SCREENING FOR PREGNANCY COMPLICATIONS AT 11-13 WEEKS' GESTATION

ARGYRO SYNGELAKI

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ABSTRACT

Background: The current approach to prenatal care, which was established more than 80 years ago, is characterised by a high concentration of visits in the third-trimester of pregnancy which implies that firstly, most complications occur at this late stage of pregnancy and secondly, most adverse outcomes are unpredictable during the first or even the second trimester.

Objectives: The objective of this thesis is to provide evidence that most pregnancy complications are predictable as early as 12 weeks' gestation. The pregnancy complications examined include fetal aneuploidies, fetal structural defects, preeclampsia, preterm birth, gestational diabetes mellitus and fetal macrosomia.

Methods: I have critically examined fourteen articles reporting on screening for pregnancy complications at 11-13 weeks' gestation, where more than 90,000 singleton pregnancies were prospectively assessed at 11-13 weeks' gestation as part of a routine prenatal visit for screening for trisomy 21. We recorded a series of maternal characteristics and history, measured maternal weight and height, performed a detailed ultrasound examination of the fetus, measured maternal uterine artery Doppler pulsatility index and maternal mean arterial pressure and collected blood for analysis of biomarkers for prospective or retrospective analysis. All data were prospectively entered into our data base as well as the pregnancy outcomes as soon as they became available. Ethical approval was obtained for these studies. Multivariate regression analysis was used to define the contribution of each maternal characteristic and history in predicting each adverse outcome and those with a significant contribution formed an algorithm to estimate the background risk (*a priori* risk) for each one of these complications. The potential value of biophysical and biochemical markers in improving the performance of the *a priori* risk in predicting adverse pregnancy outcomes, was evaluated.

Results: First trimester effective screening for adverse pregnancy outcomes was provided by a combination of maternal factors and biophysical or biochemical markers. The developed predictive models could correctly identify the vast majority of aneuploidies, early preeclampsia and more than half of the cases of spontaneous preterm birth and gestational diabetes. First trimester

prediction of fetal macrosomia was less effective compared with other complications. First trimester examination of fetal anatomy was feasible resulting in a high detection of fetal non-chromosomal defects, including more than half of fetal cardiac defects.

Conclusions: Assessment of the mother and fetus at 11-13 weeks' gestation can provide effective early identification of the high risk group of pregnancies with fetal and maternal adverse outcomes.

CONTENTS

Chelemen T, Syngelaki A, Maiz N, Allan L, Nicolaides KH. Contribution of ductus venosus Doppler in first-trimester screening for major cardiac defects. Fetal Diagn Ther 2011;29:127-34.

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CHAPTER 4. EARLY SCREENING FOR PREECLAMPSIA

Publications

Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn 2011;31:66-74.

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CHAPTER 5. EARLY SCREENING FOR PRETERM DELIVERY

Publications

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CHAPTER 6. EARLY SCREENING FOR GESTATIONAL DIABETES AND MACROSOMIA

Publications

Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. Prenat Diagn 2011;31:135-41.

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CHAPTER 1. OVERVIEW

1.1 Introduction

This thesis will demonstrate that many serious pregnancy complications are detectable or predictable from the 12th week of gestation. In the hospitals where I am a research fellow in Fetal Medicine and Honorary Sonographer, all women are offered a first trimester assessment where a series of maternal characteristics, obstetric and family history are recorded, maternal weight and height are measured, a detailed ultrasound examination of the fetus is carried out, maternal uterine artery Dopplers and measurement of mean arterial pressure are performed and maternal blood is obtained for prospective and/or retrospective analysis for various biomarkers. This assessment is carried out by a large team of doctors and sonographers, including myself.

Since February 2008, I have been prospectively collecting all these information as well as pregnancy outcomes as soon as they became available. I have been responsible for the quality assurance of these data, training the doctors and sonographers in the ultrasound measurements of the mother and fetus. I have been producing individual operator distributions of each ultrasound measurement in a regular basis and I was providing further training to those whose distribution was incorrect. Furthermore, I have been reviewing with a group of other researchers all maternal notes where a pregnancy complication was reported to verify the accuracy of the information. Through the guidance of Professor Kypros Nicolaides, who is the director of the units where I work and a Professor in Fetal Medicine with more than 1,200 publications in peer-review international scientific journals, I learnt how to define a research question, apply the research methodology, obtain ethical approval, and conduct a research study assuring high quality of data. I have also been working closely with Professor David Wright, a Professor in Statistics, who helped me to understand

statistics and taught me how to conduct a statistical analysis. After collecting thousands of data we came to realize that actually there is a strong relationship with most of maternal factors, biophysical and biochemical markers examined at 11-13 weeks' gestation with subsequent adverse fetal and maternal outcomes and this is exactly what this thesis is highlighting.

A first trimester visit can become the basis of a more individualised care where every woman will be assessed and a risk for each pregnancy complication can be calculated. The vast majority of women would be provided with a low risk and these can follow routine antenatal care. A few women that have a high-risk for complications will be directed to a more specialized pathway with close surveillance, where early therapeutic interventions may lead to the prevention of the disease or detection at the early stages of the disease so that adverse consequences can be prevented.

This thesis will stimulate other researchers to expand the number of conditions that can be identified in early pregnancy and investigate new biophysical and biochemical markers that will improve the accuracy of the *a priori* risk based on maternal characteristics, medical and obstetric history. Moreover, early identification of high-risk groups will stimulate further research that will define the best management plans and develop new strategies for the prevention of disorders.

1.2 Early screening for aneuploidies

Aneuploidies are major causes of perinatal death and childhood handicap. The prenatal detection of aneuploidies relies on invasive testing, such amniocentesis or chorionic villus sampling (CVS), which is associated with a risk of miscarriage and therefore these tests are carried out only in pregnancies considered to be at high-risk. In the last 40 years prenatal screening for aneuploidies has focused on trisomy 21. The method of screening has evolved from maternal age in the 1970's, with detection rate (DR) of trisomy 21 of 30%, to a combination of maternal age and second-trimester serum biochemistry in

the 1980s and 1990s, with DR of 60-70% (Wald 2003, Malone 2005). In the last 20 years, the combination of maternal age, fetal nuchal translucency thickness (NT), serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) has increased the DR to 90% at a false positive rate (FPR) of 5% (Nicolaides 1992, Brizot 1994, Snijders 1998, Bindra 2002, Kagan 2008, Wright 2010). A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities, with a relative prevalence to trisomy 21 at 11-13 weeks' gestation of 1:3 and 1:7, respectively (Snijders 1994, 1995).

Studies in the last 10 years have shown that improvement in the performance of first-trimester screening can be achieved by firstly, the inclusion in the ultrasound examination the assessment of the nasal bone, flow in the ductus venosus and across the tricuspid valve (Cicero 2001, 2006, Maiz 2009, Huggon 2003, Kagan 2009), secondly, inclusion of maternal serum placental growth factor (PLGF) and α-fetoprotein (AFP) in the biochemical assessment (Pandya 2012, Bredaki 2011) and thirdly analysis of cell-free (cf) DNA in maternal blood.

