Seeing the unseen

The importance of prenatal screening

Lugthart, M.A.

Publication date
2024

CHAPTER

11

ENGLISH
Summary

Background

"Seeing the Unseen". Through prenatal screening, we attempt to reveal, discover and comprehend the unseen aspects of the fetus. It offers opportunities, reduces risks, and evaluates fetal health. Over the past decade, the field of prenatal screening has made tremendous progress. Whereas invasive procedures were previously limited to a selected group, screening for chromosomal abnormalities is now offered to all pregnant individuals. Where first trimester combined testing (FCT) was used before, nowadays the more accurate method involves analyzing cell free DNA (cfDNA) in maternal blood, using non-invasive prenatal testing (NIPT). For the detection of structural anomalies, ultrasounds are offered in the first- and second trimester of pregnancy. Fetal ultrasound enables detailed evaluation of the fetal anatomy with detection major fetal anomalies as well as subtle markers of chromosomal and genetic syndromes.

If a subtle marker or fetal anomaly is detected, expecting parents are offered invasive genetic testing in the form of amniocentesis or chorionic villus sampling. At first, numeric chromosomal anomalies (aneuploidy), such as trisomy 13, 18, 21, and sex chromosome anomalies are assessed, using Quantitative Fluorescent-Polymerase Chain Reaction (QF-PCR). If no abnormalities are found, follow-up tests such as Chromosomal Microarray Analysis (CMA) or Whole Exome Sequencing (WES) can be considered. We know that some structural anomalies, especially heart defects, can be associated with genetic abnormalities, which is also of importance for subsequent pregnancies. Identifying a fetal anomaly early in pregnancy provides parents with the opportunity to contemplate genetic testing and make autonomous reproductive choices, which include the option of terminating the pregnancy.

Nonetheless, prenatal screening, along with prenatal diagnostics, introduces uncertainty in some situations. A subtle marker does not always lead to an adverse pregnancy outcome, and not every anomaly can be detected through fetal ultrasound. Therefore, it is essential to discuss not only the importance but also the challenges and uncertainties of prenatal screening with expecting parents, ensuring they have realistic expectations. Because sometimes, the unseen remains unseen.

Part I – Subtle markers and chromosomal anomalies

During the first trimester, every pregnant individual is offered an ultrasound to determine the due date by measuring the fetal crown-rump length. Ideally, this dating scan is performed around 11 weeks of gestation. However, in the current era of NIPT, it is often performed from 9 or 10 weeks onwards because a due date is a requirement for conducting NIPT. During this dating scan, an increased NT can be detected. An increased NT is associated with congenital and/or chromosomal anomalies, but reliable reference values are only available between 11 and 14 weeks of pregnancy.

To date, there are no established normal values for increased NT early in pregnancy, i.e., before 11 weeks. Therefore, in **Chapter 2**, we present the results of a retrospective cohort study in which we evaluated pregnancy outcomes in fetuses with an early increased NT, defined as a NT equal to or greater than 2.5 mm before 11 weeks of gestation. We also explored the impact on pregnancy outcome if the NT normalized or remained thickened, by performance of a repetitive measurement in the correct time frame. An adverse pregnancy outcome was observed in 66.7% of pregnancies (80 out of 120), mostly caused by aneuploidy. Even if NT normalized after 11 weeks, we still found an adverse pregnancy outcome in 24% of cases. These findings were likely subject to selection bias, due to the retrospective nature of the study with the most severe cases probably being referred to the Amsterdam UMC and included in the study. Thus, we evaluated our results in a prospective cohort, as described in **Chapter 3**. This prospective study included 109 fetuses with an increased NT before 11 weeks of gestation and a follow-up period of 4 weeks after delivery. We found a lower adverse pregnancy outcome than in our retrospective cohort, 35.8% compared to 66.7%. The group of fetuses with a NT measurement above 4.5 mm exhibited the poorest outcomes. Even if the NT normalized after 11 weeks and no further sonographic abnormalities were present, we still observed an adverse pregnancy outcome in 8.5% of cases. Based on the elevated risk of an adverse pregnancy outcome even after NT normalizes, we advise to measure the NT at the dating scan if it is enlarged by eyeballing. In case of NT ≥2.5 mm before 11 weeks gestation (CRL=45 mm), we advise to refer to a Fetal Medicine Unit for further detailed sonography and pre-test invasive counseling before offering solely cfDNA based NIPT.

