduce the number of invasive tests performed. In addition, the early detection of T21 is comparable when the cut-off point remains unchanged. It is better when the quality parameters of the CT are improved (by lowering the cut-off point in the CT algorithm without increasing the intrinsic quality). NIPT can be offered to all pregnant women. There is no indication to limit NIPT to at-risk pregnancies.

    - The use of NIPT test as a first-tier prenatal screening test for T21 results in the lowest number of foetuses carrying T21 missed by the screening procedure. In contrast, the number of invasive tests and the number of false positive results for which the pregnant woman will be offered an invasive test is higher than that obtained when NIPT is carried out as a second-tier test. However, this figure remains well below the current situation and the difference with NIPT as a second-tier test has no clinical relevance.
    - The use of NIPT as a second-tier test, following a positive CT, results in the greatest reduction in the number of invasive tests performed. In contrast, the number of foetuses carrying T21 identified by the screening process will not necessarily be higher. It will still be inferior to the number of identified foetuses carrying T21 when NIPT is used as the first-tier test.

2. The added value of NIPT as a second-tier test, following the CT, is limited as a result of the relatively lower performance of the CT, especially that of the ultrasound scan. The Council therefore advises that this limitation should be partially compensated by improving the quality of the ultrasound examination.
3. The prenatal test for trisomy 21 can be set up by introducing NIPT as the first-tier test followed by an invasive test performed in the event of a positive result.

A comparison between current prenatal screening for Down syndrome and the use of NIPT justifies recommending the latter. Ideally, it should be implemented as the first-tier test. Compared to the method currently used for prenatal screening, the number of pregnant women wrongly referred for an invasive test on the basis of a false positive result of the CT drops significantly. Concomitant with this drop is a decrease in the number of foetuses carrying T21 that are missed, which will become rare. The use of NIPT as a second-tier test is another good option, but second best. When it is performed as a second-tier test following the CT, NIPT significantly reduces the number of invasive tests, but its added value is limited by the relatively poorer performance of the CT. Therefore, the number of foetuses carrying T21 that the prenatal screening misses, is even likely to increase.

4. NIPT cannot replace the current techniques to monitor pregnancies, such as the ultrasound scan, since NIPT only searches for a few chromosomal anomalies. Besides these anomalies, prenatal care, and especially ultrasound screening, can find many other anomalies (including non-genetic anomalies).

5. Progressive introduction: Ideally, introducing NIPT into the healthcare system should be phased in stages. A pilot phase is recommended for the introduction of NIPT into the healthcare system in order to perform a thorough assessment of all aspects of its implementation.

6. Practical prerequisites for the introduction of NIPT:
   - information and genetic counselling in cascade:
     - the need for women and couples to have access to adequate information describing the different screening techniques, their limitations and the options they provide;
     - the opportunity to benefit from genetic counselling;
   - the existence of standardized indications for testing, prescribing rules and protocols;
   - quality assurance for carrying out the different processes: counselling, laboratory tests, foetal ultrasound, risk calculation program, invasive procedures;
     - on-going training and the setting up of a certification program for sonographers in relation to prenatal screening. Refunding should be limited to tests performed in accredited centres;
     - on-going training for gynaecologists and the setting up of a certification program for carrying out invasive tests. These tests should only be performed in accredited testing centres;
     - accreditation of genetics laboratories that perform the NIPT in recognised genetics centres;
   - upgrading the knowledge of
   - GPs and specialists with respect to these new technologies;
   - an annual record of the number of NIPT performed, the criteria used for testing, basic clinical data of the mother, the test result, the result of the invasive diagnostic test, prenatal monitoring and follow-up at birth;
   - the setting up of a system for monitoring the consequences of introducing NIPT into the healthcare system on test performance and health outcomes.

7. The professional and ethical prerequisites for introducing NIPT into the healthcare system

- fair access to the new test, regardless of the economic and cultural environment;
- the possibility for pregnant women and parents-to-be to decline prenatal testing and to decide whether or not to continue the pregnancy, regardless of their choice and the results of these tests concerning T21;
    - whilst continuing to receive adequate support and without the healthcare system exerting any kind of pressure on the decision-making process;
    - finally, whilst complying with the existing legislative framework which governs abortion procedures and the medical termination of pregnancies in Belgium.

