Challenges in prenatal screening and diagnosis in the Netherlands

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1 General introduction

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Prenatal screening in the Netherlands: Introduction of a national screenings program

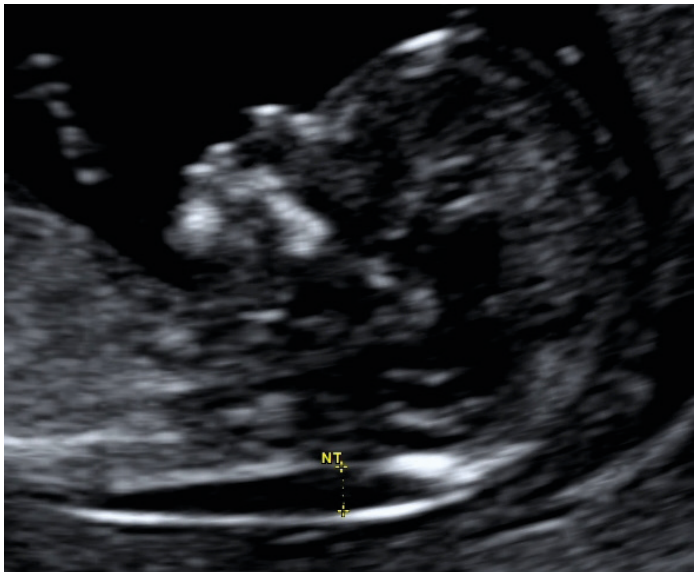
"Pregnancy care" consists of care around pregnancy and birth, starting already from the preconception period. During pregnancy future parents can opt for prenatal screening in order to let assess the risk of congenital anomalies in their fetus. A distinction should be made between prenatal screening in a low risk population and prenatal diagnosis in a high risk population. Prenatal screening does not give a definitive diagnosis but it gives an estimate of the probability that a fetal anomaly will be present. In contrast, prenatal diagnosis confirms or excludes the suspected problem in a pregnancy. Pregnant women may opt for prenatal diagnosis because in case of specific congenital anomalies (e.g. heart anomalies) it can be beneficial for the child to be born in a tertiary center, where postnatal care can be optimized for the baby. Alternatively, in case of congenital anomalies parents have the option of termination of the pregnancy.

In the Netherlands the national prenatal screenings program was introduced in January 2007. This includes the combined test (CT) which is on average performed at around 12-13 weeks of gestation and the structural anomaly scan performed at around 20 weeks of gestation. As a consequence all pregnant women are nowadays asked during antenatal visit by their caregiver if they want to be informed about prenatal screening. Only if they opt to be informed they will be counseled by their midwife or gynaecologist, or rarely by the general practitioner. This set-up was chosen to guarantee the right of 'not to know'. Based on the information provided, parents are expected to be able to make an informed choice on whether or not they want to opt for prenatal screening (RIVM).[1]

The 20 week anomaly scan was officially introduced as screening for neural tube defects, although women are informed beforehand that other structural anomalies can also be encountered.[2] The uptake of this scan is high, more than 95 percent, which is comparable to other European countries (RIVM 2011).

The CT is based on the measurement of the fetal nuchal translucency (NT, see Figure 1), maternal age and maternal serum markers (ß-HCG and PAPP-A) and has a detection rate of about 90% for a false positive rate of 5%.[3,4,5,6-8] The CT was introduced initially as risk-assessment for trisomy 21 only, but from May 2010 the screening was extended to include also trisomy 13 and 18.[3] During this scan, besides chromosomal abnormalities, non-chromosomal abnormalities can be found. Women/couples are not systematically informed about the possibility of finding structural abnormalities at the time of the CT. Nor are they informed that an enlarged NT can also be associated with structural abnormalities, especially cardiac defects.

In contrast to the 20 week anomaly scan, the uptake of the CT in the Netherlands is low, around 25-30% and this varies among different regions. The CT uptake in recent years is even lower than the uptake of invasive prenatal diagnosis in women aged 36 or older before the national screenings program was introduced.[9,10] A study performed in



■ Figure 1 – Nuchal Translucency (NT)

the Netherlands before the advent of the national screening program and involving the offer of NT screening free of charge to pregnant women attending a number of community midwife practices, showed a much higher uptake of the CT i.e. 86%. The large majority of women, including those who declined the offer, were in favor of its standard offer.[9]

At the start of the national screenings program an age-related reimbursement policy was introduced for the CT: women aged 36 years and older had free access to the test whilst younger women had to pay approximately 150 euros. In contrast, all women had and still have free access to the structural anomaly scan. After a plea of professional organizations to abolish this age-related access to prenatal screening, all women now have to pay for the CT.