Triploidy is a rare and lethal chromosomal anomaly characterized by the presence of an extra set of chromosomes, either maternal or paternal in origin. In **Chapter 4**, we describe the results of a retrospective study that investigated the ultrasound features of a triploidy, and explored the possibility of assessing parental origin through ultrasound. We included 120 cases, of which parental origin had been examined via DNA analysis in 38.3% of cases (46 out of 120). Asymmetrical growth restriction with a significant discrepancy between head and abdominal circumference was pathognomonic for a triploidy of maternal origin (digynic triploidy). Placental molar changes, increased nuchal translucency, and symmetrical fetal growth restriction were indicative of triploidy of paternal origin (diandric triploidy). In 65.2% of cases (30 out of 46), a prediction through ultrasound features was made regarding parental origin, and this prediction was accurate in 100% of cases. Based on these findings, we concluded that if an accurate prediction via ultrasound is possible, DNA analysis to determine parental origin is redundant.

The results of a literature review and meta-analysis on the risk of malignant transformation of a partial molar pregnancy (paternal triploidy) are described in **Chapter 5**. This malignant transformation is referred to as gestational trophoblastic neoplasia (GTN). Additionally, we investigated whether

management with medication poses a higher risk of GTN than curettage. The pooled GTN incidence in histopathological diagnosed partial molar pregnancies was 1.59% (95% CI 1.05-2.13), compared to 0.51% (95% CI 0.01-1.01) in cytogenetically diagnosed triploid partial molar pregnancies. Some of the histopathological diagnosed partial molar pregnancies were incorrectly diagnosed, as they were complete molar pregnancies. Complete molar pregnancies have a higher a priori chance of developing GTN than partial molar pregnancies. The actual incidence of GTN therefore emerges from our analysis of cytogenetically diagnosed partial molar pregnancies and is very rare (0.51%). It was not possible to compare the GTN incidence between the two management methods due to the very small number of cases managed with medication (n=6). From this, we can conclude that there is a gap in literature regarding the risk of developing GTN after management with medication.

In Chapter 6, we present the results of a retrospective cohort study that included 57 patients with a partial molar pregnancy. Mifepristone and misoprostol was used as a management method in 47.4% (27 out of 57) of the patients and 52.6% (30 out of 57) underwent a curettage. GTN did not occur in any of the 57 included patients. Curettage was used more often in the first trimester, and mifepristone and misoprostol in the second trimester. We did not find a significant difference between groups in postpartum hemorrhage or incomplete removal, but in total more adverse outcomes occurred after mifepristone and misoprostol as opposed to curettage (44.4% versus 13.3%). In first trimester curettage, we found a lower incidence of adverse outcomes as opposed to mifepristone and misoprostol (14.2% versus 50%). We concluded that mifepristone and misoprostol, as well as curettage, are equally safe in terms of not posing an increased risk of GTN. However, each method has its own risk profile in terms of side effects, which should be discussed with the patient involved. The counseling should include the pros and cons of both options, and highlight the lower risk of adverse outcomes associated with first trimester curettage.

Part II – Detection of structural anomalies in first- and second trimester

Chapter 7 provides a protocol outline that summarizes the current literature on the predictive value of ultrasound screening for congenital anomalies. The primary objective of this review is to assess the diagnostic accuracy of screening for fetal anomalies in the first- and second trimester of pregnancy. Given that the majority of severe structural anomalies occur in pregnancies without known or pre-existing risk factors, we focused on low-risk populations. This includes examining the number of cases detected before birth, as well as the percentage of false-positive diagnoses. The sensitivity of a second trimester ultrasound alone is compared to the performance of both a first- and second trimester ultrasound. The final article has been submitted, but is not included in this thesis.