In Chapter 2, I have included four publications; the first one demonstrates how the performance of screening for trisomies 21, 18 and 13 can be further improved by the addition of other ultrasound and biochemical markers. The second publication examines the performance of cfDNA testing in maternal blood in screening for these aneuploidies in a routine population undergoing screening for trisomies 21, 18 and 13 at 11-13 weeks' gestation. The third publication examines the performance of screening for trisomies by an approach which combines the traditional method of screening with cfDNA testing. In the fourth publication we investigate the proportion of other chromosomal abnormalities that could be missed if combined testing was replaced by cfDNA testing as the method of screening for trisomies 21, 18 and 13.

1.21 First trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing

In this paper we examined the performance of screening for trisomies 21, 18 and 13 at 11-13 weeks' gestation using specific algorithms based on combinations of maternal age, gestational age, fetal NT, fetal heart rate (FHR), ductus venosus pulsatility index for veins (DV PI), and maternal serum free β-hCG, PAPP-A, PLGF and AFP. This model could detect 93.3% of trisomy 21 cases and 95.4% for trisomies 18 and 13 at a FPR of 1.3% (Wright 2014).

Strengths and limitations

We derived data for NT, FHR and DV PIV from more than 85,000 prospectively screened pregnancies and serum free ß-hCG and PAPP-A from more than 70,000 pregnancies. These included more than 300 cases of trisomy 21 and more than 100 cases of trisomy 18, but only 39 of trisomy 13. The study population for PLGF was more than 25,000, including 138 cases of trisomy 21, 53 of trisomy 18 but only 11 of trisomy 13. For AFP we examined less than 10,000 pregnancies and only 65 cases of trisomy 21, 18 of trisomy 18 and 14 of trisomy 13. Consequently, because of the relatively limited data available, the modelled measures of screening performance are subject to a high degree of uncertainty due to sampling and non-sampling errors that are not easily quantified. However, the consistency between the modelled and empirical rates was reassuring.

1.22 Non-invasive prenatal testing for fetal trisomies in a routinely screened first-trimester population

In this paper, we investigated the performance of non-invasive prenatal testing (NIPT) by analysis of cfDNA in maternal blood in detecting fetal trisomies in a routinely screened population undergoing routine screening for aneuploidies at 11-13 weeks' gestation. We found that the performance of screening for trisomy

21 and trisomy 18 by NIPT using chromosome-selective sequencing in a routine population is effective with DR of >99% and FPR <1% (Nicolaides 2012).

Strengths and limitations

The study population of 2,049 singleton pregnancies was derived from women undergoing first-trimester screening for aneuploidies as part of their routine antenatal care in an inner city maternity hospital. The observed number of trisomies was as expected on the basis of the maternal age distribution of the study population, which was similar to the national average in England, UK (Office for National Statistics 2010).

A limitation of the study was that we did not perform karyotyping in all cases and the assumption of euploidy was based on the lack of phenotypic features of aneuploidy in the neonates. This was an inevitable consequence of the nature of the study which was based on a population undergoing routine screening for aneuploidies, rather than a high-risk population undergoing invasive testing.

1.23 First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing

In the third paper we examined the performance of screening for trisomies by an approach which combines the traditional method of screening with cfDNA testing. We explored the consequences of screening for aneuploidies by two strategies; first-line screening by cfDNA testing and cfDNA testing contingent on the results of combined ultrasound and serum biochemistry. We proposed that cfDNA testing in maternal blood should be offered on the basis of the results of first-line testing by combinations of NT, FHR, DV PIV, and maternal serum β-hCG, PAPP-A, PLGF and AFP (Nicolaides 2014).

Strengths and limitations

The strategy of cfDNA testing contingent on the results of combined ultrasound and serum biochemistry can substantially improve the performance of screening but also retain the advantages of the combined test which include firstly, diagnosis of aneuploidies within the first trimester with the option for earlier and safer termination of pregnancy, and secondly, early detection of major defects and prediction of a wide range of pregnancy complications which allows for earlier therapeutic intervention and better pregnancy management.

The limitation of this study is that the estimates on performance of screening by cfDNA were based on a series of assumptions. The first assumption was that cfDNA testing can detect 99.5% of cases of trisomy 21, 98% of trisomy 18 and 92% of trisomy 13, with respective FPRs of 0.1%, 0.1% and 0.3%. These are the summary values of published studies which mainly examined high-risk pregnancies (Gil 2014). The second assumption is that the failure rate of cfDNA testing to provide a result is 5%. This is based on our finding from clinical implementation of cfDNA testing at 10 weeks' gestation (Gil 2013). The third assumption is that invasive testing is carried out in firstly, those with a positive result from cfDNA testing and secondly, those where cfDNA testing fails to give a risk for trisomies and the combined test risk is 1:100 or higher. However, in practice it is likely that some women in the low-risk group from cfDNA testing would still desire to have a diagnostic test to provide certainty of exclusion of trisomies 21, 18 and 13 but also of other aneuploidies. This is particularly important in cases with fetal abnormalities and those with high NT.

1.24 Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal abnormalities

In this paper we investigated the proportion of other chromosomal abnormalities that could be missed if combined testing was replaced by cfDNA testing as the method of screening for trisomies 21, 18 and 13. The prevalence of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other chromosomal abnormalities was examined in pregnancies undergoing first-trimester combined

screening and CVS. This study in pregnancies undergoing CVS for fetal karyotyping after first trimester combined screening for trisomies 21, 18 and 13 has demonstrated three findings. Firstly, trisomies 21, 18 and 13 account for about 80% of the detected clinically significant chromosomal abnormalities. Secondly, the distribution of some or all marker levels, including maternal age, fetal NT, FHR and serum free ß-hCG and PAPP-A, in the various abnormalities are significantly different from those in the normal pregnancies. Thirdly, the prevalence of trisomies 21, 18 and 13, monosomy X, triploidy and other abnormalities at high-risk of adverse outcome is higher in the group with estimated risk for trisomies 21, 18 or 13 of $\geq$1:100, compared to those with risk of <1:100, and in those with fetal NT $\geq$3.5 mm, compared to those with NT<3.5 mm. Consequently, these aneuploidies are preselected, to varying degrees, by the first trimester combined test. Screening by cfDNA testing, contingent on results of combined testing, improves detection of trisomies, but misses a few of the other chromosomal abnormalities detected by screening with the combined test (Syngelaki 2014).

