Screening in early pregnancy; more than Down syndrome alone

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Thesis, Utrecht University, the Netherlands

ISBN: 978-90-5335-324-0

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Lay-out and printing: Simone Vinke, Ridderprint BV, Ridderkerk, the Netherlands
Cover illustration: Anne & Anna

The studies presented in this thesis were performed at the Division Women and Baby of the University Medical Center Utrecht in collaboration with the laboratory for Infectious Diseases and Perinatal Screening of the National institute of Public Health and the Environment.

The author gratefully acknowledges financial support for printing of this thesis from the: Division Woman and Baby, University Medical Centre Utrecht; PerkinElmer and BMA BV (Mosos).

The studies presented in this thesis were carried out at the University Medical Centre Utrecht and the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Screening in early pregnancy; more than Down syndrome alone

Screening in de vroege zwangerschap; meer dan alleen Down syndroom

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 26 oktober 2010 des middags te 4.15 uur

door

Esther Johanna Wortelboer
geboren op 23 januari 1975 te Laren

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Aan mijn ouders

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Chapter 1

General introduction and aims of this thesis

General introduction and aims of this thesis

The presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic. Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment.
Public health paper nr 34 1968, JMG Wilson, G Jungner principle and practice for disease

Prenatal screening in the Netherlands

Experience with screening for neural tube defects (NTD) in the Netherlands dates back to 1977. Figure 1 shows the stepwise evolution of prenatal screening in the Netherlands.

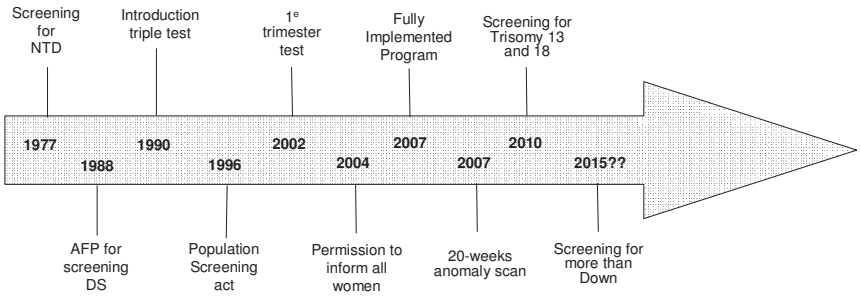

Figure 1: the stepwise evolution of prenatal screening in the Netherlands

In 1988 AFP was first used as a marker for screening for Down syndrome[1-3]. The triple test was introduced in 1990[4]. The official Dutch governmental policy was to offer women who had reached the age of 36 a diagnostic invasive test for Down syndrome. The 'Population Screening Act' was introduced in 1996 to protect people from undesirable health-screening programmes. So, offering pregnant women information on a risk estimation test for Down syndrome (DS) or NTD was only allowed on her explicit request[5-8]. The laboratory of the Dutch National Institute for Public Health and the Environment (RIVM) was the only Dutch laboratory that was allowed to perform these tests with implicit governmental approval, and basically to centralize knowledge concerning risk estimation tests for Down syndrome. Since 2004 it was allowed to inform all pregnant women on the possibility of having a screening test for Down syndrome, with the first-trimester combined test (maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A) and the free β subunit of human chorion gonadotrophin (fβ-hCG) between 8-14 weeks of the pregnancy, first-trimester ultrasound measurement of the fetal nuchal translucency and maternal age) as test of choice. Since January 2007, a nationwide screening program is running by the Ministry of Health, Welfare and Sports in which the Centre for Population Research (CvB) was indicated as the coordinating organ. Currently, 24% of all pregnant women are involved in

the screening for Down syndrome[9]. This was certainly not more in previous years. The detection rate for the first-trimester combined test during the period 2004 - 2006 was 75.9% at a cut-off level of 1 in 250 at term, with a screen-positive rate of 3.3%[10]. The performance of this test has improved over the years but there is still room for further improvement. The government license for this screening program is strictly restricted to screening for trisomy 21. There is currently no screening program for trisomies other than Down syndrome but implementation in the near future is foreseen[11]. Recently, (May 2010) a license under the Population Screening Act was issued, allowing screening for trisomy 13 and 18 in the Netherlands. Eighty percent of all trisomy 13 and 18 cases can be detected using a specific first-trimester algorithm, implying the importance of additional markers to improve the performance of the first-trimester test.
Since January 2007 there is the possibility for every pregnant woman to have a 20-week ultrasound scan. This anomaly scan is to establish whether the child has spina bifida or anencephaly. The scan also extensively examines the development of the child's internal organs.

The risk of a number of pregnancy complications, such as preeclampsia, preterm birth and low birth weight are major determinants of perinatal morbidity and mortality and recent literature has shown that some of these risks can be determined during the first-trimester of pregnancy[12,13]. Identifying pregnant women at risk for preeclampsia is still one of the most important challenges in prenatal care, since preeclampsia is a serious complication of pregnancy that affects approximately 1-2% of all pregnant women worldwide. It is a leading cause of maternal and perinatal morbidity and mortality, especially when it occurs at a gestational age (GA) less than 34 weeks. It occurs mainly in nulliparous women, who are a-priori at low risk and without a past obstetrical history. Therefore, the development of an early screening test in an unselected population in the first-trimester is of importance to identify a group of high-risk patients for an important pregnancy-related disease among the vast group of low-risk pregnancies, before these women have exhibited any symptoms of the disease yet. These women would be able to benefit from intensive antenatal care, thereby trying to reduce the severity of maternal symptoms and reducing the need for preterm delivery[14-18]. While back in 1968 Wilson and Jungner, provided a concise concept to guide screening of large parts of the population they couldn't have guessed how much more complex the world would look like 40 years later. Both the diseases for which can be screened, as well as the tests used, has grown more and more complex. Still, and even in this thesis, their criteria are the standard to which screening and its innovations are measured.

Aims and outline of this thesis

While over the last two decades tremendous progress has been made, with the Dutch screening program presently as one of the best organized in the world, fundamental issues remain. The screening performance currently and in the past did not meet the performance criteria set by international scientific literature. While, the screening is restricted to DS only, the screening test may also be applied

for the screening for other pathologies. For a screening test that exists for decades, surprisingly little is known on the natural occurrence of the parameters, in relation to the physiology of the pregnancy and other biometric and physiological parameters. Therefore, the aims of this thesis were;

1. How can we improve the performance of Down syndrome screening in the Netherlands?

2. Can we screen for more than just Down syndrome alone? Should the screening test for Down syndrome be adapted to detect other pregnancy complications in early first-trimester?

3. And, if so, which biochemical and sonographic markers should be applied and what is their physiology?

Part I: Old and New markers for Down syndrome
In part I an overview of the Dutch history of the screening for Down syndrome, using the triple test, is given (Chapter 2). Secondly, the results from the current first-trimester screening test over the years 2004 to 2006, and changes in its performance are presented (Chapter 3). Finally, a new serum marker ADAM12 is introduced and its potential as an additional marker for the first-trimester combined test in the current national screening program is studied (Chapter 4).

Part II: Screening in early pregnancy for more than Down syndrome alone
In part II an extensive overview of the literature on first-trimester screening for the early prediction of preeclampsia is presented (Chapter 7). Next to this three studies were performed in order to fulfil the second aim of this thesis, including a study on the relationship between first-trimester biomarkers and fetal growth in diabetic pregnancies (Chapter 5, 6 and 8).

Part III: The physiology of all these markers
There is much to be learned about the physiology of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF in human pregnancy. The aim of the study presented in Chapter 9 was to determine changes in maternal serum concentration of these markers in time. In this chapter results are presented of a longitudinal, prospective research describing the developmental trends of biochemical markers, uterine artery Doppler flow velocities and maternal blood pressure in early pregnancy and to study inter relationships in uncomplicated pregnancies. The principle aim of this chapter was to provide a baseline of normal values for future investigation and risk assessment.

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Part I:

Old and New markers for Down syndrome

Chapter 2

Fifteen years of triple tests in the Netherlands; the life cycle of a screening test

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Prenatal Diagnosis 2008 Oct; 28(10): 950-5

Abstract

Objective This report provides an overview of 15 years prenatal screening for Down syndrome (DS).

Methods Between 1991 and 2005 blood samples for the triple test were sent for analysis to our laboratory. Test results were considered screen-positive for neural tube defects (NTD) if the serum alpha-1-fetoprotein ≥ 2.50 MoM for singleton pregnancies or screen-positive for DS if the calculated risk was at least 1 in 250.

Results As many as 42,554 tests were performed. Data on the pregnancy outcome was available for 30,290 screening tests (71.2%). In 1991, most requests (93%) came from the university hospitals; thereafter a shift towards midwives occurred. Until 2001, the number of requests rose to 3,500 a year. Most samples were collected between 15 and 17 weeks of gestation. The median age of women for whom a test was requested increased from 30.5 to 34.5. The detection rate (DR) for DS remained stable over the years (80%), with a false positive rate of about 13%. The DR for Trisomy 13, 18 and NTD was 50%, 68% and 70%, respectively.

Conclusion Based on the results of this study, the triple test may be considered a fairly good second trimester screening test. Here it is shown that health practitioners got more acquainted to the test through the years. This may have served as a tool for the swift introduction of a formal national screening program that started in January 2007.

Introduction

In 1984 an association between low maternal serum alpha-1-fetoprotein (AFP), and fetal chromosomal abnormalities was first described[1]. In later years it was found that the combination of low AFP, with low unconjugated oestriol (uE3) and high free β subunit of human chorion gonadotropin (fβ-hCG) levels in maternal serum was more predictive for a pregnancy with a Down syndrome (DS) fetus[2]. This association in combination with maternal age, was translated into an algorithm to calculate DS risks, which is now known as the triple test[3,4].

Experience with screening for neural tube defects (NTD) in the Netherlands dates back to 1977. Figure 1 shows the stepwise evolution of prenatal screening in the Netherlands. In 1988 AFP was first used as a marker for screening for DS[5-7]. The triple test was introduced in 1990[8]. The official Dutch governmental policy was to offer women who had reached the age of 36 a diagnostic invasive test for DS[9-12]. Offering a pregnant woman information on a risk estimation test for DS or NTD was only allowed on her explicit request. The laboratory of the Dutch National Institute for Public Health and the Environment (RIVM) was the only Dutch laboratory that was allowed to perform these tests with implicit governmental approval, and basically to centralize knowledge concerning risk estimation tests for DS.

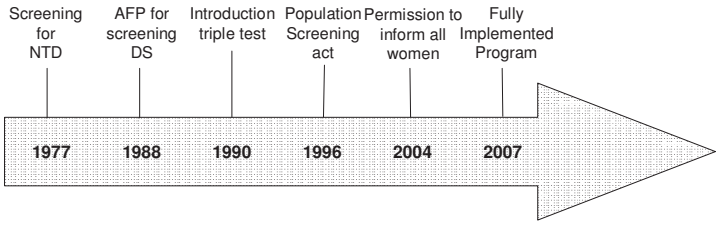

Figure 1: Stepwise evolution of prenatal screening in the Netherlands

Through the years, a growing number of women of 36 years and older chose an invasive test only when prenatal screening indicated a higher risk. This resulted in a higher number of detected abnormalities without an increase in number of invasive tests[8]. In 1996 the Population Screening Act was introduced in the Netherlands, which indicated that screening for DS could only be performed under a governmental license. Given the rules of the Population Screening Act it became increasingly unclear for health professionals whether they were still allowed to honour the explicit request of pregnant women to have a triple test performed. Especially the Health Council, an advisory board to the Dutch Ministry of Health, tried to combine the demands of the Population Screening Act with the needs of pregnant women and health professionals[7,9-13]. In three concordant reports, it advised to introduce a screening test for DS. More specifically the Health Council advised the first-trimester combined test, for all pregnant women, regardless of their age, with special reference to counselling of pregnant women, training and education of health professionals, and quality assurance. Since 2004 it was allowed to inform all pregnant women on the possibility of having a screening test for DS, with the first-trimester combined test as test of choice. This resulted in a steady decline of the number of triple tests, with currently almost insignificant

numbers. The Health Council advice was adopted by the ministry of Public Health and the policy is fully implemented as of January 1, 2007[16].

This study gives an overview of 15 years of prenatal screening for DS with the triple test, with its rise and ultimate demise, presenting its performances and analyzing trends with time. The prolonged period of monitoring enables the evaluation of the influence of governmental policy and the experience of the health practitioners on the uptake and performance of the test. We also discuss whether the struggle by the health care providers and policy makers to ensure a valid and robust screening program may have led to a swift introduction of a formal national screening program.

Methods

Sera and patients

Between 1991 and 2005 gynaecologists, midwives and general practitioners, except those in the northern part of the Netherlands, sent blood samples for the triple test to the laboratory of the Dutch National Institute for Public Health and the Environment (RIVM). All samples were accompanied by a form with information relevant for the risk calculation (e.g. request origin, date of birth, gestational age (GA) (to be decided by the requesting health professional on either a dating scan or first day of last menstrual period (LMP)), maternal weight, insulin-dependant diabetes mellitus (IDDM) status, number of fetuses and since 1996 the nuchal translucency if known). Blood sampling was done between 14 and 23 weeks of gestation. Information for requestors indicated that blood sampling for the triple test had to be carried out at 14-23 weeks of gestation, and at least 15 weeks when it was a NTD screening request as well. The samples were stored at 4ºC until analysis.

Risk estimation

Analysis of AFP, hCG (or the ß subunit of hCG) and uE3 was performed with a commercially available radioimmunoassay (RIA) kits (Amerlex, Johnson & Johnson, USA) until March 2001. From March 2001 onward the analysis of the sera was performed with the autoDELFIA method (PerkinElmer, Turku, Finland). The sample analysis process was assessed weekly and the quality of the entire risk calculation process was assessed by participating in both internal and two external (Biorad, EQAS, Irvine, California; UK-NEQAS, Edinburgh, UK) quality control programs for AFP, hCG, uE3 and the risk calculation, providing a robust means of quality assurance.

Risk calculations were performed with the risk calculation program 'Alpha' (Logical Medical Systems, London, UK). The risk calculation process has been described extensively elsewhere[17,18].

Since March 2003, the risk calculation software Lifecycle Elipse (PerkinElmer, Turku, Finland), was used. The settings of the Alpha software were adopted to assure that the Lifecycle Elipse software performed similarly. From 1995 onward the multiple marker screening programs for DS was extended to include Edwards syndrome (trisomy 18). A risk of 1 in 200 was used as a cut-off value for trisomy 18 screening.

The occurrence of trisomy 13 and neural tube defects (NTD) was also analyzed. All test results were considered screen-positive for NTD if the maternal serum AFP ≥ 2.50 MoM for singleton pregnancies and for DS if the calculated risk was at least 1 in 250. The cut-off risk of 1 in 250 was adopted from a 1997 guideline of the Dutch Association for Obstetrics and Gynaecology that referred to a Royal College Obstetricians and Gynaecologists-guideline[19].

Data analysis

Women were asked to fill in a short questionnaire about the pregnancy outcome and a second request to report the outcome was done by mail, if need be. Data from the triple test and pregnancy outcome were combined. Only the data with outcome were included in the analysis. To calculate the performance of the triple test, pregnancies with IDDM (n = 477), multiple pregnancies (n = 715) and pregnancies with a previous Down pregnancy (n = 160) were excluded. Thus, data of 30,290 pregnancies were available for epidemiological analysis.

Results

During 15 years of DS screening 42,554 triple tests were performed at our laboratory. Data on the pregnancy outcome was available for 30,290 screening tests (71.2%). Figure 2 shows the number of triple tests per year between 1991-2006, subdivided per requestor category. A maximum was reached in 1996 of about 4000. In 1991, 93% of the requests came from the university hospitals, mainly from the University Hospital in Utrecht while in 1996 this was only 56%. The number of applications from general hospitals and midwives increased.

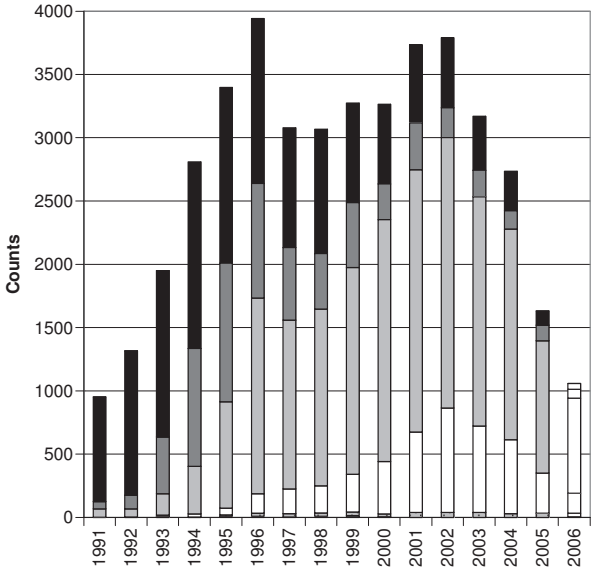

Figure 2: Number of triple test requests (n=42,554) and requestors per annum over 1991-2006. Sections in the bars represent the contribution of individual groups of requestors

Column from top to bottom, University Hospital in Utrecht, other university hospitals, general hospitals, midwives, general practitioners, other. The 2006 data are added to illustrate the ongoing trend

Before 1994 the GA at blood sampling was stated in completed weeks, thereafter in weeks and days. Figure 3 shows the distribution of the GA at blood sampling. All triple tests were performed between 14 and 21 weeks of gestation. Most of the samples were collected between 15 and 17 weeks of gestation, with two peaks at exactly 15 and 16 weeks of gestation in every period. The data of figure 3 also show that GA at blood sampling tended to be earlier in pregnancy as time progressed, starting sharply at 15 weeks of gestation.

Figure 4 shows maternal age at the expected date of delivery (EDD). Median maternal age of women for whom a triple test was requested increased with time from 30.5 years in 1991 to 34.5 years in 2005.

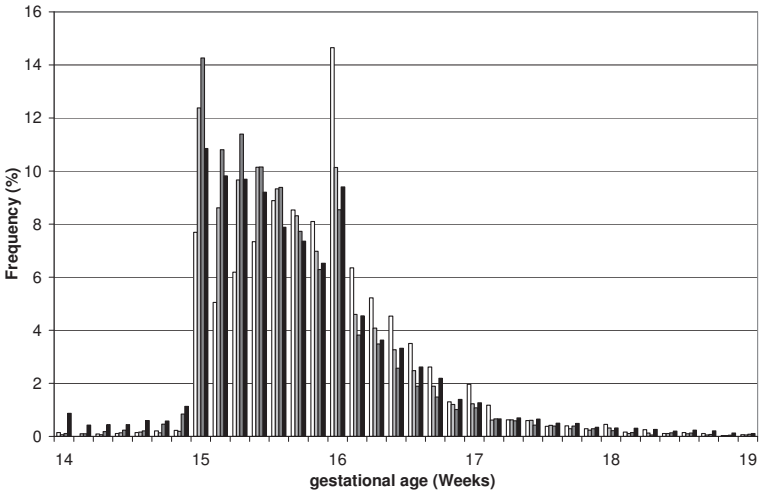

Figure 3: Gestational age distribution for the triple test 1993-2005 (n=42,554) White = 1993-1994, light grey = 1995-1999, dark grey = 2000-2005 and black = 2003-2005

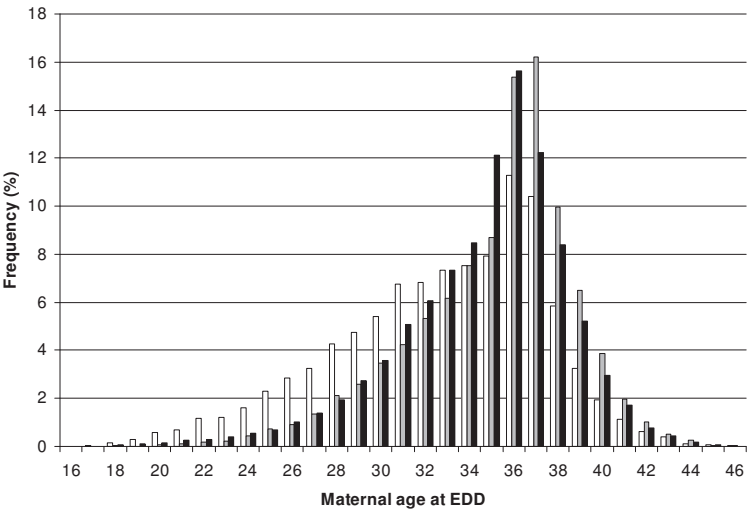

Figure 4: Maternal age at expected date of delivery distribution for requests for a triple test (n=42,554), every column represents a couple of years

White denotes 1991-1995, grey: 1996-1999 and black: 2000-2005

Table 1 shows the performance of the triple test. 105 DS pregnancies were found during the study period. The detection rate of DS cases was approximately 80% and stable over the years. The odds of being affected given a Positive Result (OAPR) were slightly higher in 2000-2005 compared to previous years.

Table 1: Performance indicators of the triple test 1991-2005

	1991-1995	1996-1999	2000-2005
Total number of tests with outcome	8,068 (75%)	8,242 (61%)	13,980 (67%)
Total of Downs	24 (0.3%)	33 (0.4%)	48 (0.3%)
FPR	11.3	14.4	13.3
DR	83.3	87.9	75
PPV	2.2	2.4	1.9
NPV	99.9	99.9	99.9
OAPR	1:45	1:41	1:52

Data of pregnancies with outcome report n=30,290
FPR, False Positive Rate; DR, Detection Rate; PPV, Positive Predictive Value; NPV, Negative Predictive Value; OAPR, Odds of being Affected given a Positive Result

Figure 5 shows the relationship between maternal age at the expected date of delivery, test requests and the FPR for 3 different time periods. The FPR increased with maternal age. From 1991 until 1999 the performances per age group were about the same, whereas the FPR was slightly lower from 2000-2005. In the age category 35-39, the odds of being affected given a positive result (OAPR) were 1:35, 1:35 and 1:50 in 1991-1995, 1996-1999 and 2000-2005, respectively.

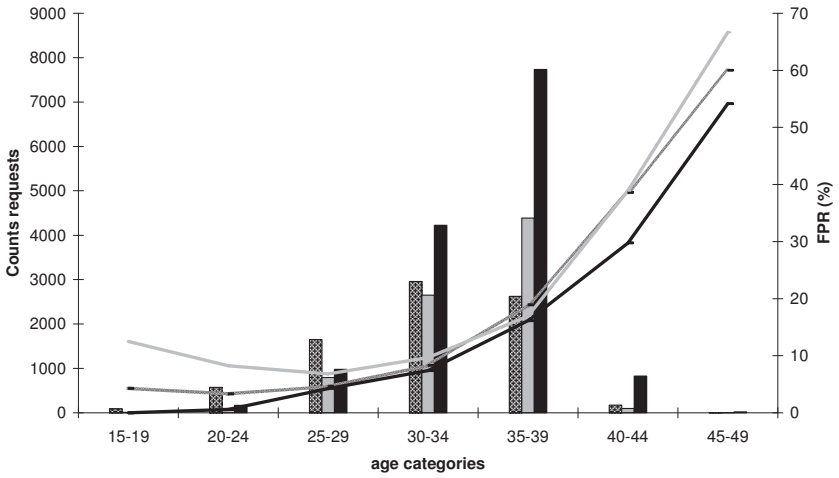

Figure 5: Relationship between the maternal age at EDD, number of requests and the FPR. Data of pregnancies with outcome report n=30,290
Dotted column and line denotes 1991-1995, grey: 1996-1999, and black: 2000-2005

Within the screened population 6 of the 12 trisomy 13 fetuses (50%) and 19 of the 28 trisomy 18 fetuses (68%) were identified.
In 1991-1995, 1996-1999 and 2000-2005, 8, 9 and 7 fetuses with a neural tube defect were found, respectively. In these periods the sensitivity for NTD was 87.5%, 66.7% and 57.1%, respectively. The incidence of NTD within the study population was on average 0.8 per 1000 fetuses (0.1%, 0.1% and 0.05% for 1991-1995, 1996-1999 and 2000-2005, respectively).

Discussion

The aim of this study was to provide an overview of 15 years triple test and to analyze trends, with time. Between 1990 and 2005, 42,554 tests were performed at the laboratory of the RIVM; with around 3,000-4,000 triple tests per year between 1995 and 2003 and a decrease thereafter. The RIVM and the University Hospital Groningen processed more than 90% of all triple tests in the Netherlands[14]. The number of tests performed in Groningen was roughly 2,000 tests a year. So, between 1995 and 2003 the total annual number of triple tests in the Netherlands was about 5,000-6,000 (of around 190,000 pregnancies per year).