In **Chapter 8**, in a large retrospective cohort study, we investigate whether the implementation of cfDNA by NIPT for aneuploidy and the subsequent decline and abolition of FCT affected the first trimester detection of certain fetal anomalies. From 2011 to 2020, we identified all fetuses with prenatally detected severe cranial- and brain anomalies (anencephaly, holoprosencephaly, encephalocele), abdominal wall defects (gastroschisis, omphalocele, and others), and severe congenital heart defects (atrioventricular septal defect (AVSD) and hypoplastic left heart syndrome (HLHS)). In total, we included 705 cases and divided them into two groups: the period before (396 cases) and after (309 cases) the introduction of cfDNA. We observed a decrease in first trimester detection (<14 weeks) of gastroschisis, AVSD and HLHS in the after group, respectively 54.5% versus 18.5%, 45.9% versus 26.9%, and 30.0% versus 3.4%. After excluding chromosomal anomalies identifiable through cfDNA testing, this decline persisted for non-chromosomal AVSD (43.3% versus 9.5%), leading to a possible earlier gestation at termination. More patients were referred after ultrasounds performed between 11-14 weeks of gestation, often because anomalies were observed during the FCT ultrasound. Without this ultrasound, certain fetal anomalies would have been detected later in pregnancy, suggesting the value of a scan dedicated to fetal anatomy in the first trimester.

With a prevalence of 5-8 per 1000 births, congenital heart defects (CHD) are the most common congenital disorder. It can severely jeopardize a child's chances of survival and is the leading cause of child mortality. Approximately one-third of these cases involve severe CHDs, necessitating surgical interventions within the first year of life. In **Chapter 9**, we investigated the value of an additional first trimester scan on the prenatal detection of isolated severe CHDs involving 264 cases. We assessed prenatal detection rate and pregnancy outcomes of an additional first trimester screening scan compared to only a second trimester scan in fetuses diagnosed with isolated severe CHDs. A first trimester scan was defined as a scan between 11+0 and 13+6 weeks of gestation. The prenatal detection rate significantly differed, with 70.0% (106 out of 151) in the group undergoing both a first- and second trimester scan, compared to 58.0% (66 out of 113) in the group with only a second trimester scan. The median gestational age at detection also significantly varied, with a median gestational age of 19+6 weeks (interquartile range (IQR) 15+4 - 20+5) in the group with a first- and second trimester scan, compared to 20+3 weeks (IQR 20+0 - 21+1) in the group with only a second trimester scan. Furthermore, there were significantly more terminations in the group with both a first- and second trimester scan, 48% (48 out of 99) compared to 27% (18 out of 66). Parents primarily opted for termination if they were pregnant with a fetus diagnosed with hypoplastic left heart syndrome (HLHS) or another univentricular heart defects. Based on these findings, we can conclude that adding an extra scan in the first trimester can positively impact the prenatal detection of pregnancies complicated by an isolated severe CHD. Additionally, conducting two ultrasounds appears to lower the threshold for pregnancy termination, although it does not result in earlier terminations of the pregnancy.

While it is commonly known that there is an association between aneuploidy and CHDs, submicroscopic deletions or duplications, known as copy number variations (CNVs), have also been reported in 10-15% of children with a CHD. Chromosomal Microarray Analysis (CMA) is the recommended test for this purpose. Unfortunately, it is not uncommon for some CHD to be diagnosed with a genetic syndrome after birth since sequence variants go unnoticed by CMA. Exome sequencing (ES) could provide a solution for this. In **Chapter 10** we present data of a retrospective study of the PRECOR database, investigating the prevalence of chromosomal anomalies and sequence variants, as well as the potential yield of ES in fetuses with a severe CHD. Thanks to the collaboration between Leiden University Medical Center (LUMC) and Amsterdam UMC through the Center for Congenital Heart Defects Amsterdam-Leiden (CAHAL), all fetuses and infants diagnosed with a severe CHD within this region have been registered in the PRECOR registry since 2002. In total, 919 fetuses with a severe CHD were detected, and after excluding aneuploidy, a genetic diagnosis was found in 15.7% of cases (111 out of 708). In 5.8% (41 out of 708), sequence variants were found that would go unnoticed with CMA. In conclusion, structural chromosomal abnormalities and sequence variants are identified in a significant portion of CHD after excluding aneuploidy. Therefore, it is essential that expecting parents are offered sequential exome sequencing after obtaining a normal CMA result, if time allows for it. This is especially important when the CHD is accompanied by other structural anomalies, considering the diversity of genetic syndromes that can significantly impact the diagnosis and neonatal outcome.