Strengths and limitations

The main strength of this study is the large number of pregnancies examined. We used data from 14,684 singleton pregnancies undergoing invasive test for fetal karyotyping and 74,561 singleton pregnancies undergoing routine screening for aneuploidies with combined testing. The main limitation of our screening study relates to ascertainment of pregnancy outcome, especially for the group classified as euploid, which was essentially based on the absence of any suspicious clinical findings in the neonatal period. In the case of sex chromosome aneuploidies we estimated the potential impact of such ascertainment bias. However, in the case of other abnormalities, both for those at high-risk of adverse outcome and more so for those at low-risk, it is impossible in the absence of karyotyping all neonates to define their true prevalence and it is likely that this has been considerably underestimated and the ability of the combined test to detect them has been overestimated. The estimates we derived on the prevalence of other chromosomal abnormalities at

high-risk of adverse outcome are based on assumptions that will be difficult to validate.

1.3 Early screening for fetal defects

Fetal non-chromosomal structural defects are the most common cause of perinatal mortality. Cardiac defects account for about 20% of all stillbirths and 30% of neonatal deaths (Office for National Statistics 2007). In the United Kingdom, the National Institute for Clinical Excellence (NICE) has issued guidelines on routine antenatal care recommending that pregnant women should be offered two ultrasound scans in pregnancy (NICE 2008).

The primary aims of the first scan at 11-13 weeks are to establish gestational age from the measurement of fetal crown-rump length (CRL), to detect multiple pregnancies and determine chorionicity and to measure fetal nuchal translucency (NT) thickness as part of combined screening for trisomy 21. The primary aim of the second scan, which is carried out at about 20 weeks, is the detection of structural fetal abnormalities. With this approach, the vast majority of fetal detects are detected only in the second trimester of pregnancy.

In Chapter 3, I have included three publications which aimed to define the performance of the 11-13 weeks scan in detecting fetal non-chromosomal abnormalities and secondly, to demonstrate how the detection rate of major cardiac defects can be improved by the examination of the blood flow in the tricuspid valve and ductus venosus after assessing a population of >45,000 singleton pregnancies.

This is particularly important as the current method of screening for cardiac defects, which relies on family history of such defects, maternal history of diabetes mellitus and maternal exposure to teratogens can identify only about 10% of affected fetuses (Allan 1995).

1.31 Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks

In this paper, we examined the performance of the 11-13 weeks scan in detecting non-chromosomal abnormalities. This was a prospective study were the findings were compared to those of the 20-23 weeks scan and postnatal examination. We concluded that at 11-13 weeks some abnormalities are always detectable, some can never be and others are potentially detectable depending on their association with increased NT, the phenotypic expression of the abnormality with gestation and the objectives set for such a scan (Syngelaki 2011).

Strengths and limitations

The strengths of this study were the large number of pregnancies examined, the specific check list used for the ultrasound examination of fetal anatomy and that the sonographers had received appropriate training to perform such examination.

This was the largest study is the literature to describe the performance of the 11-13 weeks' scan in detecting structural defects in a low risk population. The vast majority of previous publications reporting on the detection rate of fetal defects at this gestation were in a small group of high risk pregnancies and this could not allow a fair comparison of our results with these studies.

1.32 Contribution of ductus venosus Doppler in first trimester screening for major cardiac defects

In this paper, we determined whether assessment of ductus venosus (DV) flow at 11-13 weeks' gestation improves the detection rate of cardiac defects achieved by screening with fetal NT thickness. We found that reversed flow in

the DV is common among fetuses with cardiac defects and assessment of DV flow improves the performance of NT screening (Cheleman 2011).

Strengths and limitations

The strengths of this study are that we examined a large population of singleton pregnancies, including 85 with major fetal cardiac defects. Furthermore, all operators had received extensive training for the measurement of fetal NT and assessment of DV and had obtained certifications of competence in doing so.

A limitation of this study was that in all live births the diagnosis of cardiac defects was based on clinical examination only in the neonatal period. It is therefore likely that some defects, such as coarctation of the aorta and transposition of the great arteries, the diagnosis may have been missed. Another limitation of this study is the method of diagnosing or excluding a cardiac defect in cases of pregnancy termination or fetal death. We selected the pragmatic end-point of sonographically detectable defect by a paediatric cardiologist specialist in fetal echocardiography. Ideally in these cases the antenatal findings should have been validated by post-mortem examination but this was not performed in all cases.

1.33 Contribution of fetal tricuspid regurgitation in first trimester screening for major cardiac defects

In this paper, we investigated the potential value of assessment of the blood flow across the fetal tricuspid valve in the prediction of major cardiac defects at 11-13 weeks' gestation. We used the same population as the previous paper and we found that tricuspid regurgitation is very common in fetuses with major cardiac defects at 11-13 weeks' gestation and this assessment improves the detection rate of these abnormalities when combined with the measurement of NT thickness and assessment of the DV flow. For fixed FPRs of 1%, 3% and 5%, the estimated DRs of major cardiac defects in screening by fetal NT alone were 25.9%, 30.6% and 35.3%, respectively, and these were increased to

36.5%, 48.2% and 54.1%, respectively, in screening by a combination of NT and both ductus venosus and tricuspid flow (Pereira 2011).

Strengths and limitations

The strength if the study is that we developed an algorithm combining fetal NT with flow in the DV and across the tricuspid valve to estimate the patient-specific risk for major cardiac defects. The use of specific risk cut-offs which will depend on available resources and clinics will direct patient to specialists in fetal echocardiography which will allow an early detection of major cardiac defects.

The potential limitations of this study are the same as the previous publication examining the contribution of DV assessment in screening for major cardiac defects at 11-13 weeks' gestation (Cheleman 2011).

1.4 Early screening for preeclampsia

Preeclampsia (PE) is a major cause of maternal and perinatal morbidity and mortality affecting 2-3% of all pregnancies (WHO 2005, CEMACH 2008, Duley 2009). In the last decade extensive research has been devoted to screening for PE with the aims of firstly, reducing the prevalence of the disease through pharmacological intervention in the high-risk group (Bujold 2010, Roberge 2012) and secondly, minimizing adverse perinatal events for those that develop PE by determining the appropriate time and place for delivery (Koopmans 2009).

The traditional approach to screening for PE is to identify risk factors from maternal demographic characteristics and medical history. In the UK, the National Institute for Health and Clinical Excellence (NICE) has issued guidelines recommending that women should be considered to be at high-risk of developing PE if they have any one high-risk factor or any two moderate-risk factors (NICE 2010). However, the performance of such approach, which

essentially treats each risk factor as a separate screening test, has a screen positive rate of 11.2% and the DRs of all PE, PE requiring delivery at <37 and at <34 weeks' gestation are 35%, 40% and 44%, respectively (Wright 2015). In chapter four, I have included three papers which essentially demonstrate effective methods of screening for PE from as early as 12 weeks' gestation. Early identification of the high-risk group for subsequent development of PE can potentially improve outcome by directing such patients to specialist clinics for close surveillance.

1.41 Prediction of early, intermediate and late preeclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks

In this paper, we developed models for the prediction of early PE, requiring delivery before 34 weeks, intermediate PE with delivery at 34-37 weeks and late PE delivering after 37 weeks. These models were based on maternal factors, biophysical and biochemical markers at 11-13 weeks' gestation. The performance of these models was effective with estimated DRs of 91.0% for early PE, 79.4% for intermediate PE and 60.0% for late PE, at a fixed FPR of 5% (Akolekar 2011).