In 1996 the Population Screening Act[15] was introduced in the Netherlands. This caused some confusion, and may have underlain the notable, temporary decline in the number of triple tests directly after 1996. The first-trimester combined test was introduced in the Netherlands around 2000-2002. This test was considered to perform better than the triple test[16]. Not surprisingly, the number of first-trimester screening tests rose from roughly 600 in 2002 to more than 20,000 in 2005. This probably explains the decline in requests for the triple test after 2002.

Whereas from 1990 onward the triple test requests originated from the university hospitals, in later years the requests came from midwife practices and general hospitals (Figure 2). This may well reflect the fact that health practitioners got more acquainted through the years to a test that at its introduction was considered experimental. Apparently this did not influence the performance of the test (Table 1, Figure 5). At the time when the total number of tests was still increasing (around the year 2000) the relative and absolute numbers of requests from the University hospitals were already decreasing due to the fact that these hospitals were the first to move to first-trimester screening tests.

The information leaflet for requestors stated that a triple test for DS screening could be performed at a GA of 14 weeks and later. If a NTD risk had to be calculated as well, the GA needed to be at least 15 weeks. Through the years, the range of GA became narrower, with ultimately a highest incidence at 15 weeks and 0 days. Apparently triple test results were preferred as early as possible, but late enough to enable NTD risk estimation. . In the course of the study period, and probably through education, GA was indicated more precisely. From a perspective of the quality of the screening test, a concentration of GA

around weeks 15-17 is both an advantage and disadvantage. The quality of the dataset for weeks 15-17 is high, with a high reliability of the statistically modeled medians of the biochemical parameters. Data beyond 17 weeks, however, become scarce which hampers reliable statistical regression.

Through the years, the median maternal age at which a triple test was requested increased gradually (Figure 4). The median maternal age of the general pregnant population rose from 29.7 years in 1991 to 31.5 years in 2005. Surprisingly, compared to the period 1991-1995, there was a relative decrease in requests from women younger than 32 years. This may reflect a change in the consciousness of being 'at risk', around a maternal age of 32 years. Figure 4 may show that the triple test served two purposes in a country with the policy of offering an invasive test only to pregnant women of 36 years and older. For younger women (32-36 years) the triple test was the only possibility for prenatal testing (it was only allowed to offer an invasive test to women younger than 36 years if they had a 'high risk' triple test result). For women of 36 years and older, the triple test served as a means to avoid an invasive test that might potentially harm the fetus.

There is currently no nation-wide registration of test results and the pregnancy outcomes in a national database but a plan to do so is currently developed. A total of 105 DS affected pregnancies was found in our study population (Table 1). Using the method described by Wald and Hackshaw and taking into account the maternal age distribution and the percentage of tests with reported outcome of the study periods, the expected numbers of DS cases for 1991-1995, 1996-1999 and 2000-2005 were 23, 31 and 53, respectively. This agrees quite well with the reported number of DS cases in table 1. This indicates that the amount of ascertainment bias is probably limited[20].
A detection rate (DR) of 80% combined with a FPR of 13% does not match the expected performance of the triple test (about 65% DR for a 5% FPR). However, it reflects the high maternal age of the study population (Figure 5), combined with a fixed cut-off risk of 1 in 250. When the performances were evaluated for different age-groups, as shown in figure 5, the FPR was still almost the same. In the 35-39 age-groups a slight but not significant decline of the FPR from 18.9 in 1991-1995 to 16.1 in 2000-2005 occurred. Surprisingly, the OAPR, declined from 1:35 to 1:50 during this period. This may be explained by the introduction of the first-trimester combination test which probably led to a decline of DS cases in our study population.

Generally it is considered that the triple test can detect 60% of aneuploidies[4]. In our hands the triple test detected 50% of trisomy 13 and 68% of trisomy 18 fetuses. This is comparable to the results of other studies[21,22]. Little is known about trisomy 13, probably because there are fewer pregnancies affected with trisomy 13 in the second as compared to the first-trimester. This finding is consistent with that of a Canadian study, in which a detection rate of 60% for trisomy 13 was found[22]. The incidence of NTD in our study was comparable to that in the rest of Europe[23,24]. The apparent decrease of sensitivity for neural tube defects might be ascribed to the increased use of ultrasound, enabling an early detection

of anencephalies. Thus, only cases in which the defect was hard to visualize were available for detection by the triple test.

We have described the life cycle of the triple test. Based on the results of this study, the triple test may be considered a fairly good second trimester screening test. However, the odds of being affected given a positive result (OAPR) was 1 in 41. This is inferior to the OAPR of the first-trimester combined test (1 in 14)[13], and this has resulted in the recent decline and almost disappearance of the triple test. For late-bookers there still is the possibility for second trimester screening, provided by one dedicated Dutch laboratory.

Governmental policy has strongly influenced the offer and uptake of prenatal screening in the Netherlands. Between 1977 and 2003 the Dutch government chose not to regulate the Down screening, as it was politically unacceptable to endorse a program using a test with limited performance and with termination of pregnancy as one of the final outcomes. The long term experience with a smaller, not governmentally approved program, improvement of the available tests, and valuable advice of the Dutch Health Council, ultimately led to a solid, governmentally approved screening program with special reference to quality assurance and patient information[25]. Health practitioners got gradually acquainted to the triple test, and this has facilitated the fluent transition to first-trimester testing. The triple test served as a preparation for the current formal national screening program for DS that started in January 2007.

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Chapter 3

First-trimester Down syndrome screening performance in the Dutch population; how to achieve further improvement?

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Prenatal Diagnosis 2009 Jun; 29(6): 588-92

Abstract

Objective To study the performance of the first-trimester combined test in the period 2004 to 2006 compared to a previous period to investigate changes with time and reasons for suboptimal performance.

Methods Serum samples were analysed for pregnancy-associated plasma protein A (PAPP-A) and the free β subunit of human chorionic gonadotropin (fβ-hCG). Nuchal translucency (NT) was measured between 10-14 weeks. Tests were considered screen-positive if their calculated Down syndrome (DS) risk was at least 1 in 250 at term.

Results 20,293 singleton pregnancies were included in the analysis. The median maternal age fell from 35.7 to 34.3 years. The overall median weight-corrected MoM values of PAPP-A and fβ-hCG were 1.12 and 1.03, respectively. The median MoM value of NT was 0.89 and increased from 0.82 to 0.96. Sixty-six DS cases were detected by the screening test. The detection rate (DR) for DS was 75.9%, with a false positive rate of 3.3%.

Conclusion The performance of the first-trimester test has improved over the years. A better performance of the NT measurement was the main reason, although NT assessment should further be improved. In addition, a better setting of the medians for the biochemical parameters may contribute to a higher DR.

Introduction

In the Netherlands, screening for Down syndrome (DS) is exclusively done with the first-trimester combined test (maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A) and the free β subunit of human chorion gonadotrophin (fβ-hCG) between 8-14 weeks of the pregnancy, first-trimester ultrasound measurement of the fetal nuchal translucency and maternal age). Since mid 2002, the Dutch National Institute for Public Health and the Environment (RIVM) has been involved in the performance of the first-trimester screening test, on request of the Dutch Health Care Inspectorate. Since January 2007, a nationwide screening program was set by the Ministry of Health, Welfare and Sports in which the Centre for Population Research (CvB) was indicated as the coordinating organ. Currently, 24% of all pregnant women are involved in the screening for DS. This was certainly not more in previous years.

Previous studies have shown that nuchal translucency measurement between 11 and 14 weeks, combined with maternal age is an effective screening method for DS, with a detection rate (DR) of 75% for a false positive rate (FPR) of 5%[1]. When combined with fß-hCG and PAPP-A, the DR of chromosomal abnormalities may increase to 85-90% (FPR 5%)[2]. In contrast, in the Netherlands the performance of the first-trimester screening test showed a DR of only 71%, with a FPR of 4.7% in the period 2002 to 2004[3]. The reliability of the quality of the NT measurement depends mainly on the experience and expertise of the sonographer. It was shown that the quality of the NT measurement was hampered and suggestions for quality assurance of the NT were made[4]. A formal national screening program for DS started in January 2007.

This report presents the results from 2004 to 2006, to determine changes of the performance of the first-trimester combined test in the Netherlands, and to identify the factors involved in a possible improvement.

Methods

The results of the first-trimester screening tests, between May 2004 and July 2006, from the Dutch National Institute for Public Health and the Environment (RIVM) were analysed. Venous blood samples were taken from pregnant women at a gestational age (GA) of 8.0 to 13.6 weeks. From the blood samples, serum was prepared and sent to the laboratory of the RIVM. The samples were stored upon arrival at 4°C until analysis. The fetal nuchal translucency was measured in all participating centres between 10-14 weeks GA or a crown-rump length (CRL) between 45-84 mm. It was assumed that these measurements were carried out in accordance with the FMF protocol[5] and that the sonographers were FMF-certified. All samples were accompanied by a form containing information on maternal age and GA, (e.g. dating scan details and first day of last menstrual period (LMP)), maternal weight, insulin-dependent diabetes mellitus (IDDM), number of fetuses, NT and crown-rump length (CRL). To calculate the multiple of the

median (MoM) values of the biochemical parameters, the GA at blood sampling as stated on the form by the requestor (gynaecologist or midwife) was used. In most cases, the GA was stated by the requestor, otherwise it was calculated at the laboratory of the RIVM (preferably by using dating scan details, or else by using the LMP)[6]. Maternal serum concentrations of PAPP-A and fβ-hCG were measured using commercially available kits and the AutoDELFIA analyzer (PerkinElmer, Turku, Finland).

The risk for DS at term was calculated with the software package 1T-risks (version 1.7, 1999, PerkinElmer, Turku, Finland). The risk calculation process has been described extensively elsewhere[7,8]. In short, a statistical relationship between the median concentration from PAPP-A and fβ-hCG and the GA, and from NT and CRL values, is used to express measured marker concentrations as multiples of the median (MoM). As a reference for the median NT (MoM = 1.0) the converted formula of Nicolaides et al. was used ($\log(NT) = -0.3599+0.0127*CRL-0.000058*CRL^2$)[2]. PAPP-A and fβ-hCG MoMs were corrected for maternal weight by reciprocal-linear regression. Log transformations of these MoM values were compared to Gaussian distributions for DS pregnancies and unaffected pregnancies[2,9]. For every MoM-value a likelihood ratio (LR) can be established. The product of the LR and the age risk, written as an odds-ratio, produces the DS risk[10,11]. Test results were considered screen-positive if the calculated risk for DS was at least 1 in 250 at term.

The quality of the risk calculation process was assessed on a daily and monthly basis by participating in both internal and external (UK-NEQAS, Edinburgh, UK) quality control programs for PAPP-A, fβ-hCG and the risk calculation.

Participating centres could calculate the risks for DS, using their own risk calculation software. In that case, serum samples were sent to the RIVM, where the concentrations of PAPP-A and fβ-hCG were measured and the risk based on the biochemical parameters and MoM values was calculated and sent back to these centres. In the database of the RIVM, a non-combined risk was registered, so these were excluded in this study. Alternatively, serum samples were sent to the RIVM accompanied by the NT measurement, and a combined risk was calculated. Only the combined risk calculations as calculated at the RIVM were included in the present analysis. In addition to non-combined risk calculation, multiple pregnancies, a pregnancy with previous DS and IDDM pregnancies were excluded, as well as pregnancies with a NT measurement at a CRL outside the 38-84 mm range. Pregnancy outcome (chromosomal disorders, date of birth, birth weight) was evaluated by questionnaires and collected through self-reporting of the participating women. Six months after the expected delivery date, a reminder letter is sent to these women to collect missing data. This way 75% of all pregnancy outcomes were collected. Due to strict privacy rules as stated by the Personal Data Protection Act the researchers were not allowed to request the missing pregnancy outcomes.

Results

During the period of study, from May 2004 until July 2006, the laboratory received 41,877 requests for the first-trimester screening test. As shown in figure 1, 20,293 cases were ultimately available for epidemiological analysis.

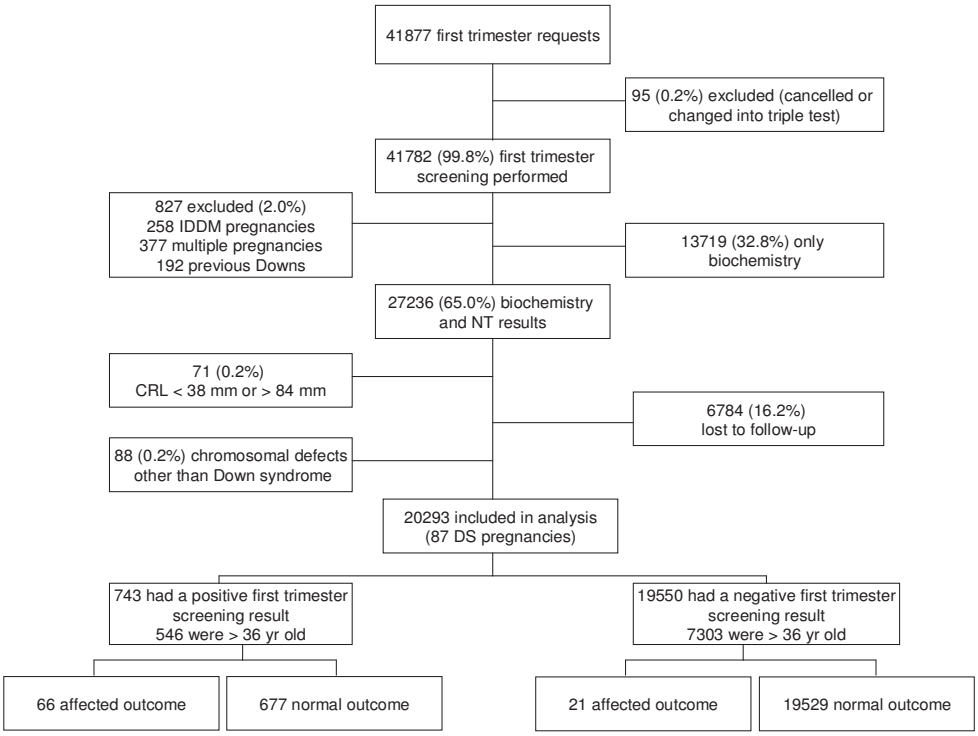

Figure 1: Flow chart indicating the number of requests eligible for the epidemiological analysis

The median maternal age for 2004, 2005 and 2006 was 35.7, 34.7 and 34.3, respectively (range 15 - 48). During these years a shift occurred towards younger ages. The median gestational age at sampling of all first-trimester requests in 2004, 2005 and 2006 was 11.4, 12.0 and 11.5 weeks, respectively. The distribution of GA was biphasic with medians at 10 and 12.3 weeks, respectively. The overall median (5^{th} - 95^{th} percentile) weight-corrected MoM values of PAPP-A and fβ-hCG were 1.12 (0.38 - 2.98) and 1.03 (0.40 - 2.91), respectively. The median MoM value of NT was 0.89 (0.52 - 1.40). Figure 2 shows the trend of the median MoM value of 20,293 NT measurements during this study period. The median MoM NT was 0.82 at the start of the study period and increased to 0.96 in July 2006. The overall median MoM NT was 0.89 (0.52 - 1.40).

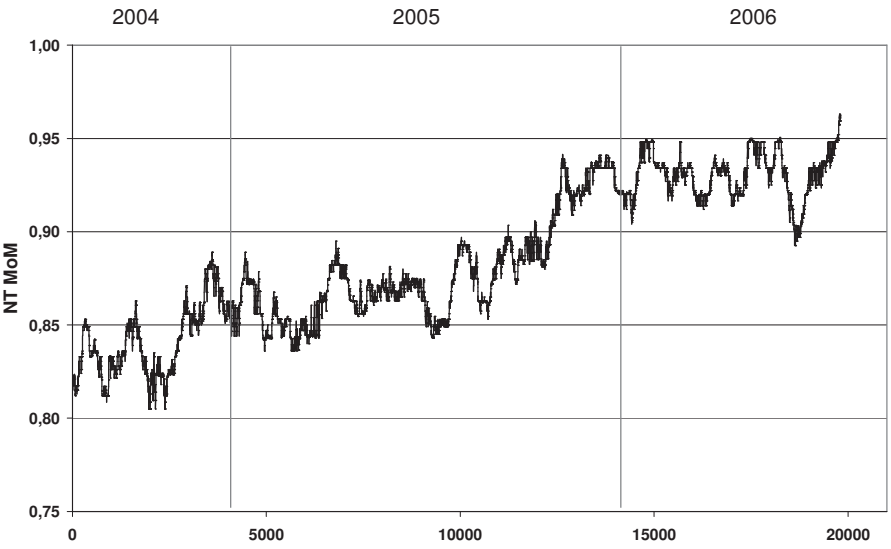

Figure 2: Median NT MoM measurements during the study period

Of the 20,293 cases, there were 743 'high risk' screening results and 19,550 cases with 'low risk' results. There were 87 DS cases of which 66 DS cases were detected and 21 were missed. Table 1 shows the screening characteristics of both subgroups. The MoM values are not corrected for weight or smoking status. There was 1 smoker in the undetected subgroup and 7 in the detected group. Pregnancy outcomes were reported to the laboratory for 75% of all the combined requests of the first-trimester screening test. The estimated risk for DS based on maternal age, fetal NT, and maternal serum PAPP-A and fβ-hCG was 1 in 250 or higher in 3.3% of the normal pregnancies and in 75.9% of those with DS. The Odds of being affected given a positive result (OAPR) was 1 in 10.

Table 1: Screening characteristics of the Down syndrome cases, not corrected for weight or smoking

Case	n	Median maternal age at expected date delivery	Median weight in kg (range)	GA at sampling in days (range)	MoM PAPP-A	MoM fβ-hCG	CRL at NT (range)	MoM NT
Detected	66	37.4	66 (48 - 105)	85 (67 - 95)	0.59	1.97	60 (40 - 78)	1.76
Not detected	21	34.6	67 (55 - 80)	86 (73 - 95)	0.92	1.54	59 (45 - 78)	1.0

The screening characteristics of the trisomy 13, 18 and triploidy cases are summarised in Table 2. The median PAPP-A and fβ-hCG MoM for the trisomy 13, 18 and triploidy cases were lower than in the normal population, the NT was higher.

Table 2: Screening characteristics of the trisomy 18 and 13 cases and triploidy cases

Case	n	Median maternal age at expected date delivery	MoM PAPP-A	MoM fβ-hCG	MoM NT
Trisomy 18	24	38.1	0.20	0.18	1.1
Trisomy 13	10	37.0	0.28	0.82	2.0
Triploidy	4	36.8	0.06	0.22	1.7

Discussion

The detection rate for the first-trimester combined test during the period May 2004 - July 2006 was 75.9% at a cut-off level of 1 in 250 at term, with a screen-positive rate of 3.3% based on the percentage of outcome 75%. This is a notable improvement as compared to 2002 - 2004 (DR 71% with a FPR 4.7%[3]), especially, since the median maternal age (34.9 years of age), was considerably lower than during the earlier period (36.6 years of age). We have no indication that the missing outcomes lead to significant ascertainment bias. The improved screening performance is reflected in a better OAPR of 1:10, as compared to 1:14 in the previous period[3]. The balance between DR and FPR is comparable to reported results from other population screening programs in surrounding countries, such as France[12], Scotland[13] and Belgium[14] and are also close to results from theoretical modelling such as in the SURUSS report. Still, the performance was lower as compared to the published results of prospective studies[15,16].

The reasons for this may include the following:

1) With regard to the biochemical markers, the overall median MoM of PAPP-A and fβ-hCG were 1.12 and 1.03 MoM, respectively. The weight-corrected PAPP-A MoM fluctuated considerably during the study period and was too high for prolonged periods of time. Recently, a method has been published to quantify the effect of a biased median MoM on the performance of the screening test[17]. Using this method, the biased median weight-corrected PAPP-A MoM of 1.12 would result in a performance loss of the FPR of about 2%. According to this paper, a better setting of the PAPP-A median and weight correction equation to correct the median PAPP-A may lead to an improvement of the performance.

2) The NT measurement is a reliable parameter for DS screening, provided it is carried out according to protocol. The protocol of the Dutch Association of Obstetrics and Gynaecology (NVOG), adopted by the Centre for Population Research (CvB), essentially the same as the FMF protocol. According to this protocol, a sonographer should perform at least 150 NT measurements per year and needs to be qualified for measuring NT through proper and certified education. NT measurements are often a point of discussion, only a measurement with the CRL between 45 and 84 mm is allowed. Previously, we have shown that in the years 2005 - 2006 the average median NT-MoM for FMF-certified sonographers was 0.98 MoM as compared to 0.91 NT-MoM for sonographers without a FMF

certificate[18]. This indicates that the FMF-certified sonographers performed better concerning the NT measurements. Figure 2 shows that the NT measurement was below the 1.0 MoM level for the whole study period (on average 0.89), with improvements with time. For a reliable risk calculation for DS, the overall median NT-MoM should be 1.0. It may be concluded that the overall quality of NT measurements in the Netherlands has improved with time, but still is unsatisfactory. Extra training should be offered.

3) Another reason for the relative low DR may be the fact that according to the Centre for Population Research (CvB), women with a NT > 4 mm may directly be offered invasive testing, without additional blood sampling for determination of biochemical markers. These women, at high risk for DS, were, therefore, not included in the data of this screening programme and this may have contributed to a lower DR. Quantitative data on this group are lacking.

4) Various co-variables are known to influence the first-trimester markers (PAPP-A and fβ-hCG). Studies describing the effects of smoking on biochemical markers, report that the median values of PAPP-A and fβ-hCG were lower in smokers as compared with non-smokers[19,20]. Only 7.1% of our study population were smokers so correction for this effect is unlikely to have greatly affected the performance, of the biochemical markers. Furthermore, we did not correct for ethnicity. The distribution for Caucasian, Asia, Negro and other ethnicities was about 96%, 1.0%, 1.1% and 1.5%, respectively. Because of the low penetration of the subgroups it is unlikely that the test performance would be considerably improved by correction for ethnicity. Additionally, estimation of GA in the first-trimester has not yet been standardized in the Netherlands. We have previously suggested that the performance of the test may improve by estimating the GA on a dating scan with one nationally accepted CRL-curve[6].

5) Furthermore, other measures to improve screening performance have been reported in the literature, such as sequential screening in the first-trimester or screening in both the first-and second-trimester. The test performance will probably improve by the implementation of other ultrasound markers such as fetal nasal bone or ductus venosus flow into the screening algorhymts. Another option is the addition of new biochemical screening markers, such as ADAM12 or PlGF. More research has to be done about these additional markers before implementation in het Dutch screening program.

As shown in Table 2, 10 cases of trisomy 13 and 24 cases of trisomy 18 were found within the study population. The MoM-values in the cases were comparable to those described in literature[21]. So, an algorithm for the risk calculation for those aneuploidies early in pregnancy may be applied. Indeed, it has recently been decided to screen for trisomy 13 and 18 within the Dutch program in the near future.

In conclusion, in the Netherlands the performance of the first-trimester test has improved over the years.

The odds of being affected given a positive result (OAPR) of 1 in 10 indicates a marked improvement in comparison with the odds presented in our previous report (1 in 14). Although the quality of the NT measurement increased impressively, there is still room for further improvement. A better setting of the medians for the biochemical parameters may also improve the detection rate. Correction for smoking and ethnicity currently seems to only minimally improve the screening results.

Acknowledgments

We thank Mr. M. Jonker, Ms. E.M van Logchem and Mr. I. Belmouden for their excellent technical assistance.

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Chapter 4

ADAM12s as a first-trimester screening marker of trisomy

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Prenatal Diagnosis 2009 Sep; 29(9): 866-9

Abstract

Objective To evaluate the potential of maternal serum A Disintegrin And Metalloprotease 12-S (ADAM12s) as an additional marker for the Combined test in the Dutch first-trimester national Down syndrome (DS) screening program.

Methods Serum samples were collected between 2004-7 as part of the national program. A total of 218 singleton cases of trisomy 21 (DS), 62 trisomy 18 (Edwards syndrome) and 29 trisomy 13 (Patau syndrome) were identified. All cases were matched with controls for gestation, maternal weight and maternal age. The serum concentration of ADAM12s was determined 'blind' to outcome and expressed in multiples of the gestation-specific median for controls (MoM).

Results The median ADAM12s was 1.00 MoM in controls, and in the DS cases at 8, 9, 10, 11, 12, 13 weeks it was 0.45 (n=3), 0.73 (22), 0.74 (53), 0.85 (37), 0.92 (71), 1.06 (32) MoM, respectively. The median for trisomy 18 was 0.85 MoM and for trisomy 13 0.63 MoM.