The nuchal translucency plays an important role in prenatal screening. It consists of a subcutaneous accumulation of fluid behind the neck of the fetus and is generally visible by ultrasound between 11 and 14 weeks of gestation. The NT is part of normal development and its size is influenced by gestational age.[11] It is considered abnormal only when it exceeds a certain cut-off.[3] Many different definitions and cut-offs for an increased NT have been used in the past.[12] Although debate continues as to which cut-off should be used to offer further ultrasound investigation, the 95th or the 99th centile. Currently the second one (3.5 mm) is considered as an ultrasound abnormality. Measurement of the nuchal translucency is performed by certified and skilled sonographers, but it still remains a difficult measurement to perform accurately.[13,14] To further improve the detection rate of trisomies, but especially to lower the false positive rate, the ductus venosus, tricuspid valve Doppler and the nasal bone were added in (1998-2001) as additional markers to the combined test.[15-23]

Once a high risk of a trisomy is found after the combined test (>1:200) or when there is suspicion of a structural anomaly, women are counseled for prenatal diagnosis. Whether or not they opt for prenatal diagnosis depends for most women on the procedure related risk.[24]

1. LOW UPTAKE OF THE COMBINED TEST

Although the CT is offered to all the women in the Netherlands, its uptake is low, and varies among different regions, suggesting that possible cultural differences in attitude towards Down syndrome and termination of pregnancy (TOP) may play a role.[9,10,25] Furthermore, the difference in uptake in comparison with other European countries with a national screening program, such as Denmark (>90%) or with regional screening policies such as England and France (88%)[26-29] is striking. In contrast to other screening policies, the aim of the Dutch prenatal screening is not to reach the highest uptake as possible, but to make sure that future parents make an informed decision regarding screening. So it is important to find out what possible reasons are for the low uptake of the CT in the Netherlands.

2. IMPROVING THE QUALITY OF THE NUCHAL TRANSLUCENCY MEASUREMENT

The NT is the most effective marker of trisomy 21 and is able to detect about 75-80% of the affected fetuses for a false positive rate of about 3-5%.[6,7] Moreover, an enlarged NT is associated with other chromosomal anomalies, genetic syndromes and structural anomalies.[30] It is therefore important that the NT is measured precisely. The Fetal Medicine Foundation (FMF) has developed international guidelines to promote a standardized measurement technique aimed at obtaining accurate manual NT measurements. Aim of the guideline is to achieve uniformity among different operators and guarantee a valid risk assessment.[31] However, the acquirement of the correct midsagittal plane, the selection of the area containing the maximum NT and the placement of the calipers are still prone to error with the manual technique, which may compromise the performance of screening.[13,14,32] Quality control programs performed in the setting of prenatal screening indicate that some sonographers tend to underestimate the NT-measurements, probably trying unconsciously to avoid unfavorable risk assessment results. In this respect the introduction of a technical tool aimed at standardizing the NT measurement may reduce such a measurement error. In order to minimize variability in the measurement of the NT, a semi-automated NT measurement has been developed recently (sono-NT, GE Medical Systems). Standardization through semi-automated measurement is thought to lower the standard deviation (SD) of the distribution of NT measurements, increase its precision, and enhance the correct discrimination of normal from trisomic fetuses, especially when the operators are less experienced.[33-35,36] However, it is not yet evaluated if possible differences between the manual and semi-automated measurements are not only significant in terms of precision but also in terms of changing the individual risk status.

3. COUNSELING REGARDING THE RISK OF MISCARRIAGE AFTER INVASIVE PROCEDURES

Once an increased risk or an increased nuchal translucency is found during the combined test, women are counseled on the possibility of prenatal diagnostics. About 20-30% of the fetuses with an increased risk at the CT has a chromosomal abnormality.[37] When the NT is increased the presence of an abnormal karyotype varies from approximately 7% for a NT between the 95th and 99th centile (3.5 mm), to 20% for a NT of 3.5-4.4 mm, 50% for a NT of 5.5-6.4 mm, and 75% for a NT of 8.5 mm or more.[37]

After an increased risk parents are counseled and offered invasive prenatal diagnosis. One of the factors influencing women's decision to opt for or decline chorionic villus sampling (CVS) or amniocentesis (AP) is the procedure related risk.[24] Estimates of procedure related fetal loss rates differ considerably in literature, varying from 0.5% to 1.0% or even more, for both CVS and AP.[38-52] Most of these studies are cohort studies, have mixed populations and are not recent. There are only a few randomized (controlled) trials. Tabor et al compared women undergoing an AP with a control group, showing a 1% higher fetal loss rate in women undergoing midtrimester amniocentesis.[43] The fetal loss rate (FLR) following CVS has never been compared in a randomized controlled trial (RCT) with a control group. The FLR of CVS has been compared to AP in a RCT and the procedure related risk was found comparable.[42,44,48,53] When parents are counseled regarding the procedure related risk of CVS or AP, most often 1% (or 1:100) is quoted according to the study of Tabor et al.[54]