Strengths and limitations

The main strengths of this study were firstly the large number of pregnancies examined prospectively in a narrow gestation range between 11-13 weeks' gestation and secondly, the use of logistic regression analysis to derive the *a priori* risk for each of the PE groups from maternal characteristics. In this model, maternal characteristics and history were incorporated into a combined algorithm derived by multivariate analysis and the effects of variables were expressed as odds ratios for early, intermediate and late PE. This alternative method of screening of PE is superior than the one proposed by NICE as it has a higher detection rate and most importantly can provide women a patient-specific risk for PE. The limitation of this study was that the gestational age at

delivery in the case of PE was treated as a categorical variable rather than a continuous.

1.42 A competing risks model in early screening for preeclampsia

In this paper, we developed a survival time model for the prediction of PE in which the gestation at the time of delivery for PE was treated as a continuous variable. We used maternal characteristics and biophysical markers, including uterine artery pulsatility index (PI) and mean arterial pressure (MAP) at 11-13 weeks' gestation. The Bayes' theorem was used to combine the prior information from maternal characteristics with the uterine artery PI and the MAP. We found that screening by maternal characteristics, uterine artery PI and MAP can detect 90% of PE cases requiring delivery before 37 weeks and 57% of all PE cases at a fixed FPR of 10% (Wright 2012).

Strengths and limitations

This study demonstrated that PE is a spectrum disorder the degree of which is reflected in gestational age at the time of delivery, rather than considering PE as two or three different diseases. Multivariate screening for PE with maternal risk factors evolved into a new approach in which the gestation at the time of delivery for PE was treated as a continuous rather than a categorical variable. The major strengths of the study were firstly, prospective examination of a large number of pregnancies in which specific questions were asked to identify known factors associated with PE, secondly, the use of multivariable survival analysis to identify the factors and define their contribution in the prediction of PE and thirdly, the development of a survival-time model which allowed estimation of individual patient-specific risks of PE requiring delivery before any specified gestation. Bayes theorem was used to combine the information on maternal characteristics and medical history with biomarkers for risk assessment at different stages of pregnancy. A limitation of the study was that the performance of screening by a model derived and tested using the same dataset could be

overestimated and a cross validation to reduce this effect was not performed. Furthermore, external validation on independent data from different sources will be required to confirm these results.

1.43 Competing risks model in early screening for preeclampsia by biophysical and biochemical markers

In this paper, we developed a model for the prediction of PE based on maternal characteristics, biophysical and biochemical markers, including PAPP-A and PLGF at 11-13 weeks' gestation in which the gestation at the time of delivery for PE is treated as a continuous variable. We found that screening by this combination can achieve a DR of 96% of cases of PE requiring delivery before 37 weeks and 54% of all cases of PE at a fixed FPR of 10% (Akolekar 2013).

Strengths and limitations

The strengths and limitations of this study are the same as the one of Akolekar 2013.

1.5 Early screening for preterm birth

Preterm birth is responsible for more than 70% of all neonatal and infant deaths (Office for National Statistics 2012). Additionally, children born preterm, compared to those born at term, have a 10-fold increase in risk of cerebral palsy (Kodjebacheva 2015). Mortality and morbidity are inversely related to gestational age at delivery and are therefore more common in cases with early preterm birth (Office for National Statistics 2012, Saigal 2008, D'Onofrio 2013). The risk of preterm birth is inversely related to cervical length measured by ultrasound examination at mid-gestation (Iams 1996).

The rate of preterm birth has not decreased in the last 30 years (Goldenberg 2008). Although improvements in neonatal care have led to higher survival of

very premature infants, a major impact on the associated mortality and morbidity will only be achieved through the development of a sensitive method to identify women at high-risk of preterm delivery and an effective strategy for prevention of this complication.

In Chapter 5, I included two publications which illustrate methods of identifying women being at high risk for early preterm birth. These studies have provided evidence that spontaneous preterm delivery can be effectively identified by screening at 11-13 weeks' gestation and ongoing randomized studies, based on first-trimester screening to identify the high-risk group for subsequent early delivery, will investigate the extent to which pregnancy outcome would improve through early intervention with such measures as prophylactic use of progesterone.

1.51 Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11-13 weeks

In this paper, we developed a model for the prediction of spontaneous delivery before 34 weeks' gestation based on maternal factors and markers of placental perfusion and function at 11-13 weeks' gestation. We examined 34,390 singleton pregnancies in a prospective manner, including 365 cases that delivered spontaneously before 34 weeks of gestation. This model could identify correctly 38.2% of the preterm deliveries in women with previous pregnancies at or beyond 16 weeks and 18.4% in those without, at a FPR of 10% (Beta 2011).

Strengths and limitations

The main strengths of this study were firstly the large number of pregnancies examined, secondly the well documented maternal characteristics and outcomes, and thirdly, the use of multivariable logistic regression analysis to identify the factors associated with preterm birth which provided a patient specific risk for this condition.

The rate of spontaneous preterm delivery before 34 weeks in a heterogeneous inner city population was 1% and in half of the cases there was spontaneous onset of labor and in the other half there was preterm pre-labor rupture of membranes. These rates are similar to those in our previous multicentre study of about 60,000 singleton pregnancies involving hospitals in and around London (Celik 2008).

A potential limitation of the study was that cases with prenatal interventions for preterm birth such prophylactic cerclage or administration of progesterone because of previous preterm birth or short cervical length, were not examined separately. It is known that these interventions are effective and the inclusion of these cases in the control group may have led to an underestimation of the detection rate of our model.

1.52 First trimester screening for spontaneous preterm delivery with maternal characteristics and cervical length

In this paper, we examined the potential value of cervical length at 11-13 weeks of gestation in the prediction of spontaneous preterm delivery before 34 weeks of gestation. We examined 9,974 singleton pregnancies including 104 (1.0%) cases that delivered prematurely. We used multiple regression analysis to determine the value of cervical length over and above the maternal characteristics and obstetric history. This study demonstrated that in screening by a combination of maternal characteristics and cervical length, the estimated DR of preterm delivery was 54.8%, at a FPR of 10% (Greco 2011).

Strengths and limitations

This was the largest study evaluating cervical length at 11-13 weeks' gestation in predicting spontaneous preterm birth. Furthermore, cervical length was measured by operators who had received specific training in undertaking such

measurement and they had obtained a certification of competence in measuring cervical length, which ensured the reliability of data.