Conclusion The ADAM12s MoM values were clearly reduced in early first-trimester for all trisomies. However, the screening performance for DS did not greatly improve adding ADAM12s. ADAM12s could be an additional biochemical marker for first-trimester screening for trisomies other than DS.

Introduction

In the Netherlands, all pregnant women are informed on the possibility of having a screening test for Down syndrome (DS), with the first-trimester combined test as policy of choice. This test combines maternal age with maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A), fβ subunit of human chorionic gonadotrophin (fβ-hCG) and fetal nuchal translucency (NT) measurement by ultrasound. The performance of the Dutch screening program has been described previously[1].

A Disintegrin And Metalloprotease 12-s, the short and secreted spliceform of ADAM12s is a placenta-derived glycoprotein produced by trophoblasts, that is involved in growth and differentiation[2]. Previous studies have shown reduced ADAM12s levels in the first-trimester of pregnancies with trisomy 21[3] and in cases with trisomy 18 and other rare aneuploidies[4-6]. ADAM12s may thus improve the test performance of the first-trimester combined test[7].

In this study we determined the first-trimester ADAM12s levels in maternal serum of trisomy 21, 18 and 13 affected pregnancies and evaluated the potential of ADAM12s as an additional marker for the first-trimester combined test in the current national screening program. We studied two large series including a wide range of first-trimester gestational ages since other published series have indicated that the marker is more discriminatory at early gestations.

Methods

Serum samples were collected in 2004-7 as part of the first-trimester screening program in the Netherlands in two centers, the National Institute for Public Health and the Environment (RIVM) and the VU University Medical Center (VUMC).

In the screening program blood was taken at 8-13 (RIVM) or 9-13 (VUMC) completed weeks of gestation and immediately tested for serum PAPP- A and fβ-hCG using commercially available kits and either a DelfiaXpress (VUMC) or AutoDelfia (RIVM) analyzer (PerkinElmer, Turku, Finland). The agreement between these two assay platforms has been described elsewhere[8]. Unused material was frozen and stored at -20°C for research purposes. Pregnancy outcome was evaluated by questionnaires and collected through self-reporting of the participating women.

During the study period a total of 218 singleton cases of trisomy 21 (DS), 62 trisomy 18 (Edwards syndrome) and 29 trisomy 13 (Patau syndrome) were identified and specimens were retrieved from storage. Sera of the cases were matched with at least seven sera from singleton controls at the exact same gestational age and as accurate as possible for sample date (+/- 6 months), maternal weight (within 5-10kg) and maternal age (years) at sampling. A total of 2,466 control samples were studied. The samples had been exposed to a maximum of two freeze-thaw cycles before ADAM12s analysis. Serum ADAM12s was measured blinded for clinical outcome, using a semi-automatically performed time-resolved immunofluorometic assay (AutoDelfia) (PerkinElmer, Turku, Finland). Interassay CV for the

ADAM12s assay was below 5% at all levels.
The gestational age at sample date was indicated by the requesting health professional based on either a dating scan or first day of last menstrual period. All samples were accompanied by a form containing information on maternal age, gestation, maternal weight, insulin-dependent diabetes mellitus, NT and crown-rump length.
All markers were expressed as multiple of the median (MoM) for unaffected singleton pregnancies. Log-quadratic regression of the median concentration on median gestation for each completed week of gestation in controls, weighted for the number each week, was used. All MoM values were adjusted for maternal weight using inverse regression.

Results

Patient characteristics of cases and controls are shown in table 1. Possible confounding variables, ethnicity and smoking status were similar in cases and controls. Because of the matching procedure maternal age, gestational age and maternal weight were also similar.

Table 1: Cases and controls baseline characteristics

	Cases (n=309)	**Controls (n=2466)**	***P*-value**
Median maternal age (years)	37.2 +/- 3.8	36.8 +/- 3.2	0.15
Median gestational age at sample (days)	82 (range 59-97)	82 (range 59-97)	0.86
Median maternal weight (kg)	67 (range 48-114)	66 (range 42-109)	0.46
Ethnicity (%)			
- Caucasian	95.8	95.3	0.78
- Non-Caucasian	4.2	4.7	
Smoking (%)			
- yes	8.1	6.3	0.22
- no	91.9	93.7	

In controls ADAM12s concentrations ranged from 19-1096 µg/L; median concentrations were 195, 237, 302, 349, 415, 475 µg/L in week 8, 9, 10, 11, 12, 13, respectively. ADAM12s concentrations in trisomy 21 cases ranged from 65-917 µg/L. Table 2 shows that the median ADAM12s MoM level in trisomy 21 pregnancies was reduced at before 10 weeks of gestation but the extent of reduction diminished as pregnancy progressed until at 13 weeks the median was above 1.0 MoM. The table also shows the corresponding median PAPP-A and fβ-hCG MoM values.

We observed a strong association between ADAM12s and PAPP-A. Correlation coefficients were calculated between log transformed MoMs, after excluding outliers exceeding 3 standard deviations from the mean. There were too few DS cases at 8 weeks, but the correlation coefficient at 9 weeks was 0.69 ($P<0.0005$), 10 weeks 0.50 ($P<0.0005$), 11 weeks 0.54 ($P<0.001$), 12 weeks 0.40 ($P<0.0005$) and

13 weeks 0.29 (P=0.11). In the controls the r-value was 0.41 (P<0.0001). The corresponding r-values for ADAM12s and fβ-hCG were lower: in DS cases at 9 weeks 0.05 (P=0.82), 10 weeks 0.31 (P<0.05), 11 weeks 0.34 (P<0.05), 12 weeks 0.13 (P=0.27) and 13 weeks 0.02 (P=0.91); and in controls 0.16 (P<0.0001).

Table 2: Median maternal serum ADAM12s, PAPP-A and fβ-hCG MoM levels in trisomy 21 affected pregnancies according to gestation and the controls; SD (log_{10}MoM) shown in parentheses*

Week	n	ADAM12s	PAPP-A	fβ-hCG
8	3	0.45** (***)	0.46 (***)	0.95 (***)
9	22	0.73 (0.19)	0.51 (0.29)	1.38 (0.26)
10	53	0.74 (0.15)	0.38 (0.28)	1.61 (0.26)
11	37	0.85 (0.19)	0.48 (0.33)	1.53 (0.30)
12	71	0.92 (0.14)	0.53 (0.29)	1.92 (0.28)
13	32	1.06 (0.15)	0.52 (0.23)	2.30 (0.24)
Controls	2,466	1.00 (0.16)	1.00 (0.26)	1.00 (0.26)

** Standard deviation estimated from the 10-90th centile range divided by 2.563*
** 0.39, 0.45 & 0.80 MoM*
**** Too few to estimate*

The median MoM of maternal serum ADAM12s in trisomy 18 affected pregnancies was 0.85 MoM and for trisomy 13 affected pregnancies 0.63 MoM (Table 3), a statistically significant difference (P<0.005, 2-sided Wilcoxon Rank Sum Test). There is no obvious tendency for levels to change with gestation (Figure 1). An association between ADAM12s and PAPP-A was also present in both trisomy 18, with correlation coefficient 0.43 (P<0.001), and trisomy 13 with correlation coefficient 0.60 (P<0.001). The corresponding values for ADAM12s and fβ-hCG were 0.19 (P=0.17) and 0.63 (P<0.0005), respectively for trisomy 18 and 13.

Table 3: Median maternal serum ADAM12s, PAPP-A and fβ-hCG (MoM) and SD (log10MoM) for trisomies18 and 13*

	ADAM12s Med SD	PAPP-A Med SD	fβ-hCG Med SD
Trisomy 18 (n=62)	0.85 0.17	0.20 0.44	0.18 0.28
Trisomy 13 (n=29)	0.63 0.22	0.24 0.38	0.44 0.32

**Standard deviation estimated from the 10-90th centile range divided by 2.563*

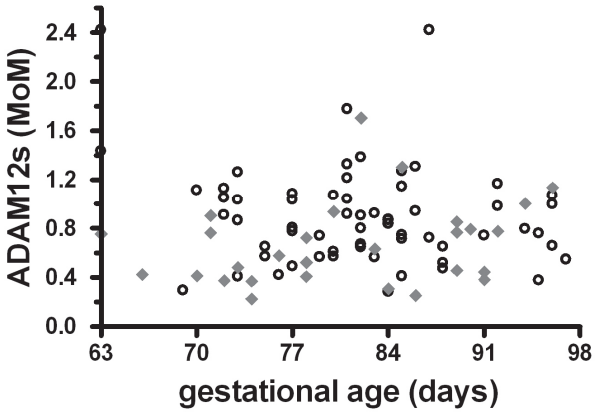

Figure 1: Individual ADAM12s levels for trisomy 18 (black dot) and trisomy 13 (black diamond) according to gestation

Discussion

ADAM12s was introduced as a promising marker for DS screening, but the promise has not been delivered yet. The initial study of Laigaard et al. showed decreased levels of ADAM12s, with a MoM value of 0.14 in eighteen early first-trimester DS pregnancies[3]. ADAM12s seemed to be most powerful at discriminating between trisomy 21 and normal pregnancies early in pregnancy, before 10 weeks. Modelling has predicted that ADAM12s and PAPP-A at 8-9 weeks, combined with NT and fβ-hCG at 12 weeks could achieve a detection rate of 97% at a 5% false positive rate[7]. However the extreme reductions in ADAM12s found in trisomy 21 affected pregnancies seen by Laigaard et al. could not be confirmed to such an extent in more recent studies[9,10]. Our large study on a wide range of first-trimester gestations was designed to provide sufficient data to determine the potential of ADAM12s for DS screening at different gestations.

Table 4 summarises the results of all the published first-trimester studies of ADAM12s in trisomy 21 affected pregnancies, according to gestational age. All of them show that levels are not different from unaffected pregnancies by 13 weeks gestation and all of those which covered a wide of gestations found the lowest values before 10 weeks. Our study shows a gradual rise of ADAM12s MoMs in trisomy 21 in the early first-trimester. Apparently the upward trend continues into the second trimester when a median of 1.36 MoM was recently reported[11,12].

Table 4: Median ADAM12s (MoM) in trisomy 21 according to gestational age in seven studies (number of cases in parentheses)

Study	n	Gestation (completed weeks)					
		6-8	9	10	11	12	13
Laigaard et al 2003	18	0.03 (7)	0.18 (6)	0.34 (2)	0.32 (3)	-	-
Laigaard et al 2006a	16	-	0.43 (1)	1.33 (5)	0.61 (9)	1.61 (1)	-
Laigaard et al 2006b	214	-	-	0.59 (3)	0.49 (39)	0.74 (108)	1.38 (64)
Spencer et al 2008b	10	0.59 (3)	0.60 (6)	1.34 (1)	-	-	-
Spencer et al 2008a	46	-	-	-	0.91 (7)	0.90 (23)	1.03 (16)
Spencer et al 2008	54	0.61 (13)	0.60 (13)	1.15 (8)	0.66 (11)	0.88 (6)	1.52 (3)
Current study	218	0.45 (3)	0.73 (22)	0.74 (53)	0.85 (37)	0.92 (71)	1.06 (32)

ADAM12s has been shown to be reduced in cases with trisomy 18 and other rare aneuploidies[4-6,13]. Laigaard et al. report a median of 0.28 MoM in 10 trisomy 18 cases[4]. A second study from this group reported two further cases, one with elevated levels and the other reduced[13]. Spencer et al. reported in 132 first-trimester cases of trisomy 18 a median of 0.83 MoM and a median of 0.66 MoM in 60 first-trimester trisomy 13 cases[5]. Our findings are remarkably close to these (0.85 MoM in 62 trisomy 18 cases and 0.63 MoM in 29 trisomy 13 cases).

Moreover notice should be given to the fact that ADAM12s might also be beneficial if screening for adverse pregnancy outcome, other than fetal anomalies. Previous studies have found that ADAM12s is decreased in cases developing pre-eclampsia later in pregnancy[14,15]. Recently, a reduction in first trimester ADAM12s levels has been found in cases later developing fetal growth restriction[16].

ADAM12s as screening marker for DS can only be used in the early first-trimester of pregnancy, preferably before 10 weeks of gestation[7,17]. The results of the current study with large numbers of DS affected pregnancies support the results previously published. In the clinical setting of an OSCAR clinic in which serum withdrawal and NT are conducted at the same day ADAM12s will not be a valuable marker. In the Dutch screening program where serum and NT can be conducted separately, and thus serum can be taken earlier in pregnancy, ADAM12s might have some potential. We assessed this by modelling with the observed ADAM12s means, standard deviations and r-values in the current study and previously published meta-analysis parameters for the first-trimester combined test markers[18]. This predicted that the addition of ADAM12s at 9 weeks to the other markers at 11 weeks would increase the detection rate for 5% false-positive rate only from 87% to 88%. The model assumes that in DS the correlation between ADAM12s and the other serum markers is the same as that observed when all are tested at 9

weeks. If PAPP-A testing was also brought forward to 9 weeks the predicted detection rate, based on the mean at that gestation estimated by Spencer *et al*. (2002) was 90%[19]. However, these results are based on modeling. Implementation in clinical practice would have far-reaching logistic and financial consequences.

Routine screening for additional abnormalities in the near future is foreseeable and
ADAM12s could be an additional biochemical marker for first-trimester screening for trisomies other than DS. Modelling with the parameters derived by Spencer and Nicolaides (2002) shows that the first-trimester Combined test has a predicted combined trisomy 18 and 13 detection rate for a 0.5% false-positive rate of 68%. With the addition of ADAM12 this increases to 70%[20].

Acknowledgments

We thank Mr. M. Jonker and Mr. I. Belmouden for their excellent technical assistance at the RIVM. At the VUMC we thank Dr. M.Levitus, Ms C. Beertsen and Ms M.Lomecky for their excellent technical assistance. Moreover we thank Dr. A.C. Muller Kobold for her cooperation in this study.

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Part II:

Screening in early pregnancy for more than Down syndrome alone

Chapter 5

Placental Protein 13 as a first-trimester screening marker for aneuploidy

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Prenatal Diagnosis 2009 Dec; 29(13): 1237-41

Abstract

Objective To determine whether placental protein 13 (PP13) could be an additional marker in first-trimester screening for aneuploidies.

Methods To evaluate differences in multiples of the gestation-specific normal median (MoMs), PP13 concentrations were measured in serum samples from Down syndrome, trisomy 18 and 13 affected pregnancies and euploid singleton pregnancies (four for each case matched for duration of storage, maternal weight and age).

Results The PP13 MoM in Down syndrome cases (n = 153) was 0.91 (not statistically significant from controls [n = 853]; $P = 0.06$; Wilcoxon rank sum test, two-tail). PP13 MoMs were decreased in trisomy 18 (n = 38; median MoM 0.64; $P < 0.0001$) and trisomy 13 cases (n = 23; median MoM 0.46; $P < 0.0001$). There was a slight upward trend in MoMs of the Down syndrome cases with gestational weeks. The PP13 MoM was significantly correlated with the pregnancy-associated plasma protein A MoM and the free beta subunit of human chorion gonadotropin (fβ-hCG) MoM.

Conclusion PP13 does not seem to be a good marker for Down syndrome. PP13 MoMs are, however, significantly lower in trisomy 18 and 13 pregnancies. The addition of PP13 to the current screening test could be valuable for improving the discrimination of aneuploid from euploid pregnancies.

Introduction

Placental protein 13 (PP13) is one of the few known proteins predominantly produced by the syncytiotrophoblast[1,2]. It is thought to play a major role in the implantation and modelling of the common foeto-maternal blood spaces through binding to proteins between placenta and endometrium[3,4].
Recently, PP13 concentrations in maternal serum have been found to be decreased in pregnancies complicated by pre-eclampsia (PE) and/or small-for-dates foetuses[5-9]. It is hypothesized that the alteration of angiogenic factors found in PE could be a result of an impaired placental function[10]. It has also been described that there is abnormal placental development in trisomy 21 (Down syndrome) and, to greater extend in trisomy 18 and 13[11]. Therefore current screening markers produced by the placenta, such as pregnancy-associated plasma protein A (PAPP-A), isoforms of human chorion gonadotropin (hCG) and inhibin, can be altered in these pregnancies. Since PP13 is also produced by the placenta, concentrations of this protein could also be altered in trisomic pregnancies. If so, PP13 might be an additional marker for aneuploidy screening.
To the best of our knowledge, this study on first-trimester PP13 concentrations in maternal serum of trisomy 21, 18 and 13 affected pregnancies is the first study in which the association between aneuploidies and PP13 is investigated.

Methods

Serum samples were collected at the National Institute for Public Health and the Environment (RIVM) between 2004 and 2006 as part of the Dutch national first-trimester Down syndrome screening programme. Samples were drawn between 8 and 14 weeks of gestational age and serum analysis of PAPP-A and the free beta subunit of hCG (fβ-hCG) was performed. For all requests maternal age, gestational age at sampling (GA), maternal weight and smoking status were recorded, as well as data on the nuchal translucency measurement. The health professionals who requested the test determined the gestational age at blood sampling and the method of calculation (either last menstrual period [LMP] or ultrasound dating). Women were asked to fill in a short questionnaire about the pregnancy outcome, including date of birth, birth weight, chromosomal abnormalities and pregnancy complications.
From this cohort, serum samples from Down syndrome, trisomy 18 and 13 pregnancies were selected and retrieved from storage. Four control sera from euploid singleton pregnancies were matched to each case, for the same day of gestation and as accurately as possible for sample date (± 6 months), maternal weight (within 5-kg weight class) and maternal age (± 2 years) at sampling. Baseline characteristics of the cases and controls are shown in Table 1.

Table 1: Baseline characteristics in cases and controls.

	controls (n=853)*		Down syndrome (n=153)		Trisomy 18 (n=38)		Trisomy 13 (n=23)	
Gestational age (days)†	81	(59-97)	81	(59-97)	82	(63-97)	78	(63-92)
Maternal weight (kg)†	67	(50-109)	67	(48-114)	68	(55-90)	67	(55-91)
Maternal age (years)†	37	(23-44)	37	(21-45)	38	(28-45)	37	(30-42)
Smoking (%)	64	(7.5)	13	(8.5)	6	(15.8)	2	(8.7)
Ethnicity (% Caucasian)	821	(96.2)	148	(96.7)	37	(97.4)	22	(95.7)

** Each case was separately matched to four control samples; three controls had to be excluded since there was not enough serum for analysis.*
† Presented as median values (range).

PP13 concentrations were measured using an automated dissociation-enhanced lanthanide fluorescent immunoassay (AutoDelfia; PerkinElmer, Turku, Finland).

PP13 levels were expressed in multiples of the gestation-specific normal median (MoMs). Normal medians were obtained by regression analysis in the controls of the median concentration for each completed week of gestation on the median days, weighted for the number of women tested. The observed MoM value was divided by the expected value for the maternal weight based on regression analysis in the controls of the median MoM on the median 1/weight in nine weight groups, weighted by the number of women. Furthermore, MoM values were divided by a correction factor for smoking and non-smoking women. Median MoM values and standard deviations of $\log_{10}$ transformed MoM values in cases and controls were estimated and statistically compared using the Wilcoxon rank sum test, two-tailed. Correlation coefficients were calculated for the associations of $\log_{10}$ PP13 with PAPP-A, fβ-hCG and gestation, after excluding outliers exceeding three standard deviations from the median. *P*-values <0.05 were considered statistically significant.

Results

Between 2004 and 2006 serum samples from 153 Down syndrome, 38 trisomy 18 and 23 trisomy 13 cases were collected. These samples, together with 853 control samples, were analyzed; three controls had to be excluded because there was not enough serum for analysis. Figure 1 shows the distribution of PP13 concentrations in the controls. The regression equation for the normal median PP13 concentrations was: $10^{(-0.316673 + 0.0494822 \times GA - 0.000294144 \times GA^2)}$, where GA is the gestational age in days.

PP13 MoM values were significantly negatively related to maternal weight ($P < 0.005$). The regression equation for the expected MoM for a given weight was: 0.42293 + 40.1339/weight.

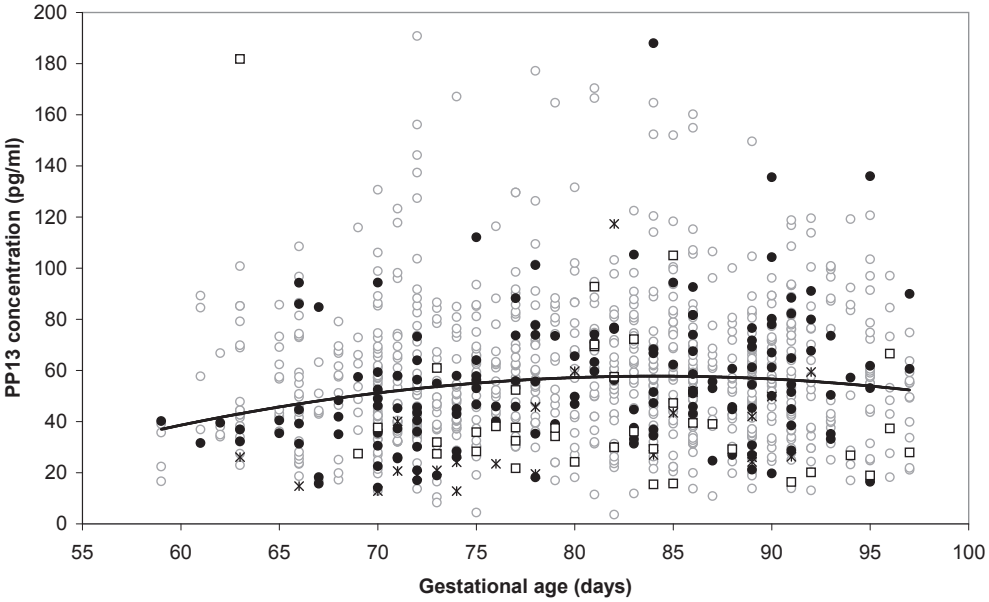

Figure 1 – Distribution of PP13 concentrations in Down syndrome (black dots), trisomy 18 (open squares), trisomy 13 (crosses) and unaffected pregnancies (open dots). The trendline represents a log-quadratic regression of the unaffected pregnancies.

Table 2 shows the median PP13 MoM values and standard deviations for cases and controls according to maternal smoking status. Smoking status was stated here as a dichotomous variable; no reliable quantitative information was available. For each type of case and for controls the median is lower in smokers, which was highly statistically significant among Down syndrome cases and controls (both $P < 0.0001$; Wilcoxon rank sum test, two-tailed). Correction for smoking was performed using the following equations: PP13 MoM/1.016 for non-smokers and PP13 MoM/0.635 for smokers. The dataset contained too few non-Caucasian women to study differences between ethnic groups.

Table 3 shows the median PP13 MoM values and standard deviations after correction for maternal weight and smoking in Down syndrome, trisomy 18 and 13 cases compared to controls. PP13 MoM levels were reduced on average in the three types of aneuploidy studied. In Down syndrome cases the reduction, with a median of 0.91 MoM, was not statistically significant ($P = 0.06$). However, there were highly significantly lower PP13 MoMs for trisomy 18 cases (0.64; $P < 0.0001$) and trisomy 13 cases (0.46; $P < 0.0001$).

Among the Down syndrome cases there was a statistically significant tendency for PP13 MoM values to increase with gestation in days (GA), with correlation coefficient 0.21 ($0.321 + 0.00841 \times GA$; $P < 0.01$). Similarly, in trisomy 13 where the r-value was 0.44 ($10^{(-1.09 + 0.0106 \times GA)}$; $P < 0.05$), whilst in trisomy 18 the trend was downwards ($r = -0.31$; $31.7 - 0.728 \times GA + 0.00425 \times GA^2$; $P = 0.06$). PP13 was significantly correlated with both PAPP-A and fβ-hCG concentrations (Table 4). Correlation coefficients tended to be higher among the trisomic pregnancies.

Table 2 – Median MoM values and log_{10} MoM standard deviations (SD) in Down syndrome (DS), trisomy 18 (T18), trisomy 13 (T13) and unaffected pregnancies (controls) according to smoking status.

