General introduction
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In the Netherlands prenatal screening (PNS) for structural and chromosomal anomalies is implemented in obstetrical care in the past decade. Since January 2007 PNS is part of population screening and all women are informed about the first-trimester combined test (FCT) for the detection of chromosomal anomalies and about the fetal anomaly scan at 18-22 weeks of gestation for the detection of neural tube defects and other structural anomalies. With the introduction of population screening an essential element in the screening program was provision of high quality information to those pregnant women and their partners who wanted to be informed. Counselling over PNS however is known to be quite difficult due to the complexity of information and the fact that the information provided must be suited for the individual pregnant woman.

The introduction of this thesis describes background information on the history of PNS and prenatal diagnostic testing (PND), on screening performance and on the screening parameters, including the different factors of influence. Furthermore the importance of prenatal counselling is discussed. The focus in this thesis is on the uptake of the FCT and PND over the past decade, and on the performance of first-trimester screening for Down syndrome (DS) for different maternal age groups. The effect of adjustments of maternal serum parameters, timing of serum sampling and adjustment of the risk algorithm on screening performance are evaluated.

Historical perspective of prenatal screening and prenatal diagnostic testing

Prenatal screening aims to identify women with an increased risk for carrying a fetus with a chromosomal abnormality or a structural defect. Invasive diagnostic tests (i.e., chorionic villus sampling or amniocentesis) can identify a chromosomal abnormality. However these tests carry an iatrogenic risk on fetal loss that exceeds the background fetal loss rate. Advanced imaging examinations in fetal medical centres can provide certainty about fetal structural defects. So prenatal screening and diagnostics offer the possibility of optimal antenatal and postnatal care and it offers parents the possibility to make an informed choice about the options available to them.

In the Netherlands PND for the detection of chromosomal abnormalities has been part of prenatal care for about four decades. PND has been offered routinely to pregnant women considered to be at an increased risk of a fetal chromosomal abnormality. The relationship of having a child with DS and advanced maternal age (AMA) has been known for a long time. Also the prevalence of trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome) increases with maternal age. Since 1985 AMA was introduced as indication for PND. From all indications for PND, AMA has been the main indication in the past. PNS for DS became available in 1991 with the introduction of the second-trimester serum screening, combining maternal age, unconjugated oestriol, free β-human chorionic gonadotrophin (fβ-hCG) and alpha-1-fetoprotein. In the last decade the FCT, combining maternal
age, fetal nuchal translucency (NT) thickness and concentrations of maternal serum fβ-hCG and pregnancy-associated plasma protein-A (PAPP-A), was introduced. Up until 2004, there was no nationwide policy for PNS in the Netherlands; pregnant women were only screened on request. Since 2004, all AMA women (in the Dutch situation defined as women aged 36 years of age or older at 18 weeks of gestation) were informed of the possibility of PNS, but women < 36 years of age received information on PNS only on their explicit request. Since January 2007 PNS with the FCT is part of population screening and all women, regardless of age, are informed about the first-trimester screening options for DS. Parallel to the offer of PNS, AMA women are still offered invasive diagnostic testing. PNS is part of insured care for AMA women, but not for women < 36 years of age, unless they have an additional assurance or a listed indication for invasive diagnostic testing. For the implementation of the screening program, 8 regional centers, covering the whole country, obtained a licence with strict regulations allocated to the counsellors, the sonographers and the laboratories. In May 2010 a license under the Population Act was issued, allowing screening also for Patau and Edwards syndrome with the FCT using a specific algorithm. Since the availability of the FCT the overall uptake has been low around 25-30%, whereas the uptake of the fetal anomaly scan has been between 90-95%. The possible causes for the low uptake of the FCT in our country will be part of the general discussion of this thesis.