1.6 Early screening for gestational diabetes mellitus and macrosomia

Gestational diabetes mellitus (GDM) is associated with increased risk of maternal and perinatal short-term and long-term complications (Casey 1997, Metzger 2008, Clausen 2008, Reece 2010, Feig 2008, Bellamy 2009). The condition is diagnosed by a positive oral glucose tolerance test, which is usually carried out in the late second trimester of pregnancy either in all pregnant women (Metzger 2010) or in a selected group of women identified by their demographic characteristics and obstetric history as being at high risk for GDM (NICE 2008). Consequently, diagnosis and treatment of affected pregnancies occur during the late second or early third trimester of pregnancy. Such late onset of treatment reduces but does not eliminate the excess risks of associated complications (Hammoud 2013, Crowther 2005, Landon 2009).

In contrast, effective early identification of the high-risk group for subsequent development of GDM is likely to have a greater impact in improving pregnancy outcome because with appropriate dietary advice and pharmacological interventions the incidence of the disease could potentially be reduced.

Fetal macrosomia is associated with increased risks for the mother, including caesarean section and trauma to the birth canal, and for the baby, including shoulder dystocia and consequent brachial plexus or facial nerve injuries, fractures of the humerus or clavicle and birth asphyxia (Ferber 2000, Grassi 2000, Henriksen 2008). In chapter 6, I have included 2 papers which describe models for the prediction of GDM and fetal macrosomia from maternal characteristics and biochemical markers at 11-13 weeks' gestation. The extent to which the performance of early screening for GDM and fetal macrosomia can be further improved by additional biomarkers is currently under investigation.

1.61 Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11-13 weeks

In this paper, we developed a model for the prediction of GDM from maternal characteristics and biochemical markers at 11 to 13 weeks' gestation in a prospective screening population. In addition, we measured maternal serum concentrations of adiponectin, follistatin-like-3 and sex hormone-binding globulin (SHBG) in a case-control study. We found that in screening for GDM by maternal characteristics, the DR was 61.6% at a FPR of 20% and the detection increased to 74.1% by the addition of adiponectin and SHBG (Nanda 2011).

Strengths and limitations

The main strengths of this study were firstly the large number of pregnancies examined, secondly the well documented maternal characteristics and outcomes, and thirdly, the use of multivariable logistic regression analysis to identify the factors associated with GDM which provided a patient specific risk for the development of the disease.

The main limitation of this study relates to the method of identifying the GDM affected pregnancies. The diagnostic OGTT was not carried out in all pregnancies, as recommended by the international association of diabetes and pregnancy study groups (Crowther 2005), but only in those with abnormal results of a random blood glucose level at 24-28 weeks' gestation. If some of the women included in our normal group actually had GDM, the performance of screening of our method was underestimated.

1.62 First-trimester prediction of macrosomia

In this paper, we explored the potential value of the parameters used in screening for aneuploidies at 11-13 weeks, combined with maternal characteristics, in providing significant prediction of macrosomia. We used

multiple regression analysis to determine the significant contributors. We found that screening for macrosomia by a combination of maternal characteristics and obstetric history with fetal nuchal translucency, and maternal serum free ß-hCG and PAPP-A at 11-13 weeks could potentially identify about 35% of women who will deliver macrosomic neonates, at a FPR of 10%. The detection rate was further improved to about 40% by the measurement of maternal serum adiponectin concentration at 11-13 weeks' gestation (Poon 2011).

Strengths and limitations

The main strengths of this study were firstly the large number of pregnancies examined, secondly the well documented maternal characteristics and outcomes, and thirdly, the use of multivariable logistic regression analysis to identify the factors associated with fetal macrosomia which provided a patient specific risk for this condition.

The performance of early screening for macrosomia was poor compared to that of screening for aneuploidies and preeclampsia. Similarly, the extent to which knowledge of the individual patient-specific risk for macrosomia by first-trimester combined screening can improve antenatal surveillance and prevention of macrosomia itself or the intrapartum complications related to macrosomia remains to be determined by future studies.

The next Chapter will address screening for aneuploidies at 11-13 week's gestation by the combined test and cfDNA test and will examine the performance and implications of each test.

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CHAPTER 2. EARLY SCREENING FOR ANEUPLOIDIES

This chapter is based on four publications. The first one examines additional markers in screening for trisomies 21, 18 and 13 and their impact in the performance of screening. The second publication examines the performance of cell free (cf) DNA testing in maternal blood in screening for aneuploidies in a routine screening population. The third publication examines the performance of screening for trisomies by an approach which combines the traditional method of screening with cfDNA testing. In the fourth publication we investigate the proportion of other chromosomal abnormalities that could be missed if combined testing was replaced by cfDNA testing as the method of screening for trisomies 21, 18 and 13.

Several studies published prior to the publications included in this thesis, reported on cfDNA testing in screening for trisomies 21, 18 and 13 but these were limited in high risk pregnancies. Our study was the first to examine cfDNA testing in screening for trisomies 21, 18 and 13 in low risk population prospectively. Based on our results, we went further to explore possible implementation strategies and their implications on the performance in of screening for aneuploidies. Furthermore, in our hospitals, we are now conducting a prospective research study with an aim to identify factors that can influence the decision of women undergoing combined screening in favor of or against CVS and in favor of or against cfDNA testing.

Publications

http://www.ncbi.nlm.nih.gov/pubmed/24356462 Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther 2014;35:118-26.

http://www.ncbi.nlm.nih.gov/pubmed/23107079 Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1-6.

http://www.ncbi.nlm.nih.gov/pubmed/24192489 Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. Fetal Diagn Ther 2014;35:185-92.

http://www.ncbi.nlm.nih.gov/pubmed/24525399 Syngelaki A, Pergament E, Homfray T, Akolekar R, Nicolaides KH. Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal abnormalities. Fetal Diagn Ther 2014;35:174-84.

The next Chapter will address screening for fetal non-chromosomal abnormalities at 11-13 weeks' gestation by an ultrasound examination. The performance of this vist in detecting stractural defects will be defined and the potential role of ultrasound markers of fetal circulation will be examined in improving screening for major cardiac defects.

CHAPTER 3. EARLY SCREENING FOR FETAL DEFECTS

This chapter is based on three publications. The first publication demonstrates that the 11-13 weeks scan can identify many non-chromosomal major abnormalities, but the performance for most abnormalities ultimately depends on their association with easily detectable markers, on a policy decision as to the objectives of the scan and the necessary allocation of resources for achieving such objectives. The other two publications investigate whether the assessment of the flow across the tricuspid valve and the ductus venosous at 11-13 weeks, can improve the performance of screening for fetal cardiac defects.

The 11-13 weeks scan evolved over the last 20 years from essentially a scan for measurement of fetal nuchal translucency and crown-rump length to one which includes a basic checklist for examination of the fetal anatomy with the intention of diagnosing major abnormalities. An advantage of early rather than late diagnosis of major abnormalities, which are either lethal or associated with severe handicap, is that the parents are provided with the option of earlier and safer pregnancy termination.