It remains a challenge to estimate and report realistic valid risk figures from 'real life' cohort studies, taking into account all variables that influence the procedure related FLR. Furthermore, some of the above mentioned studies were performed a while ago at a time when ultrasound systems were less advanced and techniques and training in invasive procedures less standardized. A recent meta-analysis showed that accurate estimates of procedure-related risks following invasive procedures in the current clinical settings are lacking.[55] Many experts believe that procedure related risks need reevaluation.[56] Our national guideline published in 2000 quotes a procedure related risks of 0.5% for CVS and 0.3% for AP, and these numbers are based on an Cochrane article from 1998, which is withdrawn, and an article based on a statistical model from 1991.[57,58] Recent numbers for the Netherlands are lacking.[54] These numbers are important in order to prevent discrepancies in information given to patients among different centers.

4. COUNSELING OF PARENTS AFTER AN ENLARGED NT

When after an increased nuchal translucency, the karyotype appears to be normal this cannot be regarded as a complete reassurance, as far as the final outcome of the pregnancy is concerned. This is especially the case in fetuses where the NT is severely increased. At present, after exclusion of chromosomal aberrations, the most challenging part of managing pregnancies with an increased NT is to establish an adequate diagnostic work-up and provide parents with realistic and correct information about outcome, especially long term neurological outcome.[59-69]

Our study group has investigated at length the associations between an enlarged NT and poor pregnancy outcome with the aim of correctly informing women on the associations and on the predictors of poor or favorable outcome, given an enlarged NT (like sex-related-differences, impact of the size of the enlarged NT, other anomalies present besides the enlarged NT, and the possibility of an underlying genetic syndrome).[9,11,23,59,70-86] There is a long and still growing list of genetic syndromes presenting with increased NT.[60,72] Among the genetic syndromes the most frequently reported in combination with an increased NT is Noonan syndrome (NS), with an incidence ranging from 2-6%.[65,69,87] NS is an autosomal dominant disorder caused in approximately 50% of the cases by a missense mutation in the PTPN11 gene on chromosome 12.[88] Mutations in the SOS1-, RAF1-, KRAS-, BRAF-, MAP2K1/2-, NRAS- and SHOC2-genes account for a smaller percentage of NS cases.[89] In some clinically diagnosed NS cases the genetic background is still

unknown. Clinical diagnosis of Noonan syndrome is often challenging because of the great variability in clinical characteristics.[90,91] The main facial characteristics are hypertelorism, downslanting palpebral fissures, epicanthic fold, ptosis and low set posteriorly angulated ears. The most common cardiovascular defects are pulmonary valve stenosis and hypertrophic cardiomyopathy. Other phenotypic characteristics are short stature, broad or webbed neck and chest deformity. Associated pathologies are haematological disorders (bleeding diathesis, juvenile myelomonocytic leukemia), lymphatic vessel dysplasias, deafness and cryptorchidism. Affected individuals show a wide range in level of intelligence, with mental retardation being present in 15-35%, usually in the mild range and mainly consisting of specific visual-constructional problems and verbal performance discrepancy.[91,92]

The use of 3D rendering of the fetal face in case of (subtle) anomalies after an increased NT and normal karyotype could be a valuable tool in the prenatal detection of fetuses with NS, since the facial characteristics may be even more pronounced prenatally than postnatally.

A protocol for the management of pregnancies complicated by an increased NT to aid doctors in the prenatal follow-up of a fetus with an enlarged NT and a normal karyotype, especially to increase the prenatal detection rate of Noonan syndrome the most frequently encountered genetic syndrome, is still lacking.

5. FACIAL MARKERS IN THE FIRST TRIMESTER AND THEIR RELATIONSHIP WITH ANEUPLOIDIES AND OTHER FORMS OF ABNORMAL DEVELOPMENT

Assessment of the fetal face in the second trimester of pregnancy has become an important part of fetal evaluation, not only for the detection of facial anomalies but also in the setting of screening for trisomies, especially trisomy 21. Established second trimester profile markers for trisomies are the nasal bone length (NBL), the prenasal thickness (PNT), the ratio between the NBL and PNT and more recently the prefrontal space ratio (PFSR).[22,93-95] Other second trimester profile parameters, e.g. the profile line (FP line) and maxilla-nasion-mandible angle (MNM-angle), have been studied as markers for facial anomalies including profile alterations in case of aneuploidies.[96-99] These are proven reproducible markers for the diagnosis of retrognathia, maxillary alveolar ridge interruption, sloping forehead, frontal bossing and flat profile. Reference values for most of these markers are available for the second trimester, however it has not yet been tried to see whether these markers can be measured in the first trimester and more importantly what their clinical significance would be.