**Screening performance**

Risk calculation with the FCT is based on the a priori maternal age risk multiplied by the likelihood ratios (LR’s) of fβ-hCG, PAPP-A and NT. LR’s are dependent on gestational age. Because the levels of the different markers vary during gestation (fβ-hCG serum levels decrease with gestation and PAPP-A serum levels and NT values rise with gestation), it is important to standardize serum and NT values for gestational age. First the observed concentration of a marker in an individual pregnant woman is expressed as a ratio to the median value at the same gestational age in a population of normal pregnancies (reference population), the so-called Multiple of the Median (MoM) value. Then LR’s are calculated by comparing the MoM values of the different markers in an individual pregnant woman with the MoM values in a reference population and a population of DS affected pregnancies. All parameters are weighted equally in the risk algorithm.

Applying this risk algorithm screening performance is reported up to a 90% detection rate (DR) at a 5% false-positive rate (FPR). In our country differences in screening performance are reported between different regions. Screening performance is influenced by the reference curves used, the age structure of the screening population, timing of serum sampling and maternal characteristics (e.g. diabetes mellitus, smoking and weight). The effort is to realize the highest DR at a FPR of about 5%. Improvement of screening performance can be achieved by including other fetal sonographic markers in the risk assessment like nasal bone, tricuspid regurgitation or ductus venosus flow. In our country adding other markers to the risk assessment is not allowed unless the licence for prenatal screening is adjusted and approved by the government. There are
several ongoing studies on new techniques for DS screening that provide promising results for the detection of fetal chromosomal anomalies (e.g. fetal DNA from maternal blood and proteomics)\textsuperscript{28-33}.

**Serum parameters and NT**

Serum sampling of fβ-hCG and PAPP-A is done between 9+0 to 13+6 weeks of gestation and the NT measurement is performed according to the guidelines of the Fetal Medicine Foundation\textsuperscript{34}. For this thesis the NT reference curve of the VU University medical center was used, allowing NT measurements to a crown-rump length (CRL) between 45-79 mm\textsuperscript{15}. As a reference for the NT, fβ-hCG and PAPP-A values the following formulas were used: \((NT) \ y = 10^{(2.086419016 – 0.0537362089x + 0.000356257x^2)}\), \((\text{fβ-hCG}) \ y = (193.588 – 2.40569x + 0.0069003x^2)\) and \((\text{PAPP-A}) \ y = 10^{(-0.951652 + 0.0723578x – 0.000265658x^2)}\), \(x\) being gestational age in days (Figure1-3).

Figure 1. NT reference curve.
All necessary data were transferred to the risk software program ELIPS / LIFECYCLE 2.2 (PerkinElmer, WallacOy, Turku, Finland), and DS risk was calculated. Gestational age (GA) was determined by CRL at time of NT. All 3 parameters are discriminative in the detection of DS. Compared to a reference population, DS pregnancies present with increased fetal NT, higher fβ-hCG and lower PAPP-A values. It has been shown that the PAPP-A levels are lowest early in the first-trimester and therefore have a better predictive value whereas fβ-hCG levels are highest later in gestation and predict best at the end of the first-trimester. It is suggested that the accuracy of low PAPP-A values early in gestation outweighs the accuracy of high fβ-hCG values later in gestation. So timing of serum sampling influences screening performance.
On the other hand the serum concentrations may be affected by different maternal characteristics and adjustments to the serum concentrations should be made prior to risk calculation. With the introduction of population screening corrections were applied for maternal weight and smoking. Ethnicity and assisted reproductive techniques (ART) also influence serum marker distribution, resulting in a decreased screening performance (lower DR or higher FPR). In 2012 a correction was introduced for ART pregnancies in the Dutch screening program. For twin pregnancies a correction is applied for both serum markers. A distinction should be made between mono- and dichorionic twins, since biochemical markers are significantly lower in monochorionic twins. The effect of early vaginal bleeding, diabetes, and fetal gender on serum marker distribution are either too small or too problematical to justify a correction.