Publications

http://www.ncbi.nlm.nih.gov/pubmed/21210483 Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011;31:90-102.

http://www.ncbi.nlm.nih.gov/pubmed/21160164 Chelemen T, Syngelaki A, Maiz N, Allan L, Nicolaides KH. Contribution of ductus venosus Doppler in first-trimester

screening for major cardiac defects. Fetal Diagn Ther 2011;29:127-34.

http://www.ncbi.nlm.nih.gov/pubmed/21606749 Pereira S, Ganapathy R, Syngelaki A, Maiz N, Nicolaides KH. Contribution of fetal tricuspid regurgitation in first-trimester screening for major cardiac defects. Obstet Gynecol 2011;117:1384-91.

The next Chapter will address screening for spontaneous preterm birth at 11-13 weeks' gestation by a combination of maternal factors, biochemical markers and cervical lenght.

CHAPTER 4. EARLY SCREENING FOR PREECLAMPSIA

This chapter is based on three publications. Preeclampsia (PE), which affects 2-3% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality. There is evolving evidence that both the degree of impaired placentation and the incidence of adverse fetal and maternal short-term and long-term consequences of preeclampsia are inversely related to the gestational age at onset of the disease.

In the first paper, we developed models for the prediction of early PE, requiring delivery before 34 weeks, intermediate PE with delivery at 34-37 weeks and late PE delivering after 37 weeks based on maternal factors, biophysical and biochemical markers at 11-13 weeks' gestation. In the second paper, we developed a survival time model for the prediction of PE in which the gestation at the time of delivery for PE is treated as a continuous variable. We used maternal characteristics and biophysical markers at 11-13 weeks' gestation. The Bayes' theorem was used to combine the prior information from maternal characteristics with the biophysical markers. Similarly, in the third paper, we developed a model for the prediction of PE which included all the factors from paper two and biochemical markers measured prospectively in a large population.

Publications

http://www.ncbi.nlm.nih.gov/pubmed/21210481 Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn 2011;31:66-74.

http://www.ncbi.nlm.nih.gov/pubmed/22846473 Wright D, Akolekar R, Syngelaki

A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther. 2012;32:171-8.

http://www.ncbi.nlm.nih.gov/pubmed/22906914 Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther 2013;33:8-15.

The next Chapter will address screening for preeclampsia at 11-13 weeks' gestation by a combination of maternal factors, biophysical and biochemical markers.

CHAPTER 5. EARLY SCREENING FOR PRETERM DELIVERY

This chapter is based on two publications. Preterm birth is the leading cause of perinatal death and handicap in children and the vast majority of mortality and morbidity relates to early delivery before 34 weeks. Delivery before 34 weeks occurs in about 2% of singleton pregnancies. In two-thirds of the cases this is due to spontaneous onset of labour or preterm pre-labour rupture of membranes and in the other one-third it is iatrogenic. Although improvements in neonatal care have led to higher survival of very premature infants, a major impact on the associated mortality and morbidity will only be achieved through the development of a sensitive method to identify women at high-risk of preterm delivery and an effective strategy for prevention of this complication.

In the first paper, we developed a model for the prediction of spontaneous delivery before 34 weeks of gestation based on maternal factors and markers of placental perfusion and function at 11-13 weeks' gestation. In the second paper, we examined the potential value of cervical length at 11-13 weeks of gestation in the prediction of spontaneous preterm delivery before 34 weeks of gestation.

Publications

http://www.ncbi.nlm.nih.gov/pubmed/21210482 Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11-13 weeks. Prenat Diagn 2011;31:75-83.

http://www.ncbi.nlm.nih.gov/pubmed/22399065 Greco E, Gupta R, Syngelaki A, Poon LC, Nicolaides KH. First-trimester screening for spontaneous preterm delivery with maternal characteristics and cervical length. Fetal Diagn Ther 2012;31:154-61.

The next Chapter will address screening for gestational diabetes mellitus and fetal macrosomia at 11-13 weeks' gestation by a combination of maternal factors, biophysical and biochemical markers.

CHAPTER 6. EARLY SCREENING FOR GDM AND MACROSOMIA

This chapter is based on two publications. Gestational diabetes mellitus (GDM) is associated with increased risk of maternal and perinatal short-term and long-term complications. The frequency of adverse pregnancy outcomes can be reduced by appropriate treatment of GDM. However, there is no internationally accepted method of screening. Fetal macrosomia is associated with increased risks for the mother, including caesarean section and trauma to the birth canal, and for the baby, including shoulder dystocia and consequent brachial plexus or facial nerve injuries, fractures of the humerus or clavicle and birth asphyxia.

In the first paper, we developed a model for the prediction of GDM from maternal characteristics and biochemical markers at 11 to 13 weeks' gestation in a prospective screening population. In addition, we measured maternal serum concentrations of several biomarkers in a case-control study. In the second paper, we explored the potential value of the parameters used in screening for aneuploidies at 11-13 weeks, combined with maternal characteristics, in providing significant prediction of macrosomia. We used multiple regression analysis to determine the significant contributors.

Publications

http://www.ncbi.nlm.nih.gov/pubmed/21268030 Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. Prenat Diagn 2011;31:135-41.

http://www.ncbi.nlm.nih.gov/pubmed/20798483 Poon LC, Karagiannis G, Stratieva V, Syngelaki A, Nicolaides KH. First-trimester prediction of macrosomia. Fetal Diagn Ther 2011;29:139-47.

The next Chapter summarizes the conclusions of all studies presented in the previous Chapters.

CHAPTER 7. CONCLUSIONS

7.1 Implications for clinical practise

The current approach to prenatal care, which involves visits at 16, 24, 28, 30, 32, 34 and 36 weeks and then weekly until delivery, was established more than 80 years ago (Ballantyne 1921). The high concentration of visits in the third-trimester implies that firstly, most complications occur at this late stage of pregnancy and secondly, most adverse outcomes are unpredictable during the first- or even the second-trimester (Nicolaides 2011a, Nicolaides 2011b). This thesis has presented evidence that many pregnancy complications are predictable at an integrated first hospital visit at 11-13 weeks combining data from maternal characteristics and history with findings of biophysical and biochemical tests. It is therefore proposed that the traditional pyramid of care should be inverted with the main emphasis placed in the first- rather than the third-trimester of pregnancy (Nicolaides 2011a, Nicolaides 2011b). Early estimation of patient-specific risks for pregnancy complications will improve pregnancy outcome by shifting prenatal care from a series of routine visits to a more individualized patient and disease-specific approach.

The role of early pharmacological interventions, in women identified by early screening as high risk for pregnancy complications is under investigation. There is evidence to suggest that aspirin, starting from the first-trimester can improve placentation and reduce the prevalence of preeclampsia. Two recent meta-analyses suggested that prophylactic use of low dose aspirin starting in early pregnancy can halve the incidence of preeclampsia (Bujold 2010, Roberge 2012). Furthermore, regular monitoring of cervical length from the first trimester of pregnancy in women beeing at high risk for preterm bith and earlier intervention with measures such as

prophylactic use of progesterone or cervical cerclage could improve maternal and neonatal outcomes (Fonseca 2007, Berghella 2011). Lastly, effective early screening for gestational diabetes mellitus (GDM) may result in early diagnosis and treatment of GDM with a significant improvement in perinatal outcomes and reduction in associated macrosomia. Furthermore, the high risk group may benefit by therapeutic interventions such as probiotic ingestion from early pregnancy (Nitert 2013).