The frontomaxillary facial angle (FMF-angle) and the NBL have been introduced in the first trimester to improve screening algorithms for trisomies and to improve detection rates and decrease false positive rates.[16,17,100,101] Measurement of the PNT, PNT/ NBL ratio, MNM-angle, FP line and PFSR in the first trimester could possibly further improve the detection of trisomies and/or facial abnormalities early in pregnancy.

Despite the rapid availability of cell free fetal DNA (cffDNA) as screening test for trisomies, the CT is still the standard of care in the majority of countries. Further improvement of detection of trisomies is still valuable in case cffDNA is not performed or when it is performed as second tier test and to enhance the first trimester detection of structural anomalies which are not trisomy related.

6. ANEUPLOIDIES AND FACIAL MARKERS IN THE SECOND TRIMESTER

Specific facial profile features of Down syndrome fetuses have been investigated and used as second and third trimester markers.[22,94-99,102-105] The nasal bone length (NBL) was the first to be extensively investigated, followed by the prenasal thickness (PT). Recent studies have shown that the ratio between these two markers (PT-NBL ratio) and the prefrontal space ratio (PFSR) yields an even better detection rate.[94,105] Furthermore, we have previously investigated the maxilla-nasion-mandible (MNM) angle and fetal profile (FP) line in both euploid and pathological cases.[97-99,106]

Several studies have compared 2D and 3D US imaging during gestation and suggested 3D to be superior by allowing a better identification of anatomical landmarks, a higher accuracy and reproducibility in measurements of structures in the fetal face and profile, including the NBL. In a previous study, it was shown that 2D images judged to be midsagittal in fact are not and need 3D multiplanar correction of in average 11.9 (Y-axis) - 4.3 (Z-axis) degrees to become truly midsagittal.[102] Clear landmarks to identify the exact midsagittal plane are missing when only 2D imaging is used, making it difficult to be absolutely sure to be in the exact midsagittal plane.[102] However, it is not clear whether addition of 3D imaging in a clinical setting 3D improves the detection rate when compared to 2D.

Aims of thesis

This thesis was designed to fill some of the gaps in knowledge that counselors, sonographers and clinicians encounter in their daily practice when dealing with prenatal screening and diagnosis. In view of the above mentioned clinical problems, we summarize the research questions as follows:

- Why has the introduction of a national policy of first trimester prenatal screening for chromosomal anomalies been so poorly utilized by pregnant women and their partners and what are the factors affecting the uptake of the CT in the Netherlands?
- One of the factors mentioned by women declining the CT is that the NT measurement is not accurate and this influences the reliability of the risk assessment. Quality controls have indicated that some sonographers tend to underestimate the NT-measurements. In this respect the introduction of a technical tool aimed at standardizing the way the NT is measured may reduce such a bias. The following question was therefore: can the NT-measurement be improved by a semi-automated measurement?
- One of the major determinants of a negative attitude towards the CT in the Netherlands is the fear, in case on an increased risk, of having to undergo an invasive procedure that may lead to an iatrogenic abortion. We felt the need to redefine the actual risk of invasive procedures in a Dutch population since recent studies on this subject are in fact still missing. The investigated issues were: what is the total fetal loss rate and procedure-related risk for CVS and AP in the Dutch setting, and which maternal-, operator-, and procedure-related risk factors can be identified?
- At present the most challenging part of managing pregnancies with an increased NT, after exclusion of chromosomal aberrations, is to establish an adequate diagnostic work-up and provide parents with realistic and correct information about outcome, es-

pecially long term neurological outcome in absence of structural anomalies. Therefore the study aim was: what are various aspects that should be investigated in the setting of an increased NT?

- As Noonan syndrome is the most frequently observed genetic syndrome in fetuses with an enlarged NT and normal karyotype, and, considering the diagnosis of this condition can be challenging: which ultrasound characteristics can guide us in the prenatal diagnosis of Noonan syndrome?
- Ultrasound measurement of facial markers has an increasingly important role in second trimester risk assessment and in the work-up of other facial anomalies, such as micrognathia. We wanted to investigate if these measurements can also be performed reliably in the first trimester of pregnancy. Therefore the next issue we have investigated was: can the PNT/NBL-ratio, MNM angle, FP line and PFSR already be measured in the first trimester of pregnancy? Are these measurements also useful markers of an abnormal development when measured in abnormal fetuses in the first trimester of pregnancy?
- The last question we wanted to answer was whether in the application of these facial markers at second trimester of pregnancy, 3D technique has an additional value with respect to the standard use of 2D technique. The question was therefore: is use of 3D technique superior to 2D technique when measuring the NB, PNT, FP line, MNM-angle, PNT/NB-ratio and PFSR, in Down syndrome screening?

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