Maternal age

Maternal age risk is part of the risk algorithm of the FCT. With increasing maternal age, the risk of carrying an affected fetus increases. Young women have a low a priori risk which might be a reason to refrain from PNS. However about half of the affected fetuses are born from younger women. Although the FCT is introduced as screening for chromosomal anomalies in our country, AMA women are also offered invasive diagnostic testing for the detection of chromosomal anomalies. Thus maternal age is used as a single screening tool, with a DR of only 30%. Nowadays, AMA is still the main indication for invasive diagnostic testing. Screening with the FCT, meanwhile, is proven to be more effective for women of all ages.

The age structure of the screening population influences screening performance. Several studies reported on increasing DR and FPR with increasing maternal age. This would imply that the FCT is a better screening tool for AMA women than for younger women. However, comparison of screening performance between different maternal age groups based on DR and FPR alone is difficult. The odds of being affected given a positive result (OAPR) is a good additional estimate to compare screening performance between different maternal age groups. Anyway, the effect of maternal age risk on the risk assessment is substantial. In older women, a significantly higher FPR is found and in younger women more affected cases will not be detected. It has been suggested that elimination of maternal age risk from the risk algorithm might improve screening performance. The absolute risk method might deal with the misunderstanding that screening performance of first-trimester screening in younger women would be less and that screening in the more older women would not be advisable due to the high background risk. Thus screening with the absolute risk method might lead to a significant shift towards more uptake of screening, resulting in a more efficient screening policy with a decrease in the uptake of invasive diagnostic testing for AMA.
Counselling

PNS for chromosomal and structural anomalies is not meant for preventive or treatment purposes, but to inform prospective parents of the options available to them in a timely manner. The goal of the active, routine offer of information about PNS, is to enable pregnant women and their partners to make an informed decision about whether or not to participate in the prenatal screening program\(^1\). An informed decision is only made when one has adequate decision-relevant knowledge and when it reflects the decision-maker’s values. It is essential that relevant, good quality information is given. Counselling is only provided by certified counsellors, because the counselling over the test characteristics is extensive and complex and is related to the age of the mother. Despite good quality information, it is possible that women do not fully understand the principle of screening. Counsellor’s attitude towards PNS might be of influence on the information given.

Aim of this thesis

With the introduction of population screening in the Netherlands the evaluation of the screening program is assigned to the Centre for Population Screening of the Dutch National Institute for Public Health and the Environment. The 8 regional screening centres are responsible for securing the quality of the implementation of the screening program. This is a continuing process of evaluation and aiming for improvements. Besides information on screening performance, it is important to register the uptake of the FCT and to evaluate the effect on the uptake of PND. Reports on monitoring of the Dutch screening program in 2009 and 2010 showed results on the number of counselling performed, and on the uptake of the FCT and the fetal anomaly scan. Results on screening performance were not available due to lack of data (mainly data on follow-up)\(^5\).

In this thesis the uptake of PND and PNS as well as the screening performance of the FCT over the last decade in different maternal age groups was evaluated. Subsequently we studied screening performance of the FCT in ART pregnancies, with serum sampling at different gestational ages and with exclusion of maternal age risk from the risk assessment. The overall aim of this thesis was to study the effect of the screening policies on the uptake of PNS and PND and to determine whether adjustments in screening policies should be made to achieve a better screening performance.

The following questions are addressed in this thesis:

- What are the effects of the different governmental policies on prenatal screening on the uptake of PNS and PND tests over the periods from 2001 – 2003, 2004 – 2006 (permission to offer the FCT to AMA women; women aged < 36 years informed on explicit request) and from 2007 – 2010 (introduction population screening)? Are differences in uptake related to maternal age?
- What is the screening performance of the FCT in different maternal age groups and at different cut-off values for increased risk?
• Is timing of serum sampling of influence on the screening performance of the FCT?
• What is the screening performance for different maternal age groups if maternal age risk is excluded from the risk algorithm compared to the currently used FCT?
• Are distributions of fβ-hCG and PAPP-A in IVF and ICSI pregnancies different from spontaneously conceived pregnancies? Are adjustments of serum marker concentrations necessary? What is the effect on screening performance of applying corrections for fβ-hCG and PAPP-A concentrations in ART pregnancies?


References


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