The papers included in this thesis have been extensively cited in the literature by other research groups examining similar research questions. The number of citations of these papers and my contribution are presented in the Appendix. The major strenght of all these large observational studies included is that we efficiently used all available data from a routine antenatal visit in a National Health System setting and the results are likely to be generalisable because they include a representative sample of the whole pregnant population.

7.2 Early screening for aneuploidies

Trisomies 21, 18 and 13 account for about 80% of the detected clinically significant chromosomal abnormalities (Syngelaki 2014). First-trimester screening by a combination of maternal age, fetal nuchal translucency (NT), fetal heart rate (HRT) and serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP-A) and the use of risk algorithms for trisomy 21, 18 and 13, can detect 90% of fetuses with trisomy 21 and 95% of fetuses with trisomies 13 and 18 at a false positive rate (FPR) of 4% (Wright 2014). The prevalence of trisomies 21, 18 and 13, monosomy X, triploidy and other abnormalities at high-risk of adverse outcome is higher in the group with estimated risk for trisomies 21, 18 or 13 of $\geq$1:100, compared to those with risk of <1:100, and in those with fetal NT $\geq$3.5 mm, compared to those with NT<3.5 mm (Syngelaki

2014).

The performance of screening for trisomy 21 and trisomy 18 by cell free (cf) DNA testing in maternal blood in a routine population is as effective with detection rate (DR) >99% and FPR <1% (Nicolaides 2012). If cfDNA testing was offered as a first-line method of screening to all pregnancies about 99% of fetuses with trisomy 21 and 96% with trisomies 13 and 18 could be detected at an overall invasive testing rate of 1% (Nicolaides 2014a). In contingent screening, detection of 98% of fetuses with trisomy 21 and about 96% of fetuses with trisomies 18 or 13, at an overall invasive testing rate of less than 1%, can be achieved by carrying out cfDNA testing in about 35%, 20% and 10% of cases identified by first-line screening with the combined test alone, the combined test with the addition of serum placenta growth factor (PLGF) and alpha-fetoprotein (AFP) and the combined test with the addition of PLGF, AFP and ductus venosus pulsatility index for veins (DV PI), respectively (Nicolaides 2014a). cfDNA testing contingent on the results of first-line screening by ultrasound and biochemical testing would potentially detect most of the cases of monosomy X and between half and one third of the few other clinically significant chromosomal abnormalities that are currently detected by invasive testing if the risk for trisomies 21, 18 or 13 from the combined test is $\geq$1:100 (Syngelaki 2014).

7.3 Early screening for fetal defects

At 11-13 weeks' gestation some abnormalities are always detectable, some can never be and others are potentially detectable depending on their association with increased NT, the phenotypic expression of the abnormality with gestation and the objectives set for such a scan (Syngelaki 2011a). Fetal NT is above the 95th percentile in 35% of the fetuses with cardiac defects and in 4.8% of those fetuses without cardiac defects (Syngelaki 2011a). Reversed a-wave in the DV is observed in 28% of the fetuses with cardiac defects and in 2% of those with no cardiac

defects (Cheleman 2011). Specialist fetal echocardiography for cases with NT above the 99th percentile and those with reversed a-wave, irrespective of NT, would detect 39% of major cardiac defects at an overall FPR of 2.7% (Cheleman 2011). Therefore, assessment of ductus venosus flow improves the performance of NT screening for cardiac defects (Cheleman 2011).

Tricuspid regurgitation at 11-13 weeks' gestation is observed in about 1% of normal fetuses and in one-third of those with major cardiac defects (Pereira 2011). Fetal NT above the 95th percentile, TR or DV reversed a-wave are observed in 35%, 33% and 28%, respectively, of the fetuses with cardiac defects and 4.8%, 1.3% and 2.1% respectively, of those without cardiac defects. Any one of the three markers is found in 58% of the fetuses with cardiac defects and in 8.0% of those without cardiac defects (Pereira 2011). Assessment of flow across the tricuspid valve improves the performance of screening for major cardiac defects by fetal NT and DV flow (Pereira 2011).

7.4 Early screening for preeclampsia

The prevalence of early, intermediate and late preeclampsia (PE) is 0.3%, 0.6% and 1.3%, respectively (Akolekar 2011). Algorithms that combine various maternal characteristics at 11-13 weeks can potentially identify 33%, 28% and 25% of pregnancies that subsequently develop early, intermediate and late PE, at the FPR of 5% (Akolekar 2011). Screening by maternal characteristics, uterine artery pulsatility index (PI) and mean arterial pressure (MAP) can detect 89% of cases of PE requiring delivery before 34 weeks and 56% of all cases of PE, at a fixed FPR of 10% (Wright 2012). Screening by maternal characteristics, biophysical and biochemical markers detected 96% of cases of PE requiring delivery before 34 weeks and 54% of all cases of PE at fixed FPR of 10% (Akolekar 2013).

7.5 Early screening for preterm birth

The rate of spontaneous preterm delivery before 34 weeks in a heterogeneous inner city population is 1% (Beta 2011). Patient-specific risk of preterm delivery can be provided by maternal factors and obstetric history (Beta 2011). This model can detect 38.2% of the preterm deliveries in women with previous pregnancies at or beyond 16 weeks and 18.4% in those without, at a FPR of 10%.

First-trimester screening for spontaneous early preterm delivery can be substantially improved by the addition of the sonographic measurement of cervical length with estimated DR of 54.8% at a FPR of 10% (Greco 2011).

7.6 Early screening for gestational diabetes and macrosomia

In screening for gestational diabetes mellitus by maternal characteristics the DR is 61.6% at a FPR of 20% and the detection increased to 74.1% by the addition of adiponectin and sex hormone binding globulin (Nanda 2011). Prediction of macrosomia can be provided in the first-trimester of pregnancy by a combination of maternal characteristics and measurements of fetal NT, free-βhCG and PAPP-A with a DR of 35%, at a FPR of 10% (Poon 2011).

7.7 Implementation and implications of screening programme

My papers on fetal aneuploidies have provided strong evidence that screening for fetal trisomies is greatly improved by the ultrasound examination of the ductus venosus flow as well as the addition of maternal serum analysis of PLGF and AFP. The latter can be added to the existing model of screening for trisomy 21 with a small additional cost as these are relatively cheap biochemical tests done by

automated machines. In contrast, universal examination of the ductus venosus flow would require a big relative change to the existing approach, as extensive training of the sonographers, an auditing process of this measurement in a national level and increase in time of the appointments would be absolutely necessary which will result to an increase in the overall cost of screening.