	Smoking	n	Median PP13 MoM	Mean log_{10} PP13 MoM	SD log_{10} PP13 MoM
DS	No	140	0.93	-0.019	0.177
DS	Yes	13	0.42	-0.315	0.203
T18	No	32	0.63	-0.163	0.242
T18	Yes	6	0.50	-0.266	0.126
T13	No	21	0.46	-0.240	0.184
T13	Yes	2	0.35	-0.490	0.124
Controls	No	789	1.02	-0.005	0.183
Controls	Yes	64	0.63	-0.201	0.207

Table 3 – Median MoM values and log_{10} MoM standard deviations (SD) in Down syndrome (DS), trisomy 18 (T18), trisomy 13 (T13) and unaffected pregnancies (controls) after correction for smoking.

	n	Median PP13 MoM	Mean log_{10} PP13 MoM	SD log_{10} PP13 MoM	*p*-value
DS	153	0.91	-0.033	0.175	0.0645
T18	38	0.64	-0.154	0.242	<0.0001
T13	23	0.46	-0.259	0.184	<0.0001
Controls	853	1.00	-0.011	0.186	-

Table 4 – Correlation coefficients of PP13 with PAPP-A and fβ-hCG in Down syndrome (DS), trisomy 18 (T18), trisomy 13 (T13) and unaffected pregnancies (controls).

	DS	*p*	T18	*p*	T13	*p*	Controls	*p*
PAPP-A	0.258	<0.01	0.658	<0.001	0.295	0.18	0.283	<0.001
fβ-hCG	0.288	<0.001	0.383	<0.01	0.532	<0.05	0.235	<0.001

Discussion

To our knowledge, this is the first report on PP13 as a potential screening marker for common aneuploidies. A case–control study was conducted using sera from Down syndrome; trisomy 18 and 13 affected pregnancies and matched controls.

The small decrease of PP13 MoM values in Down syndrome pregnancies was not statistically significant and PP13 is, therefore, not likely to greatly improve first-trimester screening for Down syndrome. However, PP13 MoM values were highly significantly decreased in trisomy 18 and 13 pregnancies. In our laboratory approximately 80% of all trisomy 18 and 13 cases are detected using a specific first-trimester algorithm, implying the importance of additional markers such as PP13 to improve performance. In the

Netherlands, there is currently no screening programme for trisomies other than Down syndrome but implementation in the near future is foreseen.

There are a few specific findings that truly characterize a trisomic placenta. In Down syndrome pregnancies a decrease or delay in syncytial formation and morphological differentiation is present[12,13]. At term, the placenta is considerably smaller in Down syndrome affected pregnancies compared to unaffected pregnancies[14], which might already be present in the first trimester. Furthermore, undervascularization and hypotrophy of the placenta have been described[15]. There is an extremely wide range in the extent of these effects of Down syndrome on placental development. In trisomy 18 and 13 these effects tend to be much larger. In trisomy 18 pregnancies the placental cell proliferation rate is increased[16]. It is possible that this increase in cell proliferation may actually be the result of increased cell death. The number of foetal capillaries per villus cross-section is reduced and this finding may offer an explanation for the early onset intrauterine growth restriction which characterizes this chromosomal abnormality[17,18].

The trophoblast is the major source of placental specific hormones and proteins such as PP13. Since the highly polarized syncytiotrophoblast secretes its hormonal products into the maternal circulation with almost no storage capacity, any alteration in syncytiotrophoblast formation should be reflected in the maternal circulation.

Maternal smoking impairs placental development by changing the balance between cytotrophoblast proliferation and differentiation[19]. This may explain the lower PP13 concentrations in smoking compared to non-smoking women, an effect that has also been described by others[20]. Therefore, correction for smoking is of importance in a screening test containing placental markers like PP13. Since in this study the method of gestational dating was unknown the distribution parameters could not be calculated for LMP and scan dating separately. In the study period dating was more consistent with ultrasound dating[21]. According to Dutch policy ultrasound dating will be the method for gestational dating in the future and thus, the distribution parameters presented in here will be fitting for the future Dutch screening programme.

PP13 was found to be significantly correlated with fβ-hCG and to greater extend with PAPP-A. In the trisomy 18 and 13 cases these correlations were slightly higher compared to controls and sometimes the correlation coefficient was even larger than 0.5. High correlations between markers can be a sign of redundancy, however, this is not necessarily true[22]. Extensive modelling of all relevant first-trimester screening markers, taking into account their mutual correlations, will indicate the true predictive value of PP13 as a screening marker (to be published elsewhere).

Previous publications have shown that low first-trimester PP13 values are predictive of early PE[7,8]. In this study, it was found that PP13 levels are significantly lower in trisomy 18 and 13 pregnancies. In these trisomies serum levels of PAPP-A and fβ-hCG are also largely decreased[23] which is not necessarily the case in pregnancies complicated by PE. Therefore, addition of PP13 to the current screening test could be valuable to make a proper distinction between normal, aneuploid and PE pregnancies. However, new algorithms would be needed to clinically implement such a screening program.

Acknowledgments:

We thank Mr. Idder Belmouden, Mr. Mark Jonker and Ms. Elske van Logchem for their technical assistance at the RIVM.

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Chapter 6

First-trimester PP13 and PlGF: markers for identification of women destined to develop early-onset preeclampsia

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BJOG 2010 (in press)

Abstract

Objective To investigate the predictive value of maternal serum pregnancy-associated plasma protein A (PAPP-A), free β subunit of human chorionic gonadotropin (fß-hCG), Placental Protein 13 (PP13), Placental Growth Factor (PlGF) and A Desintegrin And Metalloproteinase 12 (ADAM12), for first-trimester identification of early-onset preeclampsia.

Design Nested case-control study.

Setting Routine first-trimester screening for Down syndrome in the Netherlands.

Population Eighty-eight women who developed preeclampsia or haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome before 34 weeks of gestation and 480 controls.

Methods PP13, PlGF and ADAM12 were measured in stored first-trimester serum, previously tested for PAPP-A and fß-hCG. All marker levels were expressed in multiples of the gestation-specific normal median (MoMs). Model predicted detection rates for fixed false-positive rates were obtained for statistically significant markers alone and in combination.

Main outcome measures Development of PE or HELLP syndrome.

Results PP13 and PlGF were reduced in PE cases, with medians 0.68 MoM and 0.73 MoM respectively ($P<0.0001$ for both). PAPP-A was reduced (median 0.82 MoM, $P<0.02$) whilst ADAM12 and fß-hCG did not differ between controls and PE cases. In PE complicated by a small-for-gestational age fetus, all markers except fß-hCG had lower values, compared to cases with fetuses with normal weight. The model-predicted PE detection rate for a combination of PP13 and PlGF was 44% and 54%, respectively, for a fixed 5% and 10% false-positive rate.

Conclusion This study demonstrates that PP13 and PlGF in the first-trimester might be promising markers in risk assessment for early PE/HELLP syndrome but for an adequate screening test additional characteristics are necessary.

Key words: Preeclampsia, first-trimester, PP13, PlGF, ADAM12

Introduction

Identifying pregnant women at risk for preeclampsia is one of the most important challenges in prenatal care, since preeclampsia is a serious complication of pregnancy that affects approximately 1-2% of all pregnant women worldwide. It is a leading cause of maternal and perinatal morbidity and mortality, especially when it occurs before 34 weeks of gestation. It occurs mainly in nulliparous women, who are *a priori* at low risk and without an obstetric history. Therefore screening in an unselected population is important. Maternal serum markers have been investigated and found to be potentially useful as predictors of preeclampsia. Previous studies have reported that the first-trimester concentrations are reduced for Placental Protein 13 (PP13), anti-angiogenic factors such as Placental Growth Factor (PlGF), and A Desintegrin And Metalloproteinase 12 (ADAM12) in pregnancies with early-onset preeclampsia (delivery before 34 weeks of gestation) and preeclampsia later in pregnancy, with a higher predictive value in the former group[1-6]. However, the number of cases studied for early-onset PE was generally low, ranging from about 6 to 34. Recently, a detection rate of 86% of early-onset preeclampsia has been predicted at a false positive rate of 10%, by combining maternal characteristics, obstetrics history, serum PlGF and uterine artery pulsatility index (PI) at 11 to 14 weeks of gestation[6].

The aim of this study was to investigate the predictive value of the markers PP13, PlGF, ADAM12 and the current routine markers for Down syndrome, pregnancy-associated plasma protein A (PAPP-A) and free β subunit of human chorionic gonadotropin (fß-hCG), for the first-trimester identification of severe and early-onset PE. This study was performed on stored first-trimester serum samples from 88 women who were delivered for severe PE or HELLP syndrome (Haemolysis, Elevated Liver enzymes, Low Platelets), before 34 weeks of gestation together with controls.

Methods

Study population

First-trimester prenatal screening for Down syndrome is part of routine obstetric care in the Netherlands. Serum samples were collected between 2004 and 2006 as part of the routine first-trimester screening program. Samples taken at a gestational age (GA) of 8 weeks 0 days to 13 weeks 6 days were stored at -30°C for research purposes. This was a case-control study. Serum samples (cases and controls) where retrieved from storage and thawed as aliquots once or twice. For all samples maternal age, sample date, gestational age at sampling, maternal weight and smoking status were recorded. Pregnancy outcome (chromosomal disorders, date of birth, birthweight, PE, HELLP syndrome and hypertension) was evaluated by questionnaires and collected through self-reporting by the participating women. Six months after the expected delivery date, a reminder letter was sent to these women to collect missing data.

During this study period 88 pregnancies were complicated by severe PE or HELLP syndrome resulting in a delivery before 34 weeks of gestation. In 67 of these cases pregnancy related data such as blood pressure, amount of proteinuria, HELLP syndrome, fetal weight and outcome could be confirmed at the participating hospitals rather than the study questionnaire alone. This was approved by the Scientific Ethics Committee of the University Medical Center Utrecht, the Netherlands.

Definitions

Preeclampsia was defined as an increased systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure (≥ 90 mmHg) on at least two occasions 4 hours apart developing after 20 weeks of gestation in previously normotensive women with proteinuria (≥ 300 mg/24 hours) or at least one dipstick with ≥2+ on urinalysis according to the definition of the International Society for the Study of Hypertension in Pregnancy[7]. The diagnosis HELLP syndrome was made when the following laboratory abnormalities were present: AST > 70 U/L, ALT > 70 U/L, LDH > 600 U/L, platelet count < $100x10^9$/L and evidence of haemolysis. PE combined with HELLP was defined as hypertension, proteinuria and Hemolysis, Elevated Liver enzymes and Low Platelets. The participating hospitals classified the women with HELLP syndrome or PE. The cases were classified according to centile of birthweight using the Kloosterman growth chart and regarded as small-for-gestational age (SGA) if birthweight was under the 5^{th} centile[8].

Sample selection and analysis

Women with PE were individually matched to control women (n = 480) who delivered after 36 weeks of gestation, for the same GA at sample date (exact) and for the duration of specimen storage (± 4 months). Control women were women who reported "no complications during pregnancy" on the questionnaire after delivery. The concentration of PP13, PlGF and ADAM12, was measured in the thawed specimens (controls and PE samples frozen an equal amount of times, not exceeding two), whilst PAPP-A and fß-hCG had been measured during screening, all using a time resolved fluorescence assay (autoDELFIA; PerkinElmer, Turku, Finland). Before analysis extensive validation was performed for the PP13, PlGF and ADAM12 assays. Mean intra- and interassay CV for the assays were below 5% at all levels.

Statistical analysis

The first-trimester concentration of each marker was converted into a multiple of the gestation-specific normal median (MoM) following the method described by Cuckle and Wald[9]. In brief, normal medians were obtained by regression of the observed median for each completed week of gestation in the controls against the median days, weighted by the number of controls. The observed MoM value was divided by the expected value for the maternal weight based on the regression of the observed median MoM according to weight group against 1/weight, weighted by the number of controls. Similarly for markers with statistically significant difference in median MoM between controls who smoke and controls that don't smoke, the observed MoM was divided by the appropriate median MoM. The same was done for markers with statistically significant difference in median MoM between controls

according to ethnicity. The MoM values were adjusted for gestation, weight, smoking and ethnicity in order to obtain model parameter.

For each marker the standard deviation of $\log_{10}$ transformed MoMs in cases and controls was estimated by the 10th-90th centile range divided by 2.563. The correlation coefficients between log MoMs were estimated directly, after excluding outliers exceeding 3 standard deviations from the median.

Model predicted detection rates (sensitivity) for fixed 5% and 10% false-positive rates (1-specificity) were obtained for each statistically significant marker and different combinations of markers by numerical integration[10]. This assumed multivariate log Gaussian distributions fit both preeclampsia and unaffected pregnancies. The theoretical range of MoMs was divided into a number of equal sections thus forming a 'grid' in multi-dimensional space. The Gaussian distributions were then used to calculate for each section (square for two markers, cube for three etc) the proportion of preeclampsia and unaffected pregnancies in the section and the average likelihood ratio in the section. This yielded histograms of likelihood ratios in affected and unaffected pregnancies. The 95th and 90th centile of LR in unaffected pregnancies histogram was determined and the proportion of preeclampsia pregnancies histogram with these value or higher was the predicted detection rate. The model parameters were the observed medians, standard deviations and correlation coefficients. Data were analyzed using SAS (SAS Institute, Cary, NC).

Results

Baseline characteristics of the study populations are shown in Table 1. A larger proportion of cases were in nulliparous women and the median age was lower than for controls. The maternal age was not significantly correlated with the level of the serum markers. The proportion of smokers was greater and the median weight was higher, although these differences did not reach statistical significance.

In both control and PE/HELLP groups, there was a statistically significant correlation between PlGF and PAPP-A, with correlation coefficients of 0.18 (P<0.0001) and 0.36 (P=0.001), respectively. PP13 was correlated with PAPP-A, fß-hCG and ADAM12 in both groups: 0.20, 0.36 and 0.34 (all P<0.0001), respectively in the control group and 0.29 (P<0.01), 0.30 (P<0.005) and 0.31 (P<0.005), respectively in the cases. In the control group there was a negative correlation between PlGF and PP13 (-0.14, P<0.005) but not in cases (0.10, P=0.36).

Among the control women the smokers had statistically significantly lower PP13 levels with a median of 0.61 MoM (95% CI 0.50-0.72; P<0.0001) and increased PlGF (1.40 MoM; 95% CI 1.26-1.58; P<0.0001). Levels of PAPP-A were reduced with a median of 0.82 MoM, but this is not significant (95% CI 0.65-1.07; P=0.38). A similar pattern of PP13 and PlGF levels was also seen in women with PE/HELLP. Smaller effects were seen between the ethnic groups. Among the controls there were statistically significant reductions in PP13 levels (median 0.84 MoM; P<0.05) and increase in PlGF (1.28 MoM; P=0.02). PP13 and PlGF were adjusted for gestation, weight, smoking and ethnicity in order to obtain the model parameters.

Table 1: Maternal baseline characteristics in control women and those with preeclampsi

Maternal characteristics	Controls (n = 480)	Preeclampsia (n = 88)	*P*-value
Age (y)	36.0 (33.3-38.2)	34.6 (31.0-37.3)	0.002
Caucasian	466 (96.3%)	85 (96.6%)	1
Weight (kg)	66 (62-74)	69 (63-77)	0.085
Smoking	21 (4.3%)	7 (8.0%)	0.175
GA enrolment (days)	84 (78-88)	84 (77-88)	0.787
GA delivery (weeks)	40.1 (39.0-41.0)	32.1 (30.0-33.1)	<0.001
Birthweight (gr)	3500 (3210-3840)	1338 (1038-1733)	<0.001
Nulliparity *	93 (36.2%)	57 (81.4%)	<0.001

GA, gestational age
The median (interquartile range) or number (percentage) is shown.
P-values were calculated using either a Mann-Whitney U test or a Pearson's chi-square test.
** Complete data for parity was not always available*

Table 2 shows the median MoM values for each marker. There was a highly statistically significant reduction in PP13 and PlGF levels among cases (0.68 MoM and 0.73 MoM respectively, $P < 0.0001$ for both). PAPP-A levels were also reduced, though to a lesser extent with a median of 0.82 MoM ($P < 0.02$). ADAM12 and fß-hCG MoM values did not differ between controls and PE/HELLP.

Table 2: Median MoM and standard deviation (SD) of log_{10}MoM of marker levels of PP13, PlGF, ADAM12, PAPP-A and fß-hCG for control women and those with preeclampsia

Marker	Controls Median (SD)	Preeclampsia Median (SD)	*P*-value
PP13	0.99 (0.19)	0.68 (0.20)	<0.0001
PlGF	1.00 (0.14)	0.73 (0.19)	<0.0001
ADAM12	1.00 (0.13)	1.02 (0.17)	0.76
PAPP-A	0.99 (0.25)	0.82 (0.31)	<0.02
fß-hCG	0.98 (0.24)	0.98 (0.28)	0.93

MoM estimated by the 10-90th centile range on a log-scale divided by 2.563. In its simplest form, the data of this table can be crafted into a likelihood ratio (LR) for every single marker with the equation: $LR=(SDcontrols/SDcases)*exp^{(-0.5(Zcases*Zcases-Zcontrols*Zcontrols))}$ where $Z=(log_{10}(observed\ MoM)- log_{10}(Median\ MoM)/SD$.
To make an algorithm for a combination of markers dedicated software is needed.

In pregnancies complicated by a SGA fetus (birthweight under the 5th centile), there was a tendency for all markers except fß-hCG to be lower, than in cases with fetuses with normal weight (Table 3). This effect was statistically significant.

There were no clear differences in MoM values of the markers between patients with preeclampsia, PE and HELLP syndrome, or just HELLP syndrome (Table 4).

Table 3: Median (MoM) of PP13, PlGF, ADAM12,PAPP-A and fß-hCG in women with preeclampsia according to centile of birthweight using the Kloosterman growth chart and regarding infants as small-for-gestational age (SGA) if birthweight was under the 5th centile (n=85, birthweight is unknown in 3 cases

	Birthweight centile			***P*-value**	
Marker	**≥ 10th (n=53)**	**5-10th (n=11)**	**≤ 5th (SGA) (n=21)**	**Any centile**	**SGA**
PP13	0.75	0.71	0.48	<0.02	<0.005
PlGF	0.75	0.66	0.60	0.06	<0.05
ADAM12	1.10	0.92	0.78	<0.05	<0.02
PAPP-A	1.05	0.59	0.49	<0.001	<0.001
fß-hCG	1.05	0.78	0.85	0.29	0.15

Table 4: Median MoM level for each in women with preeclampsia, with HELLP syndrome or with preeclampsia according to the presence of HELLP syndrome

Marker	**Preeclampsia (n = 45)**	**HELLP (n = 21)**	**Preeclampsia/HELLP (n = 22)**
PP13	0.62	0.66	0.87
PlGF	0.75	0.68	0.72
ADAM12	0.99	1.04	1.03
PAPP-A	0.76	0.83	0.90
fß-hCG	0.95	1.08	0.94

Because we had too few complete data to include baseline characteristics (such as medical history, parity, blood pressure, etc.), none of these variables were included in the final model. Model-predicted detection rates for early PE/HELLP, for fixed false positive rates are shown in Table 5. When taken together, PP13 and PlGF predicted 44% and 54% of cases at false positive rates of 5% and 10%, respectively. Addition of PAPP-A did not improve this detection rate. Receiver Operating Characteristic curves for PP13 and PlGF were generated for all cases of preeclampsia (figure 1).

Table 5: Model predicted early preeclampsia detection rate (95% CI) for fixed false-positive rates of 5% and 10% for seven marker combinations

Marker combination	False-positive rate: 5%	10%
PlGF alone	31 (22-41)	41 (32-53)
PP13 alone	24 (16-34)	36 (27-47)
PAPP-A alone	14 (8-22)	22 (14-31)
PlGF and PP13	44 (34-55)	54 (43-63)
PlGF, PP13 and PAPP-A	45 (35-56)	55 (44-66)
PlGF and PAPP-A	32 (23-42)	43 (33-54)
PP13 and PAPP-A	26 (18-36)	37 (27-47)

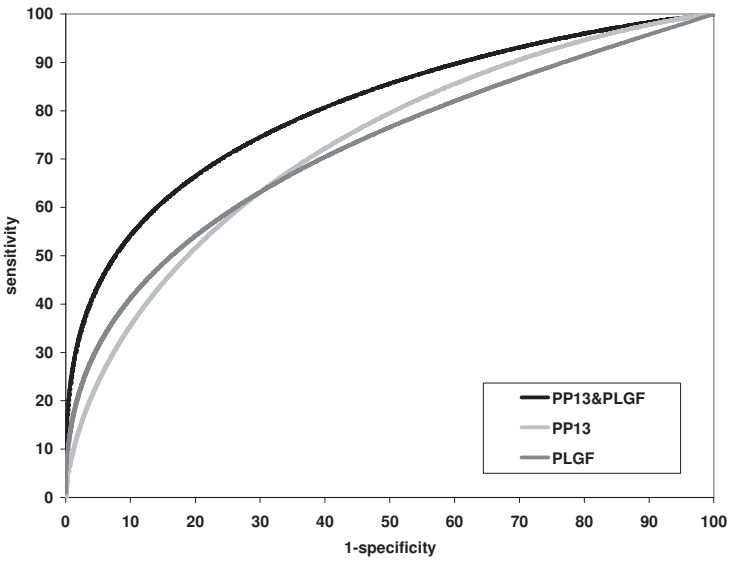

Figure 1: Receiver Operating Characteristic curves depicting the sensitivity (detection rates) and 1-specificity (false-positive rates) of the first-trimester maternal serum PP13 and PlGF, PP13 alone or PlGF alone for the identification of preeclampsia

Discussion

This retrospective study showed the predictive value of maternal serum first-trimester PP13 and PlGF for first-trimester identification of early-onset preeclampsia. The predictive value is probably even better when PE is complicated by SGA infants but more cases are needed to establish this. Risk assessment using these markers together with other variables such as blood pressure, Doppler flow velocity

waveform patterns of the uterine artery and maternal history, may result in a detection rate suitable for an applicable screening program.

The risk assessment we used is a standard method in antenatal screening. It was first used for Neural Tube Defects screening and for at least 20 years in the assessment of markers for Down syndrome. It has the advantage over regression analysis of being 'parsimonious' and avoids over-fitting of the model which makes it more robust when applied to additional study populations. This method is used in previous reports on risk assessment for preeclampsia [3,9,10].

Eighty-eight pregnancies were complicated by severe PE or HELLP syndrome, resulting in a delivery before 34 weeks of gestation. In 67 of these 88 cases and in more than half of the controls, pregnancy related data such as blood pressure, amount of proteinuria, HELLP syndrome, birthweight and outcome could be confirmed with the patient records of the participating hospitals rather than the study questionnaire alone. However, there are several limitations to our study. We have no indication that the missing outcomes may have led to significant ascertainment bias but there might have been some recall bias. In all 67 cases in which we could study patient notes, the initial diagnosis was confirmed.

Levels of PP13 and PlGF were statistically significantly reduced in pregnancies complicated by PE or HELLP syndrome for the whole gestational age range of 8 to 13 weeks of gestation. Among the controls the smokers had, on average, lower PP13 and PAPP-A and increased PlGF. So, to accomplish a better risk assessment for early preeclampsia the measured concentration should be adjusted for smoking. Furthermore, there were too few non-white women in the study population to establish an effect of ethnicity on PP13 and PlGF MoMs.

This is the first study in which the analysis of PP13 was performed using a time resolved fluorescence assay (autoDELFIA; PerkinElmer, Turku, Finland) and combined with PlGF. Both markers individually had an almost similar predictive value of 36% and 41% at a 10% false-positive rate, respectively. In PE complicated by a SGA fetus PP13 was even more reduced than PlGF (Table 3). Furthermore, as shown in table 4, there was no difference between PlGF, PP13 and the severity of PE defined by PE, PE and HELLP or HELLP syndrome alone.

The effects of PP13 on the implantation and maternal vascular remodelling are not fully understood. It is known that PP13 is produced in the trophoblast and binds to sugar residues of the extra-cellular matrix molecules, which is involved in the placental implantation. From the first-trimester onward, levels of PP13 slowly increase in healthy pregnancies. Furthermore, PP13 increases the release of prostaglandins, which are important for vascular remodelling in early placental development [11,12]. Therefore, it is plausible that a reduced level of PP13 may impair several functions that are necessary for the normal placental development and vascular remodelling. In two earlier studies in which PP13 was measured using a solid-

phase sandwich enzyme-linked immunosorbent assay (ELISA) technique, also significantly lower values were found in cases developing early preeclampsia [3,13].

PlGF is a pro-angiogenic protein, and also involved in the regulation of placental vascular development and maternal endothelial function during pregnancy. Previous studies have shown that PlGF concentrations are reduced during the clinical phase of PE [4,5,14]. Our study and recent other studies have shown that PlGF levels are already reduced in first and second-trimester, i.e. before the clinical phase of the disease [6,15,16] .

Our findings regarding ADAM12 support a previous study indicating that ADAM12 does not provide useful prediction of SGA, preeclampsia or preterm delivery[17] and contradict two previous reports showing that in pregnancies developing preeclampsia the serum ADAM12 concentration is reduced[1,2]. We only found a small, although statistically significant, reduction of ADAM12 levels in pregnancies complicated by preeclampsia who subsequently delivered a SGA neonate. This may be explained by the fact that ADAM12 is a placental product involved in the control of fetal growth.

Conclusion

This study demonstrates that maternal serum first-trimester PP13 and PlGF may be promising markers in risk assessment for early preeclampsia (/HELLP syndrome). These markers should be combined with other variables such as blood pressure, Doppler flow velocity waveforms of the uterine artery and maternal history to improve the performance of the screening for early PE. Larger prospective and longitudinal studies are necessary to support these findings. In clinical practice, early detection of preeclampsia may result in closer and intensive maternal and fetal surveillance and possibly in better outcomes.

Acknowledgments

We thank Mr. I. Belmouden, Mr M. Jonker and Ms E.M. van Logchem for their excellent technical assistance at the RIVM. Moreover we thank all the participating Dutch hospitals for their willingness to complete the data in this study; Academic Medical Center, Amsterdam; Amphia Hospital, Breda; Catharina Hospital, Eindhoven; Deventer Hospital, Deventer; Erasmus Medical Center, Rotterdam; Flevo Hospital, Almere; Gemini Hospital, Den Helder; Groene Hart Hospital, Gouda; Haga Hospital, The Hague; Jeroen Bosch Hospital, Den Bosch; Academic Medical Center, Leiden; Medical Spectrum Twente, Enschede; Onze Lieve Vrouwe Gasthuis, Amsterdam; Reinier de Graaf Group, Delft; Rijnland Hospital, Leiderdorp; Rivierenland Hospital, Tiel; Rode Kruis Hospital, Beverwijk; Sint Antonius Hospital, Nieuwegein; Sint Elisabeth Hospital, Tilburg; Slingeland Hospital, Doetichem; 'T Lange Land Hospital, Zoetermeer; TweeSteden Hospital, Tilburg; University Medical Center, Maastricht; University Medical Center St. Radboud, Nijmegen; VieCurie Medisch Center, Venlo; Vlietland Hospital, Vlaardingen; Waterland Hospital, Purmerend; Westfries Gasthuis, Hoorn; Zaans Medical Center, Zaandam.

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Chapter 7

Evaluation of seven serum biomarkers and uterine artery Doppler ultrasound for first-trimester prediction of preeclampsia. A systematic review

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Abstract

Preeclampsia (PE) affects 1-2% of pregnant women and is a leading cause of maternal and perinatal morbidity and mortality worldwide. By definition, the clinical syndrome of PE arises in the second half of pregnancy. However, many underlying contributing factors including defective placentation may already be apparent in the first- and early second trimester in many patients. In clinical practice, there is no reliable single screening method with sufficient accuracy to identify women at high risk to develop PE in the first-trimester of pregnancy. Early identification of low versus high risk pregnancy may facilitate the development of new strategies for antenatal surveillance and thus improve maternal and perinatal outcome.

It was the aim of this systematic review to study the literature on the predictive potential of first-trimester serum markers and of uterine artery Doppler velocity waveform assessment (Ut-A Doppler). Literature on seven most studied serum markers (ADAM12, fβaab-HCG, Inhibin A, Activin A, PAPP-A, PlGF and PP13) and Ut-A Doppler was primarily selected. In the selected literature a combination of these markers was analyzed, and where relevant, the value of maternal characteristics was added. In total, we included 126520 women of whom 2897 developed PE (2.3%). Measurements of both serum markers and Ut-A Doppler were performed between week 8+0 and 14+0 GA. Low first-trimester levels of PP13, PlGF and PAPP-A are significantly associated with the development of PE later in pregnancy. The detection rates (DR) of the screening tests fixed at 10% false positive rate for single markers in the prediction of early-onset PE (EO-PE) were relatively low, and ranged for PP13 from 36 to 80%, for PlGF 41-52%, for PAPP-A 22 to 41%. For abnormal uterine artery waveforms DR's varied from 33 to 83%. DR's of maternal characteristics alone ranged from 40 to 49% in EO-PE. In three studies maternal characteristics and all screening markers were combined and resulted in a DR of 90%. Therefore, a combination of serum markers with maternal constitutional characteristics and Ut-A Doppler yields high detection rates and is promising to identify patients at high risk of developing PE. However, large scale prospective studies are required to evaluate the power of this integrated approach in clinical practice.

Abbreviations

ADAM12	A Disintegrin And Metalloproteinase 12
AUC	Area Under the Curve
BMI	Body Mass Index
CI	Confidence Interval
DR	Detection Rate
EO-PE	Early Onset Preeclampsia (delivery < 34 weeks)
FPR	False Positive Rate
GA	Gestational Age
HELLP	Hemolysis, Elevated Liver Enzymes, and Low Platelets
IGFBP	Insulin-Like Growth Factors Binding Proteins
IUGR	Intrauterine Growth Restriction
LO-PE	Late Onset Preeclampsia (delivery ≥ 34 weeks)
MC	Maternal Characteristics
PAPP-A	Pregnancy-Associated Plasma Protein A
PE	Preeclampsia
PE	Preeclampsia not specified
PI	Pulsatility Index
PlGF	Placental Growth Factor
PP13	Placental Protein 13
RI	Resistance Index
ROC	Receiver Operating Characteristic
sEng	Soluble Endoglin
sFlt-1	Soluble Fms-Like Tyrosine Kinase
sVEGFR	Soluble Vascular Endothelial Growth Factor Receptor-1
TGFβ	Transforming Growth Factor Beta
Ut-A Doppler	Uterine Artery Doppler
VEGF	Vascular Endothelial Growth Factor
Fβ-hCG	Free Beta subunit of human Chorionic Gonadotropin

Introduction

Preeclampsia (PE) is a serious complication of pregnancy that affects approximately 1-2% of pregnant women worldwide[1,2]. It is a leading cause of maternal and perinatal morbidity and mortality, particularly when it occurs at a gestational age less than 34 weeks[2-5]. Although its presentation is predominantly late term with a mild clinical course, severe maternal complications of PE include renal failure, hemolysis, elevated liver enzymes and low platelets (HELLP) - syndrome, liver hemorrhage and rupture, eclampsia, cerebral hemorrhage, and maternal death. In addition, PE is associated with substantial risk of perinatal morbidity and mortality due to concomitant intrauterine growth restriction (IUGR), iatrogenic prematurity, placental abruption and stillbirth.

PE is considered to be a result from a complex interaction between placental factors, maternal constitutional factors and unsuccessful vascular and immunologic adaptation to pregnancy[6-9]. These interactions predominantly involve the cardiovascular and inflammatory system, resulting in marked maternal endothelial dysfunction and organ damage due to vascular compromise[6,7,9,10]. Most likely, PE is a heterogeneous syndrome that does not always develop by the same pathophysiological pathway[6, 7, 9,10]. The causative complex of interacting factor may differ from patient to patient. Some authors distinguish between two types of PE, maternal and placental PE. Placental PE is considered to be a result of impaired trophoblast invasion into the spiral arteries and their failure to remodel[7,11]. Narrow spiral arteries lead to placental ischemia and generate oxidative stress conditions[12]. Early-onset PE, developing relatively early in pregnancy and necessitating delivery prior to 34 weeks gestation, is more frequently associated with this defective placentation than late-onset disease. Conversely, maternal PE, i.e. to result predominantly from maternal constitutional factors like high blood pressure, obesity, impaired glucose tolerance and dyslipidemia, is considered to be the predominant type in late pregnancy[13]. However, genuine placental and maternal PE, though attractive from a conceptual point of view, may be rare and most cases of PE are likely to be of mixed etiology, i.e. resulting from interplay between factors of more than one of the three before mentioned categories.

In spite of the lack of effective preventive strategies up to now, risk assessment for PE early in pregnancy may be of benefit for both pregnancy outcome as utilization of resources in antenatal care[14,15]. Identification of women at risk, as early as the first-trimester of pregnancy, enables intensified antenatal surveillance, timely intervention and better outcomes in those who are at high risk, and less intensified antenatal care and additional testing in those at low risk. However, as yet we have no reliable single screening test to identify women who are at high risk before the clinical manifestation of PE[14-16].

Placental factors with a potential role in the pathogenesis of PE that have been studied recently for their predictive potential include A Disintegrin And Metalloproteinase 12 (ADAM12), free β subunit of human Chorionic Gonadotropin (fβ-HCG), Inhibin A, Activin A, Pregnancy Associated Plasma Protein-A (PAPP-A), Placental Growth Factor (PlGF) and Placental Protein 13 (PP13)[17-21]. Secondly, maternal (constitutional) factors such as parity, age, body-mass index (BMI), blood pressure and medical history are thought to contribute to identify high risk women. Additionally, abnormal vascular adaptation to pregnancy, such as unfavourable adaptive changes of the uterine artery (Ut-A) may contribute to the pathogenesis of

PE[22]. Based on the current knowledge of its pathogenesis, it may be expected that optimal strategies to for stratification of overall PE risk will include factors from all three categories.
The aim of this systematic review is to evaluate the literature on the single and combined potential screening value of first-trimester placental serum markers, Ut-A Doppler measurements and maternal characteristics.

Methods

Definitions of pre-eclampsia

For PE, the definition according to the International Society for the Study of Hypertension in Pregnancy was used: gestational hypertension beyond 20 weeks GA in previously normotensive women with a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg on at least two occasions 4 hours apart with the presence of proteinuria of ≥300 mg in 24-hour collection or ≥ 2+ by dipstick on a spot urinalysis with or without generalized oedema[23].
Early Onset PE (EO-PE) is commonly defined as requiring delivery before 34 weeks of gestation, Late Onset (LO-PE) requiring delivery at or after 34 weeks of gestation.

Literature search

A systematic literature search of PubMED, EMBASE, CINAHL and Cochrane was performed on 19.10.2009. In a single comprehensive search we aimed to find all primary studies reporting on the accuracy of screening tests in the first-trimester of pregnancy using serum markers in maternal blood and Ut-A Doppler. In order to find relevant articles various terms within the determinant (e.g. first-trimester and serum markers or first-trimester uterine artery Doppler) and outcome (e.g. pre-eclampsia) were combined. The domain was not included in the search filter to prevent retrieval and reporting bias. The syntax is listed in Table 1. Both determinant and outcome were searched in title and abstract in all search engines. After filtering doubles, two authors (S.K., E.W.) separately screened titles and abstracts of all selected studies to manually identify the articles investigating the value of first-trimester serum markers and Ut-A Doppler in the prediction of PE. Authors used inclusion and exclusion criteria shown in Figure 1. Of the remaining articles the full text was retrieved. Subsequently more extensive inclusion and exclusion criteria were applied.

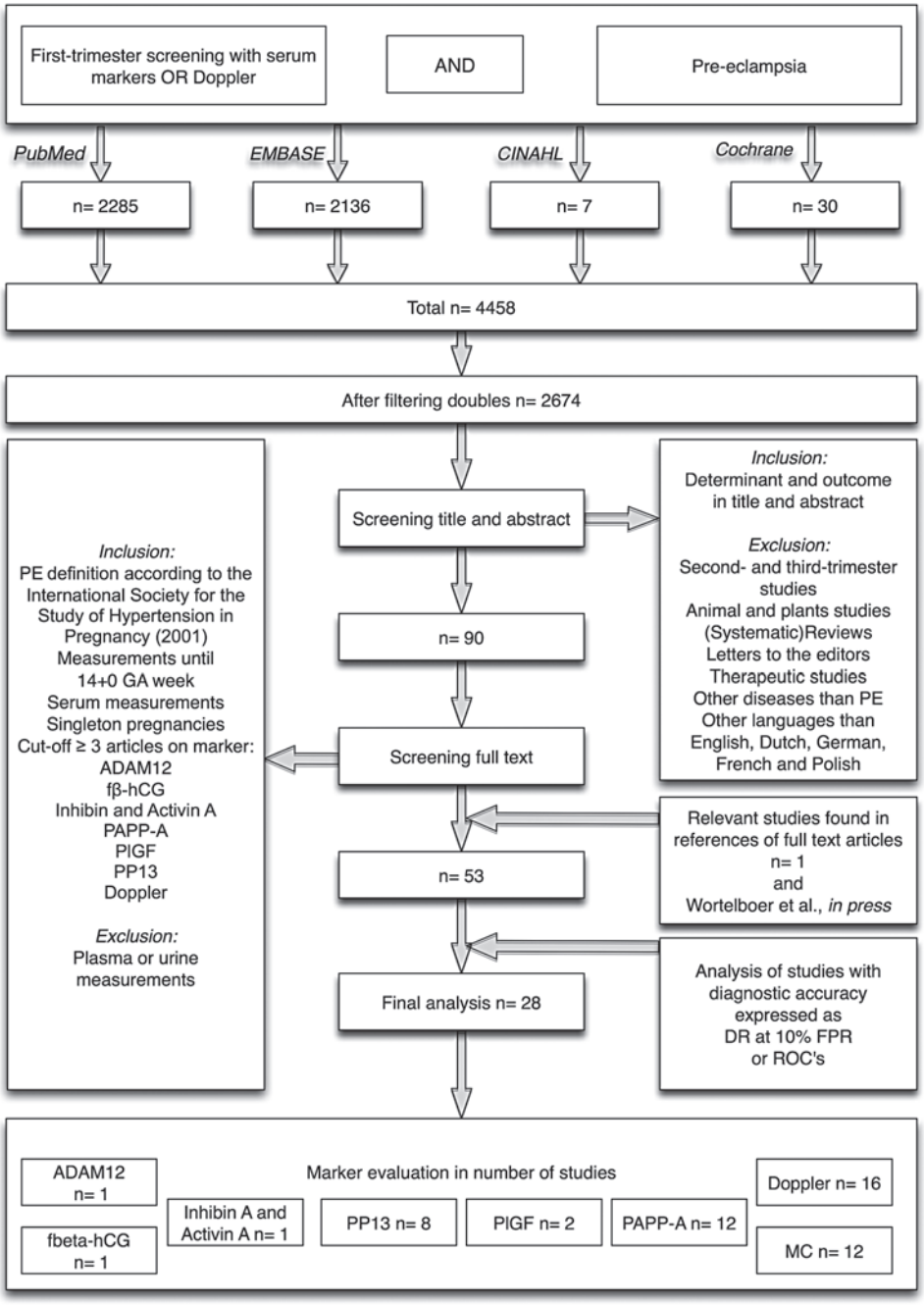

Figure 1: Flow chart of included studies on first-trimester markers used to predict PE. Abbreviations: GA gestational age, ROC Receiver Operating Characteristic

Quality assessment

The methodological quality of the selected studies was appraised independently by two reviewers (S.K. and E.W.) using the validated Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool[24] (Figure 2).

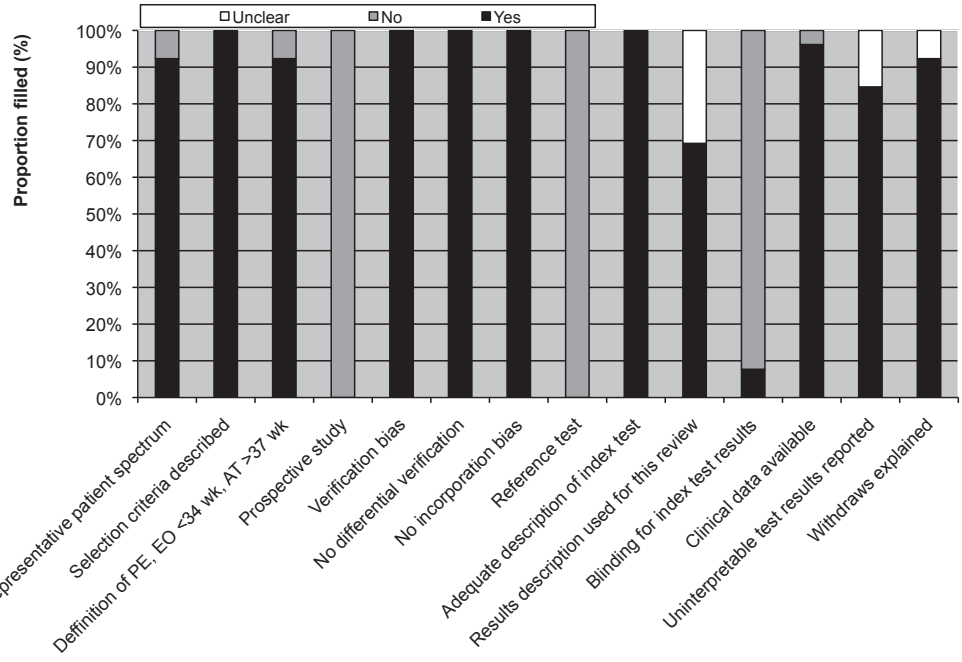

Figure 2: Summary of quality assessment (QUADAS)

Data analysis

Pregnancies complicated by PE were divided into three groups: (I) EO-PE, (II) LO-PE, and (III) PE. PE was used for those studies in which the PE subgroups-definition was different from the one applied in this review or the studies in which the PE subgroups were not specified.

In this review we were interested in the Detection Rate (DR) of the test performance at fixed 10% False Positive Rate (FPR). Where the appropriate data could be derived from the reports, it was incorporated into this review. Otherwise we personally approached the authors of the studies for additional information. When, even after various attempts we were not able to retrieve the appropriate data, we derived DR from the ROC's shown in the articles, where applicable. Otherwise the studies were discarded. If the 95% confidence interval (CI) of the DR was not cited we calculated it with a web-calculator (http://www.causascientia.org/math_stat/ProportionCI.html).

Initially, we wanted to analyze the screening performance of first-trimester serum markers and

Ut-A Doppler measurements only. In a number of selected studies however, one or more maternal characteristics were enclosed as a separate variable. Maternal characteristics in all studies contained combination of characteristics such as: maternal age, maternal weight, race, parity, cigarette smoking, family history of PE, conception, medical history and medication during pregnancy. Separate description and analysis of individual risk factors reaches beyond the scope of this review. However, when maternal characteristics were included in selected studies, they were regarded as a separate variable in the current review.

Upon final selection of the studies, authors considered the performance of:

- serum markers either individually or combined
- Ut-A Doppler measurements
- Ut-A Doppler measurements and serum markers combined
- maternal characteristics
- maternal characteristics combined with serum markers or with Ut-A Doppler

Results are presented as forest plots of DR at 10% FPR with 95% confidence intervals (CI) for EO-PE, LO-PE and PE, respectively.

Results

Figure 1 gives an overview of the selection process for relevant literature. The literature search yielded 4458 articles, after filtering doubles 2674 were left for screening of titles and abstracts. After screening for title and abstract using specific inclusion and exclusion criteria 90 articles were retrieved for full text reading. Given the large number of the proposed serum markers tested for their potential role in screening for PE, an additional exclusion criterion comprising a cut off value of ≥ three articles describing a specific serum marker in the first-trimester of pregnancy was added. As a consequence seven maternal serum biomarkers were selected: ADAM12, fβ-hCG, Inhibin A together with Activin A, PAPP-A, PlGF and PP13 together with Ut-A Doppler measurements. This step resulted in 53 studies. Among the latter ones, two studies meeting all selection criteria were added after the screening procedure. One study was found after reading the references of selected full-text articles. It was not captured by the primary search because of lack of PE or other synonyms in title and abstract. Furthermore, one article of our own research group, which is in press for publication, but not published at the time of the literature search, was included as well, since it contained a large number of EO-PE.

To be able to compare the performance of the selected markers only studies containing the diagnostic accuracy of the markers presented as DR at fixed 10% FPR, or ROC's reached the final analysis (n= 28) (Figure 1). Therefore, although markers such as ADAM12 were more than three times described in the literature, only a single study on this particular marker met the diagnostic accuracy criterion[19].. All 28 studies were case control or cohort studies where the cases were included after PE was clinically diagnosed – no prospective studies were available. Among the selected studies, eight studies evaluated PP13 separately or in combination with other markers[21, 25-31], two evaluated PlGF[17, 21], twelve considered

PAPP-A[17-19, 25, 31-37], sixteen Ut-A Doppler [17, 25, 28, 29, 34, 35, 38-47]. ADAM12, fβ-HCG and Inhibin A together Activin A were evaluated in single studies[19, 20, 33]. In twelve studies maternal characteristics were added to the analysis of the markers[17, 18, 25, 30, 34, 35, 41-43, 45, 46, 48]. Table 2 lists the finally selected studies, test characteristics and study population.

In total, we included 126520 women of whom 2897 developed PE (2.3%). Measurements of both serum markers and Ut-A Doppler were performed between week 8+0 and 14+0 GA. The study population comprised women with low prior risk, except for two studies[28, 38].

ADAM 12

ADAM12 (A Disintegrin And Metalloproteinase 12) is a placenta-derived member of the ADAM protein family[49]. It is present in the syncytiotrophoblast and is thought to be involved in placental growth and development. Gack et al., demonstrated that ADAM12 was the most up-regulated transcript in placental tissues of PE women[50]. This finding lead to speculations whether ADAM12 could serve as an early biomarker for hypertensive pregnancy diseases[50]. There are four published studies which measured serum levels of ADAM12 between 8 and 14 weeks of gestation[19, 21, 51, 52]. In these studies the percentage of PE cases was 33% (n PE= 160), 11.2% (n PE= 128), 1.4% (n PE= 64) en 15.5% (n PE= 88), respectively. Studies of Laigaard et al., and Spencer et al.,2008a have shown reduced ADAM12 levels in pregnancies complicated by PE and in both studies ADAM12 is suggested to be a potential PE marker (median ADAM12 MoM were 0.86 $p = 0.008$ and 0.49 $p < 0.0001$, respectively)[19, 51]. Contrarily, Poon et al., and Wortelboer et al., report no alteration of the first-trimester levels of ADAM12 in women developing PE[21, 52]. The median MoMs in these studies were not significantly different from controls (0.95 and 1.02, respectively). Only in one study of Spencer et al., DR of 37% for unspecified PE could be derived from the ROC curve (Appendix)[19].

Free Beta subunit of human Chorionic Gonadotropin

The free beta subunit of human Chorionic Gonadotropin (fβ-hCG) is secreted by the syncytiotrophoblast cells. Its primary function is to maintain the decidual spiral arteries and the vascular supply of the placenta during pregnancy[53]. In normal pregnancies the concentration of fβ-hCG increases exponentially until week 8-10, decreasing afterwards. In the second-trimester fβ-hCG in PE was reported to be elevated[54-56]. Studies that retrospectively evaluated fβ-hCG presented no evidence for a predictive value of this particular marker for hypertensive pregnancy disorders[18, 33, 36, 37, 57-60]. Only in one study of Ong et al., DR of 22% for unspecified PE was stated(Appendix)[33].

Inhibin A and Activin A

It is suggested that the feto-placental unit is an important source of Inhibin A and Activin A and both are involved in a feedback loop regulating hCG levels during pregnancy[61, 62]. Muttukrishna et al., showed that in the third trimester maternal serum concentrations of both markers were about tenfold higher in women with severe PE compared to controls[63]. A small number of studies showed that Inhibin A and

Activin A were significantly elevated already in the first-trimester in women with PE[20, 60, 64, 65]. In these studies the incidence of PE was 33% (n PE=52), 33% (n PE= 30), 1.2% (n PE= 9) and 21% (n PE= 64) respectively. Only in one study of Spencer et al., 2008b DR for Inhibin A and Activin A for unspecified PE were stated; 35% and 20%, respectively (Appendix)[20].

PP13

Placental Protein 13 is predominantly produced by the syncytiotrophoblast[66, 67]. It is thought to play a major role in the implantation of the blastocyst. Moreover it is possibly involved in the remodelling of the common feto-maternal blood-spaces through binding to proteins between the placenta and endometrium[68-70]. From the first-trimester onward, levels of PP13 slowly increase in healthy pregnancies. In PE pregnancies first-trimester concentrations of PP13 are significantly lower. In the second and third trimesters however, the concentrations of PP13 were higher[68]. The reasons for this are currently not known. More specifically, five studies report significant difference between median PP13 MoM of EO-PE and control pregnancies (Table 3)[21, 25, 28, 29, 71]. In these studies the DR of PP13 as a single marker was 36 - 80% (Figure 3). In PE pregnancies DRs and MoMs were comparable to those of EO-PE (Appendix)[26-28, 30, 31].

PlGF and other angiogenic factors

An imbalance between pro- and anti-angiogenic factors before and after the onset of PE is suggested to play a crucial role in its pathogenesis[8]. It is thought that the inaccurately implanted placenta becomes ischemic and subsequently secretes anti-angiogenic factors such as sFlt-1 (soluble Fms-like Tyrosine Kinase-1) also known as sVEGFR-1 (soluble Vascular Endothelial Growth Factor Receptor-1), and sEng (soluble Endoglin) into the maternal circulation which later antagonize a number of pro-angiogenic factors, such as PlGF (Placenta Growth Factor) and VEGF (Vascular Endothelial Growth Factor)[72]. It is hypothesized that as a consequence; the concentration of important angiogenic and placental growth factors in the maternal circulation is reduced, leading to impaired endothelial function and subsequently EO-PE.

These studies concentrate mostly on the second half of the pregnancy in which there is a clear difference in the concentrations of both anti-angiogenic and pro-angiogenic factors in PE pregnancies when compared to controls (elevated:[73, 74] (decreased:[74-76]. Studies in the first-trimester however, show that sFlt-1 and sEng are constant throughout the first-trimester and that their concentrations in normal pregnancy may be equal to or possibly lower than those in pregnancies destined to be complicated by PE[71, 74, 77-80].

Pro-angiogenic factors, particularly PlGF, can be measured as early as nine weeks of gestation whereas VEGF concentrations are too low to be detected in the first-trimester[74, 81]. Consequently, the majority of reports considering the pro-angiogenic factors in the first-trimester concentrate on PlGF. PlGF concentrations are found to increase throughout pregnancy in normal pregnancies starting already in the first-trimester. In pregnancies destined to develop PE the concentrations increase not as much as in healthy women or remain low throughout pregnancy[17, 71, 74, 77-79, 82].

From the perspective of first-trimester screening for PE, studies on anti-angiogenic factors so far are not conclusive. They do not warrant inclusion of these factors as new markers for risk assessment for PE. PlGF however appears to be a promising screening marker in the first-trimester. The DR of PlGF alone in first-trimester measurements for predicting EO-PE was 41-52%, and for LO-PE 33%. (Figure 3)[17, 21]. The median MoMs for PlGF were lower for both EO-PE and LO-PE (Table 3) and (Figure 4)[17].

PAPP-A

Pregnancy Associated Plasma Protein-A is produced by the developing syncytiotrophoblast[83, 84]. It regulates the bioavailability of free IGF at the placental-decidual interface during human implantation[85, 86]. It is thought to play a major role in the autocrine and paracrine regulation of trophoblast invasion in the decidua[87]. Low concentrations of PAPP-A in the first-trimester of pregnancy are highly associated with chromosomal aneuploidies. In pregnancies with a normal karyotype low PAPP-A is suggested to be an indicator of increased risk for various pregnancy complications[36]. The majority of the published studies show that low concentrations of PAPP-A are statistically significantly associated with EO-PE (Table 3)[17, 25, 31, 33, 35, 37]. For EO-PE DR ranges from 22 to 41% (Figure 3)[17, 21, 25]. The median MoMs for PAPP-A for EO-PE, LO-PE and PE are significantly lower in comparison to controls (Table 3)[17, 25, 31, 33, 35, 37].

Uterine Artery Doppler velocity waveform patterns

Ut-A Doppler has become an valuable tool for the study of utero-placental circulation[88]. Physicians can follow the development of trophoblast invasion through the registration of impedance to flow in the uterine arteries. In normal pregnancies impedance to flow progressively decreases with gestation between 6 and 24 weeks and remains constant thereafter[89, 90]. Hence, abnormal development of the placental vasculature related to PE can be detected by sustained impedance to flow in the maternal uterine vessels[88, 91, 92].

This observation has led to the idea of using Ut-A Doppler as a screening tool in predicting adverse pregnancy outcomes. Abnormal uterine artery waveforms seem to be a good predictor of PE. The DR for EO-PE varies from 33 to 83% (Figure 3)[17, 25, 29, 35, 41, 43, 45-47]. In case of LO-PE and PE the detection ranges between 21-62% (Figure 4 and Appendix)[17, 34, 35, 38-44, 47]. Median MoMs of Ut-A Doppler are in all variants of PE significantly higher compared to healthy pregnancies (Table 3)[17, 25, 31, 33, 35, 37, 43, 45, 46].

Maternal characteristics

PE is considered to be a multifactorial disease with various maternal constitutional factors contributing to its pathogenesis. Potential risk factors are: nulliparity, maternal age ≥ 40, BMI >29, previous PE, family history of PE, chronic hypertension and race[93-95]. Separate description and analysis of these risk factors is beyond the scope of this review. However a number of the analyzed articles use one or more risk factors of maternal characteristics to improve the first-trimester screening[17,18,25,30,34-36,41-43,45,46]. Maternal characteristics certainly have an additive potential to predict PE. In this review all different risk factors are analyzed together under the section called maternal characteristics). DR of maternal characteristics alone ranges from 40 to 49% in EO-PE, LO-PE and PE (Figure 3, 4 and Appendix)[17,18,25,30,34-36,41-43,45,46].

Combined screening

PP13 and PlGF in combination predicted 54% of EO-PE cases at FPR of 10% (Figure 3)[21]. PP13 and PAPP-A together resulted in a DR of 37%, while for PlGF with PAPP-A the DR was 43% (Figure 3)[21]. Combination of PP13, PlGF and PAPP-A improved the DR to 55%[21]. In case of unspecified PE combination of PAPP-A with ADAM12 resulted in a DR of 38% (Appendix)[19].

Only a few studies described the combination of Ut-A Doppler in combination with serum markers. In a study of ten women that subsequently developed EO-PE, a DR of 90% was established with a combination of PP13 and Ut-A Doppler (Figure 3)[29]. Poon et al., combined Doppler with PAPP-A and found a DR of 60% and 36% for EO-PE (Figure 3) and PE, respectively[35].

For EO-PE, maternal characteristics and various combination of serum markers produced a DR that ranged from 49% to 69% (Figure 3)[17, 25, 30, 35]. For LO-PE, maternal characteristics and various combination of serum markers produced a DR that ranged from 41% to 52%[17, 35]. In PE the DR for maternal characteristics and various markers ranged from 13 % to 49%[30, 35, 48].

The DR for EO-PE for the combination of Ut-A Doppler and maternal characteristics ranged from 50 to 81% (Figure 3)[17, 25, 34, 35, 43, 45, 46]. For LO-PE this ranged between 42-51% (Figure 4)[17, 17, 35, 35, 43, 43, 45, 46]. For PE the DR was 42-62%[34, 35, 41, 42].

In three studies maternal characteristics and all screening markers were combined[17, 25, 35]. The combination of PlGF with Ut-A Doppler and maternal characteristics was the most successful and resulted in a DR of 90% (Figure 3). In LO-PE the same combination of markers resulted in a DR of 49%[17]. In PE combination of PAPP-A, Ut-A Doppler and maternal characteristics resulted in a DR of 51%[35].

Discussion

In this study we provide a systematic review of studies on known serum markers and uterine artery Doppler for first-trimester prediction of preeclampsia. Screening for risk of preeclampsia development is considered as an important step in the early diagnostic evaluation of patients at high risk for development of maternal and perinatal complications later in the pregnancy. The findings demonstrate that fβ-hCG appeared not suitable for the prediction of PE. There is so far not enough evidence regarding the predictive value of ADAM12 or Inhibin A together with Activin A. In contrast, low first-trimester levels of PP13, PlGF and PAPP-A are significantly associated with the development of PE later in pregnancy. However, the screening potential of each individual single serum marker is limited by only modest DR's at a false positive rate of 10%. For screening of unselected populations high DR's are needed, in order not to miss a substantial number of high risks cases. Therefore single markers screening is unsuitable for clinical practice. Combinations of serum markers with maternal constitutional characteristics and/or unsuccessful vascular adaptation to pregnancy (Ut-A Doppler) yields higher detection rates and are, therefore, promising. The DR's are lower for late-onset PE as compared to early-onset PE, which seems logical given the mostly normal placentation in the former[17, 25, 35, 41, 43, 45, 46]. More promising results are expected in the prediction of EO-PE, with detection rates of up to 90% in some models. These findings

are clinically relevant, as especially EO-PE is associated with multiple maternal and fetal complications. Unfortunately, studies up to now are underpowered and retrospective. Large prospective studies are needed. Validation of combinations of markers in a different population/cohort is lacking up to now.

Limitations

This systematic review has several restrictions. First of all, the numbers of affected women in the studies included were relatively small, implying the need for large scale studies. The majority of the studies in our review did not specify the onset of PE. Only twelve studies with a relatively small number of patients evaluated the risk for early onset disease. Secondly, although the quality of included studies was generally good, they suffered from several shortcomings. In particular, none of the studies was prospective. All studies were performed after PE was clinically diagnosed. Subsequently, results may have varied between studies because of different immuno-assay kits used with different test characteristics with respect to variability and validity. In addition, discordant results may have been caused by differences in cut-off points for abnormality of the biomarker assays, differences in diagnostic indicators for uterine artery Doppler measurements (pulsatility or resistance index, PI and RI respectively), and differences in statistical models used for multiple prediction. For this reason a formal meta-analysis with estimated overall relative risks was not feasible. We dealt with this by presenting the DR at a fixed 10% FPR, which allows for recognising a trend in presented results.

Implications

Accurate prediction of PE is crucial for the improvement of prenatal care and future development of preventive treatment. Because the incidence of PE is relatively low (1-2%), a potential screening test needs to have a high detection rate with an as small as possible false positive rate. Currently, none of the serum markers meets these criteria. The detection rates of the serum markers single or in combination, are so far too low to be implemented in the first-trimester PE screening. The search for new potential markers that are capable of detecting the presence of pathologic conditions in maternal serum early in pregnancy with a high sensitivity and specificity remains still a major challenge.

The use of serum markers in combination with uterine artery Doppler appears promising. Uterine artery Doppler provides a potentially useful screening tool. Nevertheless, it remains a difficult technique and the measurements need to be standardized before it could be broadly used. Large prospective studies using standardized measurement methods are needed to evaluate the potential combination strategies.

When a potential test is developed, its effectiveness in subsequent prevention of PE still has to be proven. The role of a first-trimester screening test would be in a subsequent individualisation of the antenatal care. Identification of women at high risk for early PE could benefit from closer maternal and fetal surveillance which could potentially improve pregnancy outcomes. Prospective randomised controlled trials in screen positive women should study effects of interventions, such as the administration of low dose Aspirin [96, 97]. This could lead to prevention and/or to an earlier diagnosis of the clinical signs of

the disease and avoid serious complications through preventive measures, such as antihypertensive medication or induced delivery.

Unfortunately, so far the clinicians do not have any effective treatment of PE. Low doses aspirin reduces up to 10% the risk of early onset PE[97]. However, when started before 16 weeks of gestation it may even reduce PE with 50% (RR 0.48; 95 CI: 0.33-0.68)[96]. This stresses the importance of an early identification of patients at risk.

A potential screening model with a detection rate of at least 90% may also lead to a better utilisation of resources in antenatal care. It may help in the selection of the suitable patients for future RCT's and investigation for potential preventive medications against PE already early in pregnancy.

Based on this systematic review, we assume that despite the potency of the combined screening for PE, currently there is not enough knowledge and resources to use it, with the exception of a RCT regarding aspirin in first-trimester screen positive women. Major search for new potential screening markers and therapeutic opportunities is required.

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Table 1: Search syntax

Search syntax	*19.10.2009*
Search	Synonyms
#1	(11+0 AND 13+6) OR (11+0 AND 14+0) OR (11-13) OR (11-14) OR ("First trimester" OR "first-trimester" OR ((first OR early OR initial OR inaugural OR premier OR primal OR primary OR prime) AND ((trimester OR trimesters OR quarter OR quarters) OR (pregnancy OR conception OR conceptions OR gestation OR gestations OR gestosis OR gravidity OR gravidities))) OR (placental AND (phase OR phases OR stage OR state OR states))) AND (serum OR blood OR marker OR markers OR factor OR factors OR screening OR (Doppler OR ultrasonography OR ultrasonographies OR sonography OR sonographies OR ultrasound OR ultrasounds OR (blood AND (flow OR flows OR flowing OR flood OR inflow OR inflows OR move OR moves OR pass OR passes OR run OR runs OR stream OR streams) AND (velocity OR rapidity OR rate OR swiftness OR tempo)) AND ((artery OR arteries OR arterias OR arterial) AND (uterine OR uterus OR uteri OR womb))))
#2	(PE OR Preeclampsia OR "pre-eclampsia" OR (pre AND eclampsia) OR eclampsia) OR (((pregnancy OR conception OR conceptions OR gestation OR gestations OR gestosis OR gravidity OR gravidities) OR complex OR (syndrome OR syndromes)) AND (toxaemia OR toxemia OR toxicosis) OR ((edema OR oedema OR edemas OR oedemas OR dropsy) AND (proteinuria OR proteinurias OR albuminuria OR albuminurias) AND (hypertension OR hypertensions OR (high AND blood AND pressure))))
#3	#1 AND #2

Tabel 2: Study characteristics of included studies for prediction of preeclampsia (PE)

Early Onset PE						
Author, year	Study design	Population	Measurements GA weeks	EO-PE n (%)	Controls n	Study population
Nicolaides 2006[29]	1	LR	11 – 14	10 (2.3)	423	433
Pilalis 2007b[41]	1	LR	11 – 14	6 (0.5)	n.a.	1123
Akolekar 2008[17]	1	LR	11 – 14	29 (3.5)	609	824
Plasencia 2008[43]	1	LR	11 – 14	22 (0.7)	2595	3107
Rizzo 2008[47]	1	LR	11– 14	6 (1.7)	n.a.	348
Romero 2008[30]	1	LR	8 – 14	6 (2.0)	250	306
Akolekar 2009[25]	1	LR	11 – 14	48 (7.7)	416	624
Khalil 2009[28]	1	HR	11 – 14	14 (5.5)	210	252
Poon 2009a[35]	1	LR	11 – 14	32 (0.4)	7895	8051
Poon 2009b[45]	1	LR	11 – 14	37 (0.4)	8061	8366
Poon 2009c[46]	1	LR	11 – 14	37 (0.4)	8061	8366
Wortelboer *in press*[21]	1	LR	8 – 14	88 (15.5)	478	566

Late Onset PE

Author, year	Study design	Population	Measurements GA weeks	LO-PE n (%)	Controls n	Study population
Akolekar 2008[17]	1	LR	11 – 14	98 (11.9)	609	824
Melchiorre 2008[40]	1	LR	11 – 14	57 (1.9)	2968	3058
Plasencia 2008[43]	1	LR	11 – 14	71 (2.3)	2595	3107
Khalil 2009[28]	1	HR	11 – 14	6 (2.4)	210	252
Poon 2009a[35]	1	LR	11 – 14	124 (1.5)	7895	8051
Poon 2009b[45]	1	LR	11 – 14	128 (1.5)	8061	8366
Poon 2009c[46]	1	LR	11 – 14	128 (1.5)	8061	8366

PE not specified

Author, year	Study design	Population	Measurements GA weeks	PE n (%)	Controls n	Study population
Ong 2000[33]	1	LR	11 – 14	135 (2.5)	4297	5297
Smith 2002[48]	1	LR	8 – 14	324 (3.7)	n.a.	8839
Dugoff 2004[18]	1	LR	10+3 – 14	764 (2.3)	n.a.	33395
Gomez 2005 [39]	1	LR	11 – 14	22 (2.2)	932	999
Spencer 2005[37]	1	LR	11 – 14	64 (1.5)	3999	4390
Chafetz 2007[26]	1	LR	9 – 12	47 (11.0)	289	427
Pilalis 2007a[34]	1	LR	11 – 14	13 (1.5)	715	878
Pilalis 2007b[41]	1	LR	11 – 14	14 (1.2)	924	1123
Plasencia 2007[42]	1	LR	11 – 14	107 (1.8)	5041	6015
Spencer 2007[31]	1	LR	11 – 14	44 (0.7)	446	5867
Brameld 2008[32]	1	LR	11 – 14	60 (0.03)	n.a.	20076
Fratelli 2008[38]	1	HR	11 – 14	5 (6.5)	n.a.	76
Gonen 2008[27]	1	LR	6 – 10	20 (1.6)	1178	1239

Melchiorre 2008[40]	1	LR	11 – 14	33 (1.1)	2533	3058
Rizzo 2008[47]	1	LR	11+0 – 13+6	16 (4.5)	n.a.	348
Romero 2008[30]	1	LR	8+0 – 13+0	50 (16.3)	250	306
Spencer 2008a[19]	1	LR	11 – 14	64 (1.5)	3999	4390
Spencer 2008b[20]	1	LR	11 – 14	64 (21.0)	240	304
Khalil 2009[28]	1	HR	11+0 – 13+6	42 (16.6)	210	252
Melchiorre 2009[44]	1	LR	11-14	89 (3.0)	2445	3010
Poon 2009a[35]	1	LR	11+0 – 13+6	156 (1.9)	7895	8051

Abbreviations: LR low-risk population, HR high-risk population, n.a. not available.

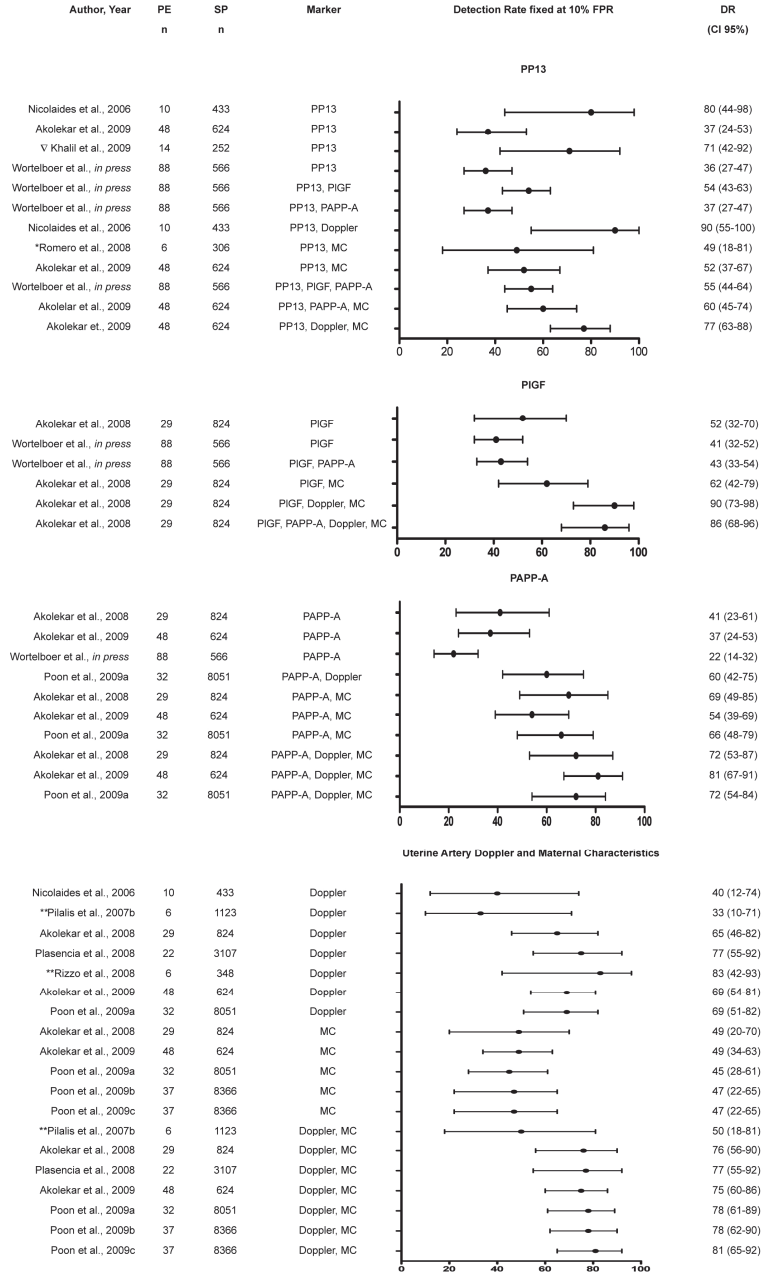


*Figure 3: Forest plot with the detection rates (DR) of the screening tests fixed at 10% false positive rates (FPR) in prediction of Early Onset PE (EO-PE) in the first-trimester, with 95% confidence intervals (CI). PE – number of pre-eclampsia cases, SP – study population. ∇ High-risk population; * Adapted from ROC curve; ** contact with the author of the study. MC – maternal characteristics – contain combination of characteristics as: maternal age, maternal weight, race, parity, cigarette smoking, family history of PE, conception, medical history, medication during pregnancy*

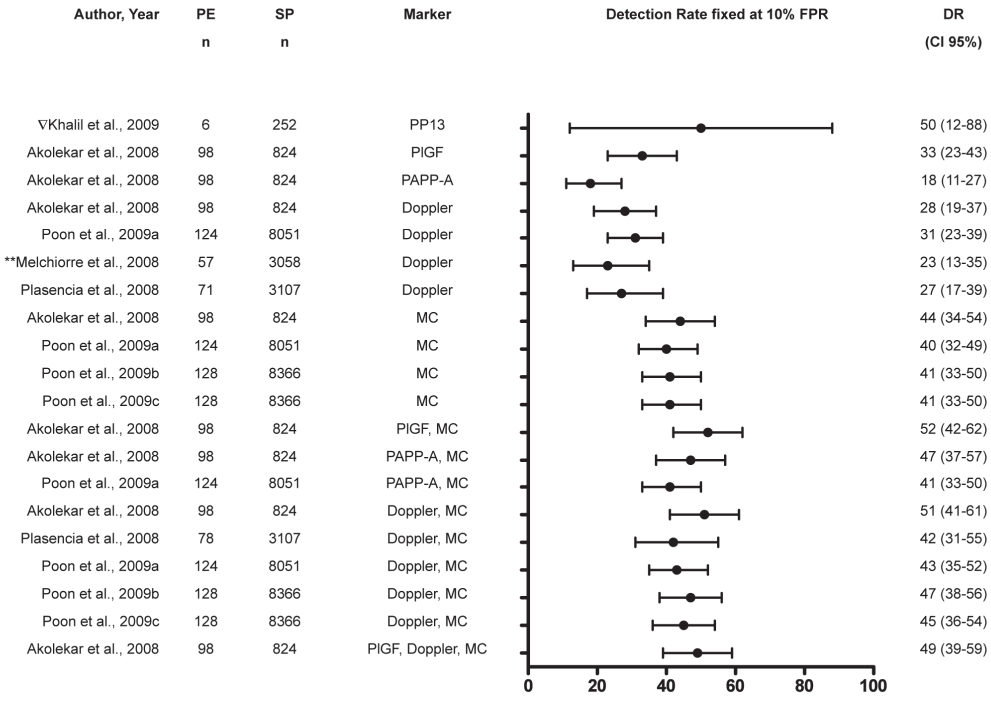


Figure 4: Forest plot with the detection rates (DR) of the screening tests fixed at 10% false positive rates (FPR) in prediction of Late Onset Pre-eclampsia (LO-PE) in the first-trimester, with 95% confidence intervals (CI). PE – number of pre-eclampsia cases, SP – study population. ∇*High-risk population; ** contact with the author of the study. MC – maternal characteristics – contain combination of characteristics as: maternal age, maternal weight, race, parity, cigarette smoking, family history of PE, conception, medical history, medication during pregnancy*

Table 3: Median MoMs of first-trimester markers

Early Onset PE

Author	Marker	GA	Pre-eclampsia n	Pre-eclampsia MoM Median	Controls n	Controls MoM Median	*P* value
	PP13						
Nicolaides 2006[29]		11 – 14	10	0.07	423	1.00	<0.001
Romero 2008[30]		8 – 13	6	0.26	250	1.00	0.002
Akolekar 2009[25]		11 – 14	48	0.83	416	1.02	<0.0167
Wortelboer *in press*[21]		8 – 14	88	0.68	478	0.99	<0.0001
	PlGF						
Akolekar 2008[17]		11 – 14	29	0.61	609	0.99	<0.0001
Wortelboer *in press*[21]		8 – 14	88	0.73	478	1.00	<0.0001
	PAPP-A						
Akolekar 2008[17]		11 – 14	29	0.53	609	1.07	<0.0001
Akolekar 2009[25]		11 – 14	48	0.55	416	1.08	<0.0167
Poon 2009a[35]		11 – 14	32	0.55	7895	1.00	<0.001
Wortelboer *in press*[21]		8 – 14	88	0.82	478	0.99	<0.02
	Doppler						
Nicolaides2006[29]		11 – 14	10	1.43	423	1.00	<0.001
Akolekar 2008[17]		11 – 14	29	1.52	609	1.03	<0.001
Plasencia 2008[43]		11 – 14	22	1.49*	2595	0.99*	<0.001
Akolekar 2009[25]		11 – 14	48	1.61	416	0.97	<0.0167
Poon 2009a[35]		11 – 14	32	1.49	7895	1.01	<0.001
Poon 2009b[45, 46]		11 – 14	37	1.51	8061	1.01	<0.0001
Poon 2009c[45, 46]		11 – 14	37	1.51	8061	1.01	<0.0001

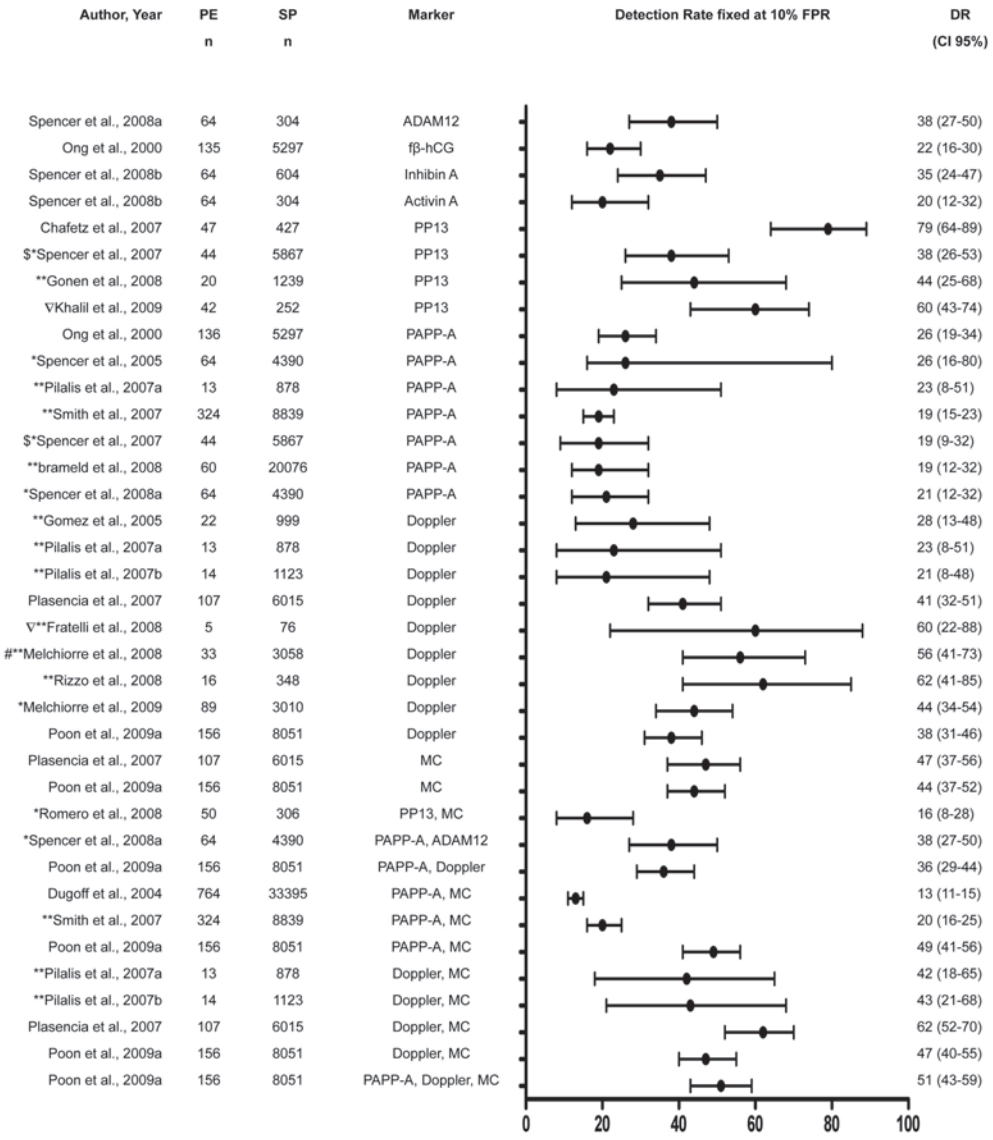
Late Onset PE

			Pre-eclampsia		Controls		
Author	Marker	GA	n	MoM Median	n	MoM Median	*P* value
	PP13						
Akolekar 2009[25]		11 – 14	160	0.96	416	1.02	NS
	PlGF						
Akolekar 2008[17]		11 – 14	98	0.82	609	0.99	<0.0001
	PAPP-A						
Akolekar 2008[17]		11 – 14	98	0.93	609	1.07	<0.05
Akolekar 2009[25]		11 – 14	124	0.84	416	1.08	<0.0167
Poon 2009a[35]		11 – 14	160	0.91	7895	1.00	0.03
	Doppler						
Akolekar 2008[17]		11 – 14	98	1.22	609	1.03	<0.0001
Plasencia 2008[43]		11 – 14	71	1.03*	2595	0.99*	0.04
Akolekar 2009[25]		11 – 14	160	1.25	416	0.97	<0.0167
Poon 2009a[35]		11 – 14	124	1.19	7895	1.01	<0.001
Poon 2009b[45, 46]		11 – 14	128	1.19	8061	1.01	<0.0001
Poon 2009c[45, 46]		11 – 14	128	1.19	8061	1.01	<0.0001

PE not specified

Author	Marker	GA	Pre-eclampsia n	Pre-eclampsia MoM Median	Controls n	Controls MoM Median	*P* value
	ADAM12						
Spencer 2008a[19]		11-14	64	0.71	4390	1.0	<0.0001
	fβ-HCG						
Ong 2000[33]		11 – 14	135	0.88	4297	1.05	0.012
	Inhibin A						
Spencer 2008b[20]		11-14	64	1.24	240	1.0	0.0006
	Activin A						
Spencer 2008b[20]		11-14	64	1.17	240	1.0	0.0276
	PP13						
Chafetz 2007[26]		9 – 12	47	0.2	289	1.00	<0.01
Spencer 2007[31]$		11 – 14	44	0.63	446	1.00	<0.001
Gonen 2008[27]		6 – 10	20	0.3	1178	1.01	<0.001
Romero 2008[30]		8 – 13	50	0.59	250	1.00	<0.001
Khalil 2009[28]∇		11 – 14	42	0.4	210	1.00	<0.001
	PAPP-A						
Ong 2000[33]		11 – 14	135	0.90	4297	1.05	<0.001
Spencer 2005[37]		11 – 14	64	n.a.	3999	1.00	0.039
Spencer 2007[31]$		11 – 14	44	0.89	446	1.00	0.042
	Doppler						
Plasencia 2007[42]		11 – 14	107	1.19*	5041	1.00	<0.001

** Calculated from log_{10} MoM to MoM*



Appendix (Figure 5) *Forest plot with the detection rates (DR) of the screening tests fixed at 10% false positive rates (FPR) in prediction of PE (PE cases/not specified in the studies) in the first-trimester, with 95% confidence intervals (CI). PE – number of pre-eclampsia cases, SP – study population. $ Delivery <35wk;∇ High-risk population; # Delivery <37wk;* Adapted from ROC curve; ** contact with the author of the study. MC – maternal characteristics – contain combination of characteristics as: maternal age, maternal weight, race, parity, cigarette smoking, family history of PE, conception, medical history, medication during pregnancy*

Chapter 8

Biomarkers of early placental function in type 1 and 2 diabetic pregnancies; relationship to fetal growth

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Submitted

Abstract

Objective To address the relationship between biomarkers of early placental function and fetal growth in pregestational type 1 and type 2 diabetes mellitus (PGDM) pregnancies we investigated the relationship between five first-trimester placental markers (fß-hCG, PAPP-A, ADAM12, PP13, PlGF), fetal nuchal translucency and macrosomia at birth.

Methods Marker concentrations were measured in first-trimester maternal serum of 178 PGDM and 186 control pregnancies. All concentrations were expressed as multiples of the median (MoM) and compared using Mann-Whitney *U* tests. Where applicable, the median MoMs of PGDM and control pregnancies were compared in relation to birthweight centiles (>90th centile – *non-macrosomic* vs. >90th centile – *macrosomic*).

Results In the PGDM group median PAPP-A (0.93; *P*=0.056) and ADAM12 MoMs (0.88; *P*=0.007) were lower than in the controls. Subgroup analyses showed that median MoMs of PAPP-A (0.65), ADAM12 (0.85), PP13 (0.81) and PlGF (0.91) were only reduced in the PGDM non-macrosomic birthweight subgroup (n=93) compared to other weight subgroups. In the PGDM macrosomic birthweight subgroup (n=69) MoMs of all markers were comparable to the control birthweight subgroups. The screening performance for macrosomia at birth in the PGDM group provided a detection rate of 30% for a 5% false positive rate (FPR) and 43% for 10% FPR.

Conclusions Fetal birthweight in PGDM offspring is partially determined by placental development during the first-trimester of pregnancy. The present increase in fetal macrosomia may be related to a better early glycemic control and placentation. Macrosomia at birth in PGDM pregnancies may be predicted by normal levels of PAPP-A, ADAM12, PP13 and PlGF already in the first-trimester of pregnancy.

Introduction

Pregestational type 1 and type 2 diabetes mellitus (PGDM) in pregnancy is strongly associated with increased fetal morbidity and mortality compared to the general pregnant population[1,2,3]. Diabetes-induced metabolic disorders are thought to interfere with embryonic organogenesis and early placentation and hyperglycaemia is thought to trigger excessive fetal growth[4]. Use of new glucose monitoring techniques, new insulins and new methods of insulin administration have enabled achievement of ever better levels of glycemic control and therefore have improved the outcomes of PGDM pregnancies significantly[5]. However, macrosomia at birth (birthweight above 90th centile) remains markedly high and – surprisingly – even seems to increase over time, with currently 28-34% of infants weighing > 97.7th centile at birth[1,6]. Currently, macrosomia at birth is one of the most concerning complications of a PGDM pregnancy. During labor, macrosomic infants suffer higher rates of shoulder dystocia, brachial plexus injuries and intrapartum asphyxia compared to non-macrosomic infants[1,2,3]. After delivery, these infants are at increased risk for hypoglycaemia, infant respiratory distress syndrome (IRDS), hyperbilirubinaemia, cardiomyopathy and hyperviscosity[2]. Macrosomia at birth predisposes to childhood obesity and to increased morbidity including insulin resistance, hypertension and diabetes[7]. Fetal growth is strongly linked to maternal factors and placental function[8]. Although the association between fetal macrosomia and PGDM in pregnancy is widely recognized, very little is known about the role of placental function in determining macrosomia. In particular, the contribution of early placentation is unknown.

In the Netherlands, the first-trimester combined test, (maternal serum concentrations of Pregnancy Associated Plasma Protein-A (PAPP-A) and the free β subunit of human Chorion Gonadotrophin (fβ-hCG) between 8-14 weeks of the pregnancy, first-trimester ultrasound measurement of the fetal nuchal translucency (NT) and maternal age), is routinely used in screening for aneuploidy. However, other potential markers emerge. Serum levels of biomarkers such as A Desintegrin And Metalloproteinase 12 (ADAM12), Placental Protein 13 (PP13) and Placental Growth Factor (PlGF) have been associated with placental growth and development[9-11]. Altered levels of these markers have been reported to be associated with fetal growth restriction and maternal preeclampsia[9-12]. Therefore, concentrations of these markers measured in maternal blood at 8-14 weeks of gestation may provide important information about early placental function and its influence on fetal growth in PGDM pregnancies.

To address the potential influence of early placental function on excessive fetal growth in PGDM pregnancies we conducted a study to investigate the relationship between five first-trimester placental markers (fβ-hCG, PAPP-A, ADAM12, PP13 and PlGF), fetal NT and macrosomia at birth.

Methods

Serum samples together with NT measurements were collected at the Dutch National Institute for Public Health and the Environment (RIVM) between 2005 and 2007 as part of the national first-trimester Down syndrome (DS) screening program. Serum samples were collected between 8 and 14 weeks of gestation age (GA) and analysis of serum concentrations f*ß*-hCG and PAPP-A was performed. Fetal NT and crown-rump length (CRL) were measured by accredited sonographers using standardized techniques. The duration of the pregnancies was determined according to either last menstrual period (LMP) or ultrasound dating. For all women taking part in the screening sample date, maternal age, maternal weight, smoking and medication use were recorded by the requestor. Pregnancy outcome, including pregnancy duration, date of birth, pregnancy complications, chromosomal disorders, child gender and weight, were collected after delivery.

From this cohort, serum samples from women with PGDM were selected and retrieved from storage. Women were classified as having PGDM if they had been registered as using insulin in the first-trimester of pregnancy. In current first-trimester DS screening there is no distinction between pregestational type 1 or type 2 diabetes. One control serum from an uncomplicated singleton pregnancy was matched to each PGDM case, for the same day of GA at sampling (±1 week), maternal weight (±5 kg), maternal age (±1 year) and for sample date (±6 months).

Serum concentrations of PP13, PlGF and ADAM12 were measured using an automated time resolved fluorescence assay (autoDELFIA or DELFIA Xpress; PerkinElmer, Turku, Finland).

Serum marker levels were expressed as multiples of the gestation-specific normal median (MoMs). Normal medians were obtained by regression analysis of the median concentration for each completed gestational week in the controls, weighted for the number of women tested. NT was expressed as multiple of the CRL-specific normal median (MoM) in the same way. When MoMs were significantly correlated with maternal weight (f*ß*-hCG, PAPP-A, ADAM12), the observed MoM value was divided by the expected value for the maternal weight based on regression analysis in the controls. When MoMs were significantly different between smoking and non-smoking women a correction factor for smoking was applied (PlGF). Median MoMs in cases and controls were calculated and statistically compared using a Mann-Whitney *U* test (2-tailed). Correlation coefficients for all markers were calculated using the $\log_{10}$ concentrations and gestation, after excluding outliers exceeding 3 standard deviations from the median. The correlations between marker concentrations and birthweight centiles were assessed by Pearson correlation coefficients. Statistical analyses were performed using SPSS software (release 17.0; Chicago, IL). Differences with *P*-values < 0.05 were considered statistically significant.

Growth charts corrected for gestational age, sex and parity according to the Dutch Perinatal Registry were used to calculate the birthweight z-scores (http://www.perinatreg.nl;[13]). Weight for GA at the 50th centile was used as the mean of the population and the average standard deviation (SD) calculated by the formula [-1SD + 1SD]/2 was used. Subsequently, the z-score was converted into an exact centile for each studied subject with a z-score to centile web calculator (http://www.measuringusability.com).

Macrosomia was defined as birthweight above the 90th centile[13]. PGDM and control groups were divided

in non-macrosomic (control non-macrosomic; PGDM non-macrosomic) and macrosomic (control macrosomic; PGDM macrosomic). In next step, entire PGDM and control group were divided according to four centile subgroups: <10th, 10-49th, 50-90th and >90th.

Model predicted detection rates (DR) for fixed false-positive rates (FPR) were obtained for each marker and different combinations of markers by numerical integration[14]. This assumes multivariate log Gaussian distributions to fit both macrosomic and non-macrosomic birthweight in PGDM pregnancies. The theoretical range of MoMs was then divided into a number of equal sections and thus forming a 'grid' in multi-dimensional space. The Gaussian distributions were used to calculate for each section (square for two markers, cube for three etc.) the proportion of macrosomic and non-macrosomic birthweights in PGDM pregnancies in the section and the likelihood ratio (LR) between them. The appropriate centiles of LR (90th and 95th centiles, respectively) of the non-macrosomic distribution were determined and the proportion of infants with a birthweight above these centiles was the predicted DR. Data were analyzed using the statistical software package SAS (SAS Institute, Cary, NC, USA).

Results

Serum from 186 PGDM and 186 control pregnancies was selected for analysis. Eight samples from the PGDM group were excluded because there was not enough serum for analysis.

The baseline characteristics of the study population are shown in Table 1. Infants from control and PGDM group had median birthweights of 3600 g and 3520 g, respectively. Median birthweight centiles were significantly higher in the PGDM group than in the control group (89th and 72nd centile respectively; $P < 0.0001$) and significantly more PGDM infants were macrosomic (42.6% and 18.3%, respectively; $P < 0.0001$), concomitant with a shorter GA at delivery (264 days vs. 280, PGDM and control group respectively; $P < 0.0001$).

The median ADAM12 MoM was significantly lower (0.88; $P = 0.007$) in the PGDM group compared to control group and for PAPP-A differences were almost significant (0.93; $P = 0.056$; Table 2). Median MoMs of fß-hCG, PP13, PlGF and NT did not differ between PGDM and control groups.

In the PGDM group the PAPP-A and PlGF MoMs were significantly correlated with the birthweight centiles (r=0.286, $P < 0.0001$ and r=0.158, $P = 0.046$, respectively). There was no significant correlation for the other markers. In the control group there were no significant correlations between any of the markers and birthweight centiles.

Table –1. Study population baseline characteristics in control and PGDM pregnancies

	Controls	PGDM	P value*
N†	**186**	**178**	
Maternal age at test (years)‡	34.6 (22-42)	34.5 (22-43)	0.790
Maternal weight (kg)‡	76.0 (49-135)	76.0 (46-144)	0.765
Smoking (%)	13 (7)	24 (13.4)	0.041*
GA at sampling (days)‡	82 (59-97)	82 (57-97)	0.906
Post-partum information available (%)	186 (100)	162 (91)	0.530
NT measurement available (%)§	120 (64.5)	119 (66.8)	0.831
Sex (%)			
male	93 (50)	84 (51.9)	0.690
female	93 (50)	78 (48.1)	0.690
GA at birth (days)‡	280 (235-294)	264 (199-290)	<0.0001*
Birthweight (g)‡	3600 (2140-5000)	3520 (755-5585)	0.03*
Birthweight centile ‡	72 (1-100)	89 (1-100)	<0.0001*
Non-macrosomic weight (%)	152 (81.7)	93 (57.4)	<0.0001*
Macrosomic weight (%)	34 (18.3)	69 (42.6)	<0.0001*

A Pearson's chi test and a Mann-Whitney U Test were used for statistical analysis.
** P < 0.05 compared to controls.*
†Each case was separately matched to one control sample. Eight samples from the PGDM group were excluded since there was not enough serum for analysis.
‡Values are presented as median (range).
§Data on the NT measurement was not always available to our laboratory, because some applicants performed a combined risk calculation on-site.

Tabel – 2. Median MoMs of all first-trimester markers in control and PGDM pregnancies

	Median MoM		
	Controls	**PGDM**	**P value***
N	186	178	
fβ-hCG	0.99	0.99	0.763
PAPP-A	0.98	0.93	0.056
ADAM12	1.00	0.88	0.007*
PP13	1.00	0.97	0.156
PlGF	1.00	0.95	0.785
N	120	119	
NT	1.00	0.99	0.970

** P < 0.05*

Table 3 shows the median MoMs for all markers according to macrosomia at birth in both control and PGDM groups. Median MoMs were on average lower in the PGDM non-macrosomic group compared to all other groups and median MoM of PAPP-A and ADAM12 in this group were significantly lower as compared to the other groups (Figure 1).

Table – 3: Median MoMs of all markers according to macrosomia at birth in control and PGDM pregnancies

	Median MoM			
	Control non-macro	**Control macro**	**PGDM non-macro**	**PGDM macro**
N	152	34	93	69
fβ-hCG	0.98	1.03	0.96	1.06
PAPP-A	0.98	0.93	0.65	1.07
ADAM12	0.99	1.04	0.85	1.03
PP13	0.97	1.04	0.81	1.01
PlGF	1.04	1.00	0.91	1.03
N	100	20	67	52
NT	0.97	1.03	0.98	1.01

Control non-macrosomic birthweight (Control non-macro), Control macrosomic birthweight (Control macro), PGDM non-macrosomic birthweight (PGDM non-macro), PGDM macrosomic birthweight (PGDM macro).

For PP13, only the difference between the PGDM non-macrosomic and control non-macrosomic groups was not significant ($P = 0.082$). The median MoM of PlGF in the PGDM non-macrosomic group was also lower compared to all other groups. However, the differences with control non-macrosomic ($P = 0.054$) and control macrosomic ($P = 0.066$) did not reach statistical significance. The median MoMs of f*ß*-hCG and NT were comparable between all birthweight groups.

When birthweight centiles were divided in four subgroups (<10th, 10-49th, 50-90th and >90th) marker values appeared to be only higher in macrosomic PGDM infants, with no trend in the non-macrosomic subgroups. To illustrate the phenomenon present for PAPP-A, ADAM12, PP13 and PlGF, as an example, the data for PAPP-A is shown in Figure 2.

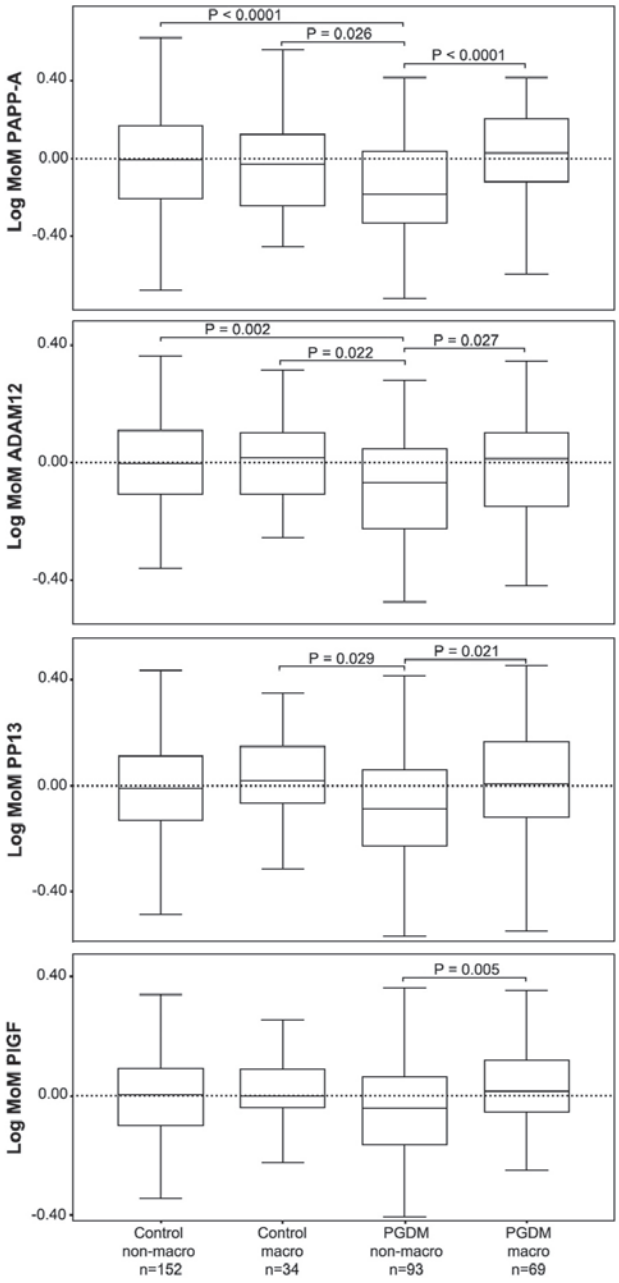

Figure 1: Boxplots showing the differences between non-macrosomic and macrosomic infants of control and PGDM pregnancies in the log MoMs of PAPP-A, ADAM12, PP13 and PlGF. Plotted are the median, quartiles and minimum/maximum values. Control non-macrosomic birthweight (Control non-macro), Control macrosomic birthweight (Control macro), PGDM non-macrosomic birthweight (PGDM non-macro), PGDM macrosomic birthweight (PGDM macro). Groups were compared with Mann-Whitney U tests. P values are indicated in the figures

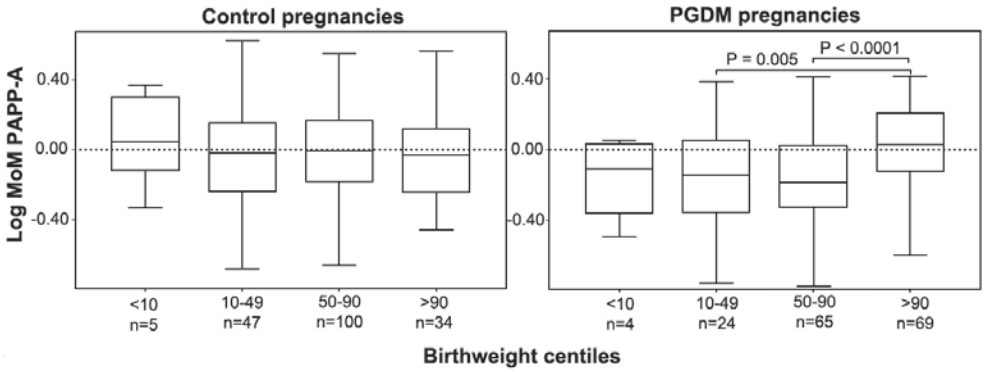

Figure 2: Boxplots showing the differences in the log MoM values of PAPP-A between four birthweight centile subgroups (<10th, 10-49th, 50-90th and > 90th centile) of control and PGDM pregnancies by plotting the median, quartiles and minimum/maximum values. Subgroups were compared with Mann-Whitney U tests. P values are indicated in the figure

The screening performance for macrosomia at birth in the PGDM group with four different serum markers, individually and in combination, is shown in Table 4. The combination of four markers together provided a DR for macrosomia in the PGDM group of 30% for a 5% FPR and a DR of 43% for a 10% FPR.

Table 4: Detection rate for 5% and 10% false-positive rate (FPR) for the prediction of macrosomic birthweight in PGDM pregnancies and with confidence intervals (CI 95%)

First-trimester markers	Detection rate	
	5 % FPR (CI 95%)	10% FPR (CI 95%)
PAPP-A	18 (10-28)	30 (21-42)
ADAM12	15 (8-25)	23 (15-34)
PP13	9 (4-18)	17 (10-28)
PlGF	7 (2-14)	14 (8-25)
PAPP-A and ADAM12	22 (14-33)	34 (23-45)
PAPP-A and PP13	18 (10-28)	30 (21-42)
PAPP-A and PlGF	25 (16-36)	38 (27-49)
ADAM12 and PP13	24 (15-34)	34 (23-45)
ADAM12 and PlGF	15 (8-25)	24 (16-36)
PP13 and PlGF	13 (7-23)	23 (15-34)
PAPP-A, ADAM12 and PP13	25 (16-36)	37 (27-49)
PAPP-A, ADAM12 and PlGF	28 (18-39)	40 (30-52)
PAPP-A, PP13 and PlGF	26 (17-37)	38 (27-49)
ADAM12, PP13 and PlGF	19 (11-30)	29 (20-40)
PAPP-A, ADAM12, PP13 and PlGF	30 (21-42)	43 (32-55)

Conclusions

This study showed that the first-trimester placental markers PAPP-A and ADAM12 were reduced in the total PGDM population. Birthweight subgroup analyses showed that levels of PAPP-A, ADAM12, PP13 and PlGF were on average lower only in the non-macrosomic PGDM group. There was no difference in marker concentrations between the general population and the PGDM macrosomic group. This suggests an impaired placentation in the PGDM non-macrosomic group, since reduced levels of PAPP-A, ADAM12, PP13 and PlGF are known to be implicated in the pathogenesis of impaired placentation[9-12]. In other words, this study indicates that normal birthweight in PGDM offspring is related to impaired early placentation and that macrosomia at birth is related to normal placentation. In both instances fetal overgrowth occurs during pregnancy (poor placentation, normal birthweight; normal placentation, increased birthweight), only in case of adequate placental development leading to macrosomia. These chain events may help to explain the weak association observed between indices of glycemic control during pregnancy (i.e. HbA_{1c}) and birthweight[15]. Apparently, events early in pregnancy contribute to eventual birthweight and maternal and fetal hyperglycemia contributes to fetal growth acceleration in all cases in the course of a PGDM pregnancy.

The present increase in macrosomia at birth in infants of PGDM women has been attributed to a decreasing prevalence of microangiopathy, thus enabling a better placentation[1]. It may also be due to a better glycemic control around the conception and in early pregnancy with smaller alterations in many of the parameters of the Insulin Growth Factor (IGF)-axis. The IGF-axis and insulin are linked at different levels and insulin in an important regulator of the IGFs[16]. The IGF-axis is associated with the degree of metabolic control and a negative association between IGF1-axis and HbA_{1c} has been observed[17]. The IGF-axis components are known to be reduced in both type 1 and type 2 diabetes with presumably decreased PAPP-A and ADAM12 concentration[17-19]. PAPP-A and ADAM12 are both part of the IGF-axis. They act as IGF Binding Protein (IGFBP)-proteases, which increase the activity of IGF through cleavage of inhibitory IGFBPs[17,20,21]. Lower protease activity leads to more inhibitory IGFBPs with subsequent less IGF-axis activity.

Optimal insulin concentrations are needed to support normal concentrations of IGF-axis components including both PAPP-A and ADAM12[17,19,22]. Therefore one could speculate that PGDM mothers of macrosomic infants have a better metabolic control, reflected in concentrations of PAPP-A and ADAM12 that are similar to those of normal pregnancies. This might further be supported by the fact that IGF-axis components appear to be crucial for correct embryonic development and growth[23]. Additionally, children with poorly controlled PGDM have lower concentration of IGF-axis components and are significantly smaller compared to children with adequate metabolic control[17,24].

In addition, while looking at the concentrations of the markers (PAPP-A, ADAM12, PP13 and PlGF) in the PGDM group, there seems to be a significant clear cut-off increase above 90th centile in the concentration of these markers in macrosomic infants (Figure 2). This finding makes these markers promising predictors of macrosomia at birth. The use of the first-trimester screening markers PAPP-A, ADAM12, PP13 and PlGF as predictors for macrosomia at birth provides a DR of 43% for a fixed 10% FPR. Two remaining

markers: f*ß*-hCG and the fetal NT appeared neither altered in PGDM pregnancies nor related to any of the birthweight groups. This is in agreement with other studies, in which no association between NT or f*ß*-hCG and diabetes, or fetal growth has been found[25]. None of the maternal characteristics such as weight or age were correlated to the macrosomia at birth in PGDM group; therefore they were not included in the prediction model.

A limitation of our study is the lack of distinction between type 1 and type 2 PGDM pregnancies. Coding only included the use of insulin during the first-trimester of pregnancy. However, at present fetal macrosomia occurs in a similar high percentage in both entities[1,3,5]. We also lacked data on placental size and histology at birth. Furthermore, in the PGDM group there were significantly more smoking pregnant women compared to the control group. It is broadly known, that smoking may influence the development and function of the placenta. Where relevant, we corrected the markers for smoking factor (PlGF). However, because of the relatively small number of smokers in both groups and lack of placenta specific information in current data, we were unable to further investigate this particular influence. Therefore, distinction between type 1 and type 2 diabetes, histological information on placental size and influence of smoking may be relevant material for future studies.

In conclusion, fetal growth and birthweight in PGDM pregnancies is partly related to markers of early placentation. The present increase in fetal macrosomia might well be related to a better glycemic control around conception and during the first trimester of pregnancy and to a better early placentation. Macrosomia at birth in PGDM pregnancies may be predicted by normal levels of PAPP-A, ADAM12, PP13 and PlGF already in the first-trimester of pregnancy.

Acknowledgements

We thank Mr. I. Belmouden and Mr M. Jonker for their excellent technical assistance at the RIVM. Moreover we thank all the participating Dutch hospitals for their willingness to complete the data in this study; Haga Ziekenhuizen, Den Haag; Medisch Centrum Haaglanden, Den Haag; Onze Lieve Vrouwe Gasthuis, Amsterdam; Leids Universitair Medisch Centrum, Leiden.

No potential conflicts of interest relevant to this article were reported. No external funding was required for this study.

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Part III:

The physiology of all these markers

Chapter 9

Longitudinal trends in feto-placental biochemical markers, uterine artery Doppler flow velocities and maternal blood pressure during the first-trimester of pregnancy

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Abstract

Objective To assess the developmental trends of biochemical markers, uterine artery Doppler flow velocities and maternal blood pressure (BP) and to study interrelationships in uncomplicated first-trimester pregnancies.

Methods This study comprised 108 women with singleton pregnancies. Venous blood samples were collected from at approximately 6-7, 8-9, 10-11 and 12-13 weeks. During the same visit BP was measured and ultrasound examination was performed to measure the crown-rump length (CRL) and Doppler flow velocity waveform patterns of both uterine arteries (Ut-A). Serum concentrations of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF were measured in thawed specimens using a (automated) time resolved fluorescence assay (autoDELFIA and DELFIA Xpress; PerkinElmer, Turku, Finland). Summary curves were created to describe normal ranges and trends with time. A linear mixed model was used with the log-transformed marker values as dependent variable. This allowed for flexible modelling of patterns over time.

Results 68 pregnancies had an uneventful course, with the birth of an appropriate-for-dates infant. In these pregnancies serum PAPP-A, ADAM12, PP13 and PlGF increased with gestational age. The Ut-A Doppler Pulsitility Index (PI) decreased and the mean arterial BP remained constant. There were no meaningful correlations between maternal age, birthweight percentile, gender and blood pressure and any of the biochemical markers. The serum markers were highly correlated except for fβ-hCG. A negative correlation was found between the biomarkers and the Ut-A Doppler PI, especially from 10 weeks onwards. Serum concentrations of ADAM12 and PP13 were reduced in a small-for-gestational (SGA) age subgroup born at term (n= 6, *P*=0.031, and *P*=0,054 (NS), respectively), whereas the Ut-A Doppler PI was significantly increased (*P*=0.02).

Conclusion There is a relation between biochemical markers of early placentation and downstream resistance to flow in the uterine arteries in low risk uncomplicated pregnancies, indicating differences in placentation. In a small series of SGA infants born at term we could demonstrate differences as compared to the normal pregnancies, with potential value for screening.

Introduction

At present, most countries perform serum screening for Down syndrome with the first-trimester combined test (maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A) and the free β subunit of human chorion gonadotropin (fβ-hCG) between 8-14 weeks of the pregnancy, first-trimester ultrasound measurement of the fetal nuchal translucency and maternal age).
Aside from these markers, other potential serum markers emerge and the scope of first-trimester screening is widening to the identification of other fetal aneuploidies and to pregnancies at risk for preeclampsia, fetal growth restriction and/or preterm delivery. PAPP-A has been suggested to be an indicator of increased risk for pregnancy complications[1] (and the same holds for serum levels of biomarkers such as A Desintegrin And Metalloprotease 12 (ADAM12), Placental Protein 13 (PP13) and Placental Growth Factor (PlGF) all have been associated with placental growth and fetal development[2-5]. Similarly, maternal cardiovascular factors and adaptation in early pregnancy have been found to be related to early intra-uterine growth restriction and preeclampsia. This holds for both blood pressure and Doppler velocity waveform patterns of the uterine artery, indicative of down stream resistance[6-8].

Relationships between these early markers during early pregnancy have poorly been studied and normal ranges and trends throughout the first-trimester are lacking for some of the most recent ones. It was the aim of this longitudinal study to assess the developmental trends of these markers in early pregnancy and to study interrelationships in uncomplicated pregnancies. Furthermore preliminary findings in complicated pregnancies will be presented.

Methods

Study design

This longitudinal observational study comprised 108 healthy low risk women with singleton pregnancies. Estimation of the gestational age (GA) was based on the first day of the last menstrual period (LMP) or on ultrasound measurement of the crown-rump length (CRL) (in case of an irregular cycle, or difference between age determined by LMP and CRL > 7 days).

Venous blood samples were collected at approximately 6-7, 8-9, 10-11 and 12-13 weeks. During the same visit an ultrasound examination was performed to measure the CRL and Doppler flow velocity waveform patterns of both uterine arteries (PI). The transducer was placed in the lower lateral quadrant of the abdomen. A sagittal section of the uterus was obtained and the cervical canal and cervical os identified. The transducer was angled to the side and colour Doppler was used to identify each artery along the side of the cervix and uterus. For this study the lowest PI measured was used since this appears to perform best as a screening tool for subsequent preeclampsia[9]. Blood pressure in both arms was measured according the protocol of the British Hypertension Society with a Maxi Stabil (Welch Allyn, Benelux) aneroid device[10], the mean arterial pressure (MAP) was defined as DP + 1/3(SP-DP). Weight and length

were measured and maternal history was documented. Data on pregnancy outcomes (chromosomal disorders, date of birth, birthweight, pregnancy complications and gender) were collected through self-reporting of the participating women using a questionnaire and verified through the medical record or by telephone call. All pregnant women signed an Informed Consent and the project was approved by the Scientific Ethics Committee of the University Medical Center, Utrecht the Netherlands.

In retrospect patients were categorized in five subgroups: (1.) women with uncomplicated pregnancies who delivered an appropriate for gestational age infant (controls; n = 68), (2.) patients who delivered a large for gestational age infant (LGA; n = 12) (3.) patients who developed gestational diabetes (GD; n = 7), (4.) patients who delivered a small-for-gestational age infant (SGA; n = 6), and (5.) patients who had a missed abortion (MA; n = 8). The categories 2-5 were initially included as healthy controls, but excluded from that group because of complications. Seven additional cases were excluded because of other complications e.g. preterm delivery or a genetic anomaly. Only 68 had a complete uneventful course and outcome of pregnancy.

LGA was defined as birthweight above the 90th percentile and SGA was defined as birthweight below the 10th percentile (http://www.perinatreg.nl;[11]). GD was diagnosed using a 50 g glucose screening, followed by a 75 g Oral Glucose Tolerance Test in screen positive women. A missed abortion was defined as an intra-uterine pregnancy with embryonic heart beat at the first ultrasound scan, but with cessation of activity later in the first-trimester.

Sample collection and immunoassays

Blood samples were centrifuged and serum was aliquoted before storage at -80ºC. Serum concentrations of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF were measured in thawed specimens using a (automated) time resolved fluorescence assay (autoDELFIA and DELFIA Xpress; PerkinElmer, Turku, Finland). Prior to analysis extensive validation was performed for all assays, mean intra- and inter-assay CV for the assays were below 5% at all levels. The lowest standard concentration of the assays were 9,4 mIU/l for PAPP-A, 2,1 ng/ml for fβ-hCG, 6 ng/ml for ADAM12 (analytical sensitivity), 7 pg/ml for PlGF (limit of quantification) and the limit of detection for PP13 was 3.8 pg/ml.

Statistical analysis

Analyses were performed to assess the change in serum concentrations of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF over time. Summary curves were created to describe the longitudinal changes in serum concentrations, uterine artery Doppler flow velocities and maternal blood pressure. A linear mixed model was used with the log-transformed marker values as dependent variable. The average pattern of the log(marker) values as a function of gestational age (in days) was represented by a restricted cubic spline function with 4 knots (at the P5, P35, P65 and P95 values of the marker). This allows for very flexible modelling of patterns over time. The between-patients variation around this average pattern

was modelled through a random effect on the intercept.
At each visit, correlation coefficients between all markers were calculated using the $\log_{10}$ concentrations, after excluding outliers exceeding three standard deviations from the median.
The correlations between the markers were assessed by Pearson correlation coefficients. Statistical analyses were performed using SPSS software (release 17.0; Chicago, IL) and the R-program. Differences with *P*-values < 0.05 were considered statistically significant.

Growth charts corrected for gestational age, sex and parity according to the Dutch Perinatal Registry were used to calculate the birthweight z-scores (http://www.perinatreg.nl). Weight for GA at the 50th percentile was used as the mean of the population and the average standard deviation (SD) calculated by the formula [-1SD + 1SD]/2 was used. Subsequently, the z-score was converted into an exact percentile for each studied subject with a z-score to percentile web calculator (http://www.measuringusability.com).

Results

The demographic and clinical characteristics of the study population are shown in table 1. GD pregnancies had a higher BMI (*P*=0.002), and MAP (*P*=0.0007) compared to controls. Furthermore, birthweight percentiles were higher in the GD-and Macro-group and lower in the SGA-group compared to the normal pregnancies (*P* =0.01, *P* <0.0001 and *P*<0.0001, respectively).

Table 1: Demographic and clinical characteristics of the study groups (N=108) with medians and (min-max)

	Normal (n=68)	**LGA (n=12)**	**GD (n=7)**	**SGA (n=6)**	**MA (n=8)**
maternal age	33.8 (19.8-42.7)	32.3 (28.1-37.0)	32.5 (25.7-38.4)	33.3 (22.4-36.1)	34.8 (32.7-40.4)
gestational age at sample	68 (40-100)	69 (42-93)	69 (44-94)	67 (43-88)	48 (42-53)
length	173 (155-184)	176 (162-183)	172 (152-175)	169 (165-187)	175 (174-175)
maternal weight	66 (50-110)	72 (63-95) *	82 (60-110) *	75 (57-120)	71 (61-80)
BMI	23 (19-35)	23 (21-31)	28 (22-40) *	25 (20-43)	20 (20-20)
birthweight	3445 (2795-4250)	4290 (3890-4900) *	3490 (3030-4185)	2943 (2740-3030) *	
birthweight centiles	47 (11-90)	95 (91-100) *	82 (20-97) *	8 (20-97) *	
gestational age at delivery	280 (260-297)	281 (268-288)	274 (251-284)	278 (276-283)	
nulliparity. n (%)	31 (46)	3 (25)	4 (57)	2 (33)	3 (38)
Smoke. n (%)	1 (1)	0	1 (14)	2 (33)	0
male/female. n/n	40/28	6/6	6/1	3/3	

LGA; large for gestational age > P90, GD; gestational diabetes, SGA; small for gestational age < P10, MA; missed abortion. Data expressed as medians (min-max or percentage)
** Statistically significant compared with normal pregnancies*

Among patients with a normal pregnancy outcome, serum PAPP-A, ADAM12, PP13 and PlGF increased with gestational age in the course of the first-trimester. Serum fβ-hCG concentration followed a bell-shaped curve (Figure 1). The lowest-PI decreased during the first-trimester and the MAP stayed constant. In women with a higher BMI the concentrations of PAPP-A and ADAM12 were significantly lower (P=0.022 and P=0.036, respectively). Furthermore, multiparous women had significantly higher PlGF concentration (P< 0.0001). While the PAPP-A and fβ-hCG assays are known to be sensitive and reliably measured as early as 6 weeks GA, for ADAM12, PP13 and especially PlGF, concentrations were generally lower than the lowest standard concentration before week 8 (ADAM12 and PP13) or week 10 (PlGF).

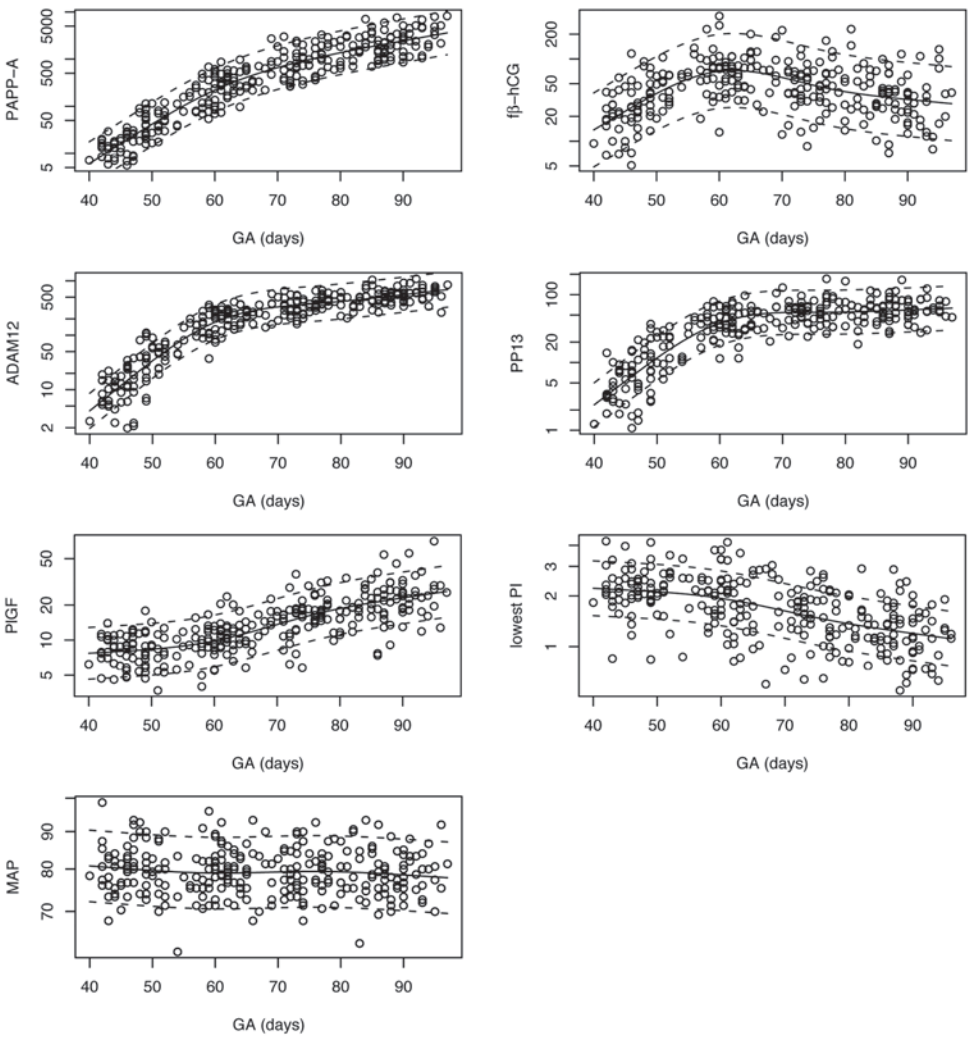

Figure 1: Maternal serum concentration of PAPP-A, fβ-hCG, ADAM12, PP13, PlGF, lowest-PI and MAP in 68 physiological pregnancies. The solid line represents the pattern over time of the mean serum concentration of the markers and the dotted lines the limits of the between-patients variation around the mean pattern

Correlations were studied for maternal age, MAP, BMI, Ut-A Doppler velocity waveform pattern and the biochemical markers for all sampling periods. Statistically significant correlations were considered meaningful. There were no meaningful correlations between maternal age, exact birthweight percentile, gender and blood pressure and any of the biochemical markers. The Pearson correlation coefficients between the serum markers in each gestational age window 6-7, 8-9, 10-11 or 12-13 weeks are shown for all normal pregnancies in figure 2. The significant correlations for each visit are marked by * . The serum markers were highly correlated except for fβ-hCG. A negative correlation was found between the biomarkers and the Ut-A Doppler flow, especially from 10 weeks onward (Table 2).

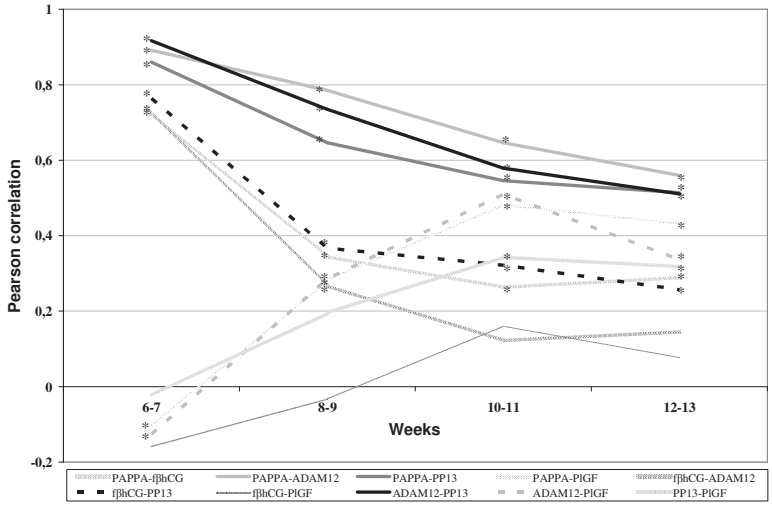

*Figure 2: Correlations, Pearson's rho between the five biochemical markers. Significant correlations are marked by * in different gestational age windows, 6-7, 8-9, 10-11, 12-13 weeks*

*Table 2: Correlations, Pearson's rho between serum markers and uterine artery Doppler flow velocity waveform patterns (lowest-PI) in different gestational age windows, 6-7, 8-9, 10-11, 12-13 weeks Significant correlations for each visit are marked by **

Gestational age in weeks	6-7	8-9	10-11	12-13
PAPP-A	0.053	-0.332*	-0.244*	-0.292*
fβ-hCG	-0.19	0.07	0.068	0.15
ADAM12	-0.034	-0.296*	-0.408*	-0.202
PP13	-0.039	-0.236	-0.366*	-0.34*
PlGF	0.013	-0.168	-0.252*	-0.263*

Figure 3 shows preliminary data of patients who had a SGA or LGA infant. Serum concentrations of ADAM12 and PP13 were reduced in the SGA-group, (*P*=0.031, and P=0,054 (NS), respectively). In contrast, the Ut-A Doppler PI was significantly increased in the SGA group (*P*=0.02). Data from the LGA group did not differ from controls. In patients who had a missed abortion PAPP-A, fβ-hCG, ADAM12 and PP13 concentrations were significantly reduced during the last visit when there was still a viable fetus present.