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5 APPENDIXES

The Council provides the following appendixes for information. The information contained in these appendixes is an integral part of the advisory report and is supported by the Council.

Appendix 1: The combined test.

Different methods can be used for screening for foetal aneuploidy in the first trimester. Currently, the most commonly used method is the combined test. The calculation is based on a combination of maternal, foetal and foetal-placental parameters:

- the age of the mother;
- Nuchal translucency (NT) thickness;
- measuring ß-hCG (human chorionic gonadotropin) and PAPP-A (pregnancy associated placental protein-A) in the maternal serum.

The age of the mother: According to the Public Service Employment report from 2012, the average maternal age at first delivery in Flemish women is 28 years. **15%** of pregnant women are aged over 35 at delivery, and aneuploidy screening based on the age of the mother implies a screen-positive group of 15%, with a Down syndrome detection rate of about **30%**. Each patient with a positive screening result should then undergo an invasive procedure (amniocentesis or chorionic villus sampling). The maternal age related risk of Down syndrome is about 30% higher at 12 weeks into the pregnancy than at full term due to the more significant rate of spontaneous miscarriages. The risk calculation should take into account these factors.

Nuchal translucency: these past 20 years, the subcutaneous build-up of excessive fluid during the first trimester of the pregnancy has been associated with an increased risk of Down syndrome. The ideal time to measure the nuchal translucency by means of an ultrasound scan is between 11 and 14 weeks into the pregnancy. A series of stringent criteria need to be applied when carrying out this measurement. The foetal nuchal translucency increases with crown-rump length (CRL) and therefore depends on the gestational age. It is expressed in MoMs (multiples of the median). The incidence of chromosomal anomalies rises with increasing thickness of the NT; it is 7% for a NT-thickness between P90 and P99 (3.5 mm); 20% when the latter ranges between 3.5 and 4.4 mm; 50% when it is between 5.5 and 6.4 mm and 75% when it is over 8.5 mm. Individual NT measurement is compared to the normal value for the CRL concerned and expressed as a likelihood ratio: the distribution of the NT measurement in pregnancies with Down syndrome compared with that in non-affected pregnancies. The likelihood ratio of the NT is multiplied by the maternal age related risk to obtain a more precise risk calculation.

Taking into account maternal age combined with the NT measurement in the first trimester generates a SE of about 75 - 80% for a false positive rate of 5%. In a normal karyotype, the risk of serious or fatal anomalies increases by a factor of 15, 40 and 80 with a NT-measurement of 3 mm; over 3.5 mm and over 4.5 mm, respectively.

ß-hCG and PAPP-A in the maternal serum: since there is no significant correlation between the level of these hormones in the mother's blood flow and the NT-thickness of the foetus, these factors can be combined to obtain a more efficient screening. The levels of these two hormones depend on the gestational age, and are therefore expressed in MoMs. In pregnancies of foetuses carrying T21, the average rates of ß-hCG and PAPP-A in the maternal serum were about 2 and 0.7 times the MoM value of unaffected pregnancies, respectively. The impact of these parameters is also expressed as a likelihood ratio.

Note that some maternal conditions can influence the values of these hormones: correction factors are then used for excess weight in the mother, smoking, twin pregnancy, a history of Down syndrome, and hormone stimulation/replacement as part of a fertility treatment.

The SE of the biochemical markers varies depending on the timing of the biochemical analyses. When the NT is measured in conjunction with the biochemical markers around the 12th week of the pregnancy ("one stop clinic assessment of risk", OSCAR), the SE is about 90% for a false positive rate of approximately 5%.

When the biochemical analysis is performed around 9 - 10 weeks into the pregnancy and the NT is measured at around 12 weeks, the detection rate rises to 92 – 93%, whilst the number of positive screening results remains the same.

This form of aneuploidy screening is applied in many centres in Belgium. The test results are considered positive when they exceed a cut-off point of 1/300.

The risk of having a foetus carrying T21 is calculated as follows:

$$R_{DownSyndrome} = R_{maternal\ age} \times LHR_{NT} \times LHR_{ß\text{-}hCG} \times LHR_{PAPP\text{-}A}$$

R= risk

LHR= likelihood ratio

A more elaborate form of the combined test increases the detection rate and/or reduces the number of false positives by taking into account additional parameters during the ultrasound examination.

These particularly sensitive ultrasound parameters **are the absence or hypoplasia of the nasal bone, the increased impedance in the ductus venosus and tricuspid regurgitation**, which are found in 65, 60 and 55% of foetuses carrying T21, respectively, and only in 2.5; 3.0 and 1.0% of non-carrier foetuses. Including these parameters into the screening increases its sensitivity to 93 - 96%, whereas the false positive rate drops to 2.5%. Nevertheless, the evaluation of each of these parameters requires additional training, which accounts for the small number of ultrasound centres in which they are used accurately. The risk assessment is performed using a software developed for this purpose and, in some cases, requires certification for both the ultrasound component as well as for the biochemical component of the process.

The following problems may occur when calculating the risk of T21:

- incorrect determination of the gestational age during the ultrasound examination;
- incorrect measurement of the CRL;
- incorrect measurement of the NT (incorrect measurement, incorrect criteria);
- a poor estimate of the nasal bone, of the tricuspid regurgitation;
- insufficient biochemical samples.

Problems in the absence of an ultrasound examination:

- incorrect determination of the gestational age for biochemical analysis;
- identification of the at-risk group for structural anomalies, syndromes and foetal mortality.

The detection of congenital anomalies in a low-risk population shows a poor SE and SP. This state of affairs is mainly due to the wide range of birth defects as well as their low prevalence. Regular gynaecologists only encounter some of these anomalies during their career, and must therefore be particularly vigilant during the ultrasound examination. The identification and isolation of an at-risk population, in which anomalies occur more frequently, increases the likelihood that they will be detected.

Removing first-trimester ultrasound screening from the standard test battery would result in a lower probability of detecting other serious and/or fatal anomalies. In the absence of

chromosomal anomalies, the risk of serious birth defects increases rapidly depending on the thickness of the NT: 2.5% for a NT between P95 and P99, up to over 45% of a population with a NT in excess of 6.5 mm. In the event of a NT ≥ P99, a targeted ultrasound examination makes it possible to detect between 30 and 36% of congenital cardiac defects. In addition, the probability of a genetic condition is 10%, the most common of which is Noonan syndrome. Consequently, an abnormal NT measurement is an indication for performing a third-tier ultrasound examination of the foetus .

$$R_{\text{Down syndrome}} = R_{\text{maternal age}} \times LHR_{NT} \times LHR_{\text{ß-hCG}} \times LHR_{\text{PAPP-A}}$$

R= risk

LHR= *likelihood ratio*

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APPENDIX 2 : Scenarios

- 100,000 pregnancies (1st Trim);
- Prevalence T21 (1st Trim) : 0.0024 (1/416);
- IVT-induced miscarriage rate: 0.010.

SCENARIO 1 : cut-off point : p(T21): 1/300

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.7254	0.9503	0.0339	0.9993

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	174	4,958	5,132
	T21 negative	66	94,802	94,868
	total	240	99,760	100,000

parameter	
number of invasive tests	5,132
number of T21	174
number of iatrogenic miscarriages	51
number of preventable miscarriages	50
detection/miscarriage rate	3.39
p (T21|negative combined test)	0.000695

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 1A : cut-off point: p(T21) : 1/300

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.8500	0.9503	0.0395	0.9996

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	204	4,958	5,162
	T21 negative	36	94,802	94,838
	total	240	99,760	100,000

parameter	
number of invasive tests	5,162
number of T21	204
number of iatrogenic miscarriages	52
number of preventable miscarriages	50
detection/miscarriage rate	3.95
p (T21|negative combined test)	0.000380

SCENARIO 1B : cut-off point : p(T21) : 1/300 –high quality CT

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.91	0.975	0.0805	0.9998

test result		diagnostics		
		T21 positive	T21 negative	total
CT	T21 positive	218	2,494	2,712
	T21 negative	22	97,266	97,288
	total	240	99,760	100,000

parameter	
number of invasive tests	2,712
number of T21	218
number of iatrogenic miscarriages	27
number of preventable miscarriages	25
detection/miscarriage rate	8.05
p (T21|negative combined test)	0.000222022

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 2 : prenatal screening : CT (cut-off point ≥ 1/300) + NIPT

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.7254	0.9503	0.0339	0.9993
NIPT	0.993	0.9984	0.955801	0.955801
NIPT after positive CT	0.720833	0.99992	0.955801	0.999329

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	174	4,958	5,132
	T21 negative	66	94,802	94,868
	total	240	99,760	100,000
NIPT	T21 positive	173	8	181
	T21 negative	1	4,950	4,951
	total	174	4,958	5,132
NIPT after positive CT	T21 positive	173	8	181
	T21 negative	67	99,752	99,819
	total	240	99,760	100,000

parameter	SCEN1	SCEN2	difference SCEN 2 - 1
number of invasive tests	5,132	181	-4,951
number of T21	174	173	-1
number of iatrogenic miscarriages	51	2	
number of preventable miscarriages	50	0	
detection/miscarriage rate	3.39	86:1	
p (T21|negative combined test)	0.000695	0.000671215	

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 3 : prenatal screening : CT (cut-off point ≥ 1/600) + NIPT

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.80989	0.9088	0.0209	0.9995
NIPT	0.993	0.9984	0.932367	0.99989
NIPT after positive CT	0.804167	0.99986	0.932367	0.999529

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	194	9,098	9,292
	T21 negative	46	90,662	90,708
	total	240	99,760	100,000
NIPT	T21 positive	193	14	207
	T21 negative	1	9,084	9,085
	total	194	9,098	9,292
NIPT after positive CT	T21 positive	193	14	207
	T21 negative	47	99,746	99,793
	total	240	99,760	100,000

parameter	SCEN1	SCEN3	difference SCEN 3 - 1
number of invasive tests	5,132	207	-4,925
number of T21	174	193	19
number of iatrogenic miscarriages	51	2	
number of preventable miscarriages	50	0	
detection/miscarriage rate	3.39	96:1	
p (T21|negative combined test)	0.000695	0.000470975	

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 4 : prenatal screening : CT (cut-off point ≥ 1/1,200) + NIPT

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.8521	0.8449	0.0130	0.9996
NIPT	0.993	0.9984	0.890351	0.999871
NIPT after positive CT	0.845833	0.999749	0.890351	0.999629

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	205	15,473	15,677
	T21 negative	35	84,287	84,323
	total	240	99,760	100,000
NIPT	T21 positive	203	25	228
	T21 negative	2	15,448	15,450
	total	205	15,473	15,677
NIPT after positive CT	T21 positive	203	25	228
	T21 negative	37	99,735	99,772
	total	240	99,760	100,000

parameter	SCEN1	SCEN4	difference SCEN 4 - 1
number of invasive tests	5,132	228	-4,904
number of T21	174	203	29
number of iatrogenic miscarriages	51	2	
number of preventable miscarriages	50	0	
detection/miscarriage rate	3.39	102:1	
p (T21|negative combined test)	0.000695	0.000370846	

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 5 : prenatal screening : high-quality CT (cut-off point ≥ 1/300) + NIPT

test type	test performance parameters			
	SE	SP	PPV	NPV
CT	0.910	0.975	0.0804	0.9998
NIPT	0.993	0.9984	0.981818	0.981818
NIPT after positive CT	0.9	0.99996	0.981818	0.999759

test result		diagnostics		total
		T21 positive	T21 negative	
CT	T21 positive	218	2,494	2,712
	T21 negative	22	97,266	97,288
	total	240	99,760	100,000
NIPT	T21 positive	216	4	220
	T21 negative	2	2,490	2,492
	total	218	2,494	2,712
NIPT after positive CT	T21 positive	216	4	220
	T21 negative	24	99,756	99,780
	total	240	99,760	100,000

parameter	SCEN1	SCEN5	difference SCEN 5 - 1
number of invasive tests	5,132	220	-4,912
number of T21	174	216	42
number of iatrogenic miscarriages	51	2	
number of preventable miscarriages	50	0	
detection/miscarriage rate	3.39	108:1	
p (T21|negative combined test)	0.000695	0.000240529	

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

SCENARIO 6 : prenatal screening first-tier NIPT

test type	test performance parameters			
	SE	SP	PPV	NPV
NIPT	0.993	0.9984	0.59799	0.99998

test result		diagnostics		total
		T21 positive	T21 negative	
NIPT	T21 positive	238	160	398
	T21 negative	2	99,600	99,602
	total	240	99,760	100,000

parameter	SCEN1	SCEN6	difference SCEN 6 - 1
number of invasive tests	5,132	398	-4,734
number of T21	174	238	64
number of iatrogenic miscarriages	51	4	
number of preventable miscarriages	50	2	
detection/miscarriage rate	3.39	60:1	
p (T21|negative combined test)	0.000695	0.000020080	

CT = combined test; NIPT: non-invasive prenatal test; SE = sensibility; SP = specificity; PPV = positive predictive value; NPV = negative predictive value

APPENDIX 3 : GLOSSARY

Amniocentesis: ultrasound guided collection of a sample of amniotic fluid by means of an aspiration needle. Usually, a volume of 10-20 ml of amniotic fluid is taken between 15 and 20 weeks into the pregnancy. Amniocytes contained in the fluid are cultured and used for foetal karyotyping.

Aneuploidy: any chromosome number that is not an exact multiple of that of a normal gamete with only one of each chromosome pair. In humans, the haploid number is 23. The most common forms of aneuploidy in humans are trisomy (the presence of an extra chromosome) and monosomy (the absence of a single chromosome).

CGH-array (comparative genomic hybridization) **- DNA microarrays** (DNA-arrays): technique that allows the identification of tiny anomalies in the number and structure of chromosomes (deletions - duplications). In prenatal medicine, this is currently only performed on samples obtained through an invasive procedure.

Chorionic villus sampling: invasive procedure used to carry out a prenatal diagnosis around the 11^{th} week of pregnancy. The chorionic villi are collected under the guidance of an ultrasound scan through the abdomen (transabdominal CVS) or cervix (transcervical CVS).

Chromosome: short rod-like structure in the cell nucleus. The chromosome contains chromatin and carries the genetic information (DNA).

Cytogenetics: study of human chromosomes and their anomalies. For many years, this was done by examining the chromosomes under a microscope. At present, the chromosomes are usually analysed by means of molecular techniques (see CGH-array).

Deletion: loss of a DNA sequence in a chromosome. This sequence can vary in size and involve a single base up to a large piece of chromosome.

DNA (deoxyribonucleic acid): molecule that encodes the genes responsible for the structure and function of living organisms and allows the transmission of genetic information from generation to generation.

Cell-free foetal DNA (cffDNA): fragment of free DNA of foetal and/or placental origin present in the maternal plasma after high-speed centrifugation. Free DNA is contained in microparticles which protect it from degradation. It should be noted that free circulating DNA (cell-free DNA - cfDNA) is also found in the body fluids of women who are not pregnant. This has been the subject of extensive research in oncology.

Genetic counselling: the providing of information and assistance to people with a potentially genetic condition or to at-risk family members. During the consultation, information is given on the consequences of the condition, the probability of developing or transmitting it, and the ways in which the condition can be prevented, managed or improved.

Genetic screening: the carrying-out of tests among a given population to identify individuals affected or likely to develop or transmit a particular condition.

Genetic diagnosis: identification of a disease or condition by analyzing the genes or chromosomes. Genetic diagnosis differs from clinical diagnosis, where the disease is identified by analyzing the symptoms.

Duplication: extra DNA sequence in a chromosome. This sequence can be of variable size and involve just a single base up to a large piece of chromosome.

Gametes: reproductive cell with a haploid number of chromosomes, namely an ovum in females or a sperm cell in males.

Genome: complete DNA sequence containing all genetic information.

Haploid: number of chromosomes in a normal gamete, i.e. 23 chromosomes in humans.

Hybridization: complementary pairing of two different strands of DNA or one RNA strand with a DNA strand.

Microdeletion: chromosomal deletion that is too small to be observed under a microscope and detected by means of molecular cytogenetics.

Microduplication: chromosomal duplication that is too small to be observed under a microscope and detected by means of molecular cytogenetics techniques.

Mosaicism: situation in which two or more cell lines are derived from a single zygote but differ from a genetic point of view due to non-disjunction or a post-zygotic mutation.

Non-disjunction: error that occurs upon cell division in the normal separation of chromosomes, resulting in an unbalanced number of chromosomes (as in trisomy 21).

Nucleotide: a molecule composed of an amine base, a 5-carbon sugar and a phosphate group. The nucleic acid is a polymer of many nucleotides.

PCR (polymerase chain reaction) : the amplification of DNA using a specific technique that allows for the analysis of minute quantities of DNA.

Polymerase: enzyme which can synthesize a new DNA strand.

RNA (ribonucleic acid): nucleic acid formed of a DNA copy which contains ribose instead of deoxyribose. A distinction is drawn between messenger RNA, transfer RNA and ribosomal RNA.

Sequencing: a method used to determine the nucleotide sequence of molecular DNA.

Trisomy: the presence of three copies of a specific chromosome.

Zygote: a fertilized ovum.

6 COMPOSITION OF THE WORKING GROUP

All experts joined the working group ***in a private capacity.*** The names of the SHC experts appointed by Royal Decree as well as the members of the Committee and the Board are available on our website (composition et fonctionnement).

The following experts were involved in drawing up the advisory report:

AERTGEERTS Bert	General medicine	KUL
ANTOINE-POIREL Hélène	Human genetics, oncogenetics	UCL
BLAUMEISER Bettina	Genetics, gynaecology	UZA, College van geneesheren voor het centrum voor menselijke erfelijkheid
CASSIMAN Jean-Jacques	Human genetics	KUL
DAELEMANS Caroline	Prenatal diagnostics	Hôpital St. Pierre
DE CATTE Luc	Gynaecology, prenatal diagnostics	KUL, VVOG
DE SUTTER Petra	Gynaecology, reproductive medicine	UGent
DE THIBAULT DE BOESINGHE Leopold	Oncology, bio-ethics	UGent
FLAMION Bruno	Pharmacogenomics	UNamur
HAUFROID Vincent	Pharmacogenomics	UCL
HORION Marc	Prenatal diagnostics	ULg
HUBINONT Corinne	Gynaecology, prenatal diagnostics	UCL, GGOLFB
HULSTAERT Frank	Medicine	KCE
LEGIUS Eric	Human genetics	KUL
LIEBAERS Inge	Medical genetics	VUB
MORTIER Geert	Medical genetics	UZA
NEYT Matthias	Health economics	KCE
PESTIAUX Dominique	General medicine	UCL
VAN NEROM Anne	*in vitro* diagnostics	WIV
VAN OYEN Herman	Epidemiology, Public Health Genomics	WIV
VANDENBULCKE Marc	Epidemiology, Public Health Genomics	WIV
VERELLEN-DUMOULIN Christine	Human genetics, bio-ethics	UCL, IPG

The working group was chaired by Mr. Herman VAN OYEN, the scientific secretary was Ms. Anouck WITTERS and Roland HÜBNER.

The general declarations of interests of the experts who approved or validated the advisory report are available on our website (page: Conflits d'intérêts).

About the Superior Health Council (SHC)

The Superior Health Council is a federal advisory body. Its secretariat is provided by the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC aims at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge.

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, staff members of scientific institutions, stakeholders in the field, etc.), 300 of whom are appointed experts of the Council by Royal Decree. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a Committee on Professional Conduct) as well as the final endorsement of the advisory reports by the Board (ultimate decision-making body of the SHC, which consists of 40 members from the pool of appointed experts). This coherent set of procedures aims at allowing the SHC to issue advisory reports that are based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

Once they have been endorsed by the Board, the advisory reports are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.shc-belgium.be). Some of them are also communicated to the press and to specific target groups (healthcare professionals, universities, politicians, consumer organisations, etc.).

In order to receive notification about the activities and publications of the SHC, please contact: info.hgr-css@health.belgium.be.