My papers on cfDNA testing for screening for fetal trisomies have demonstrated that this is the most effective screening test not only in high risk pregnancies but also in a routine low risk population. The integration of cfDNA testing in the current method of screening for aneuploidies will not only result in a significant improvement in detecting fetal trisomy 21 and but will also substantially reduce the unnecessary invasive testing and its adverse consequence of miscarriage especially in the cases of normal fetuses. Studies have demonstrated that most mothers would prefer cfDNA testing compared to an invasive test, however, the high cost of the test still remains an obstacle for the NHS to incorporate it in the current method of screening.

My papers have demonstrated that identification of many major fetal structural defects is possible at the first trimester of pregnancy. The beneficial consequence of this, is that parents can be better prepared in case they wish to continue with the pregnancy or offered the option of an earlier and safer termination of pregnancy compared to one at the end of the second trimester. The performance, however, for most abnormalities to be detected early in pregnancy ultimately depends on a policy decision as to the objectives of the first trimester scan and the necessary allocation of resources for achieving such objectives.

My papers have demonstrated that preeclampsia, preterm birth and gestational diabetes which are major causes of perinatal mortality and morbidity can be effectively predicted form the first trimester of pregnancy. Using specific algorithms that combine maternal characteristics, biochemical tests, and measurement of uterine artery Dopplers and mean arterial pressure, women can be provided an

individual patient-specific risk for these complications from as early as 12 weeks' gestation. At that stage the great majority of women would be classified as being at low-risk for pregnancy complications and a small proportion of women would be selected as being at high-risk. In the low-risk group the number of medical visits can be substantially reduced. One visit at 20-22 weeks will re-evaluate fetal anatomy and growth and reassess risk for such complications as preeclampsia and preterm delivery. Another visit at 32 or 36 weeks will assess maternal and fetal wellbeing and determine the best time and method of delivery and this will be repeated at 41 weeks for the few that remain pregnant at this stage. The high-risk group can have close surveillance in specialist clinics both in terms of the investigations to be performed and the personnel involved in the provision of care. In each of these visits their risk will be reassessed and they will either remain high-risk or they will become low-risk in which case the intensity of their care can be reduced.

The incidence of pregnancy complications has been increasing over the years, firstly because of the maternal aging population as well as the dramatic increase of maternal obesity, not only in the UK but worldwide. Even though, the existing antenatal care allows women to be seen in a regular basis, the adverse consequences of pregnancy complications have not been reduced. The proposed model of antenatal care arising from my papers emphasizes in the first trimester of pregnancy and provides women with a clear direction as to whether their pregnancies are high or low risk for subsequent development of most of complications. With the view that the high risk group can benefit by prophylactic pharmacological interventions the incidence of adverse pregnancy outcomes and their consequences can be potentially reduced.

7.8 Future direction of research

Since the date of the publications of this thesis, I have continued to research on the prediction of pregnancy complications and I have co-authored a series of papers

published in peer-review international journals not included in this thesis (Syngelaki 2015a, Syngelaki 2015b, Akolekar 2015, Wright 2015, Bahado-Singh 2015, O'Gorman 2015, James 2015, Nicolaides 2014b, Gil 2014, Nicolaides 2014c, Bahado-Singh 2014, David 2014, Ashoor 2013a, Ashoor 2013b, Gallo 2013, Nicolaides 2013a, Nicolaides 2013b, Guex 2013, Lai 2013a, Lai 2013b, Llurba 2013, Poon 2013, Savvidou 2013, Maiz 2012, Ferreira 2012, Khalil 2012, Pandya 2012, Syngelaki 2011b, Staboulidou 2011, Bredaki 2011). These papers are focusing in improving the performance of the algorithms in screening for pregnancy complications, not only at 12 weeks but also at 22, 32 and 36 weeks' gestation. Furthermore, the algorithms for screening for PE have been the basis of a large ongoing multicenter European randomised controlled trial investigating the value of screening for PE and treatment of the high-risk group with low-dose aspirin (ASPE trial). In addition, we recently published the results of a randomised controlled trial investigating the value of cervical pessary in women being at high risk for preterm birth (Nicolaides 2015). Lastly, we have just completed and submitted for publication the results of another randomised controlled trial investigating the use of metformin in reducing adverse maternal and neonatal outcomes from the first trimester of pregnancy in non-diabetic women with a body mass index >35kg/m^2 who are in particular high risk for all pregnancy complications.

My focus for the years to come will be to investigate if early diagnosis of gestational diabetes mellitus by a glucose tolerance test at 12 weeks' gestation will improve pregnancy outcomes. This will necessitate another randomised controlled trial where the control group will have a routine antenatal care and the intervention group will have early testing and early treatment.

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APPENDIX

Citations of papers (last updated on 27th of September 2015)

Reference	Citations
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Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011;31:90-102.	185
Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. Prenat Diagn 2011;31:135-41.	80
Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11-13 weeks. Prenat Diagn 2011;31:75-83.	55
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Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther 2012;32:171-8.	53
Greco E, Gupta R, Syngelaki A, Poon LC, Nicolaides KH. First-trimester screening for spontaneous preterm delivery with maternal characteristics and cervical length. Fetal Diagn Ther 2012;31:154-61.	46
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Total	1209

My contribution in each publication of this thesis

Reference	Collection of data	Writing of manuscript	Statistical analysis
Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn 2011;31:66-74.	100%	50%	30%
Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011;31:90-102.	100%	50%	100%
Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. Prenat Diagn 2011;31:135-41.	100%	50%	30%
Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11-13 weeks. Prenat Diagn 2011;31:75-83.	100%	50%	40%
Poon LC, Karagiannis G, Stratieva V, Syngelaki A, Nicolaides KH. First-trimester prediction of macrosomia. Fetal Diagn Ther 2011;29:139-47.	100%	50%	30%
Chelemen T, Syngelaki A, Maiz N, Allan L, Nicolaides KH. Contribution of ductus venosus Doppler in first-trimester screening for major cardiac defects. Fetal Diagn Ther 2011;29:127-34.	100%	50%	70%
Pereira S, Ganapathy R, Syngelaki A, Maiz N, Nicolaides KH. Contribution of fetal tricuspid regurgitation in first-trimester screening for major cardiac defects. Obstet Gynecol 2011;117:1384-91.	100%	50%	70%
Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1-6.	100%	50%	60%
Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther 2012;32:171-8.	100%	50%	10%
Greco E, Gupta R, Syngelaki A, Poon LC, Nicolaides KH. First-trimester screening for spontaneous preterm delivery with maternal characteristics and cervical length. Fetal Diagn Ther 2012;31:154-61.	80%	50%	50%
Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther 2013;33:8-15.	100%	50%	30%
Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther 2014;35:118-26.	100%	50%	10%
Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. Fetal Diagn Ther 2014;35:185-92.	100%	50%	60%
Syngelaki A, Pergament E, Homfray T, Akolekar R, Nicolaides KH. Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal abnormalities. Fetal Diagn Ther 2014;35:174-84.	100%	50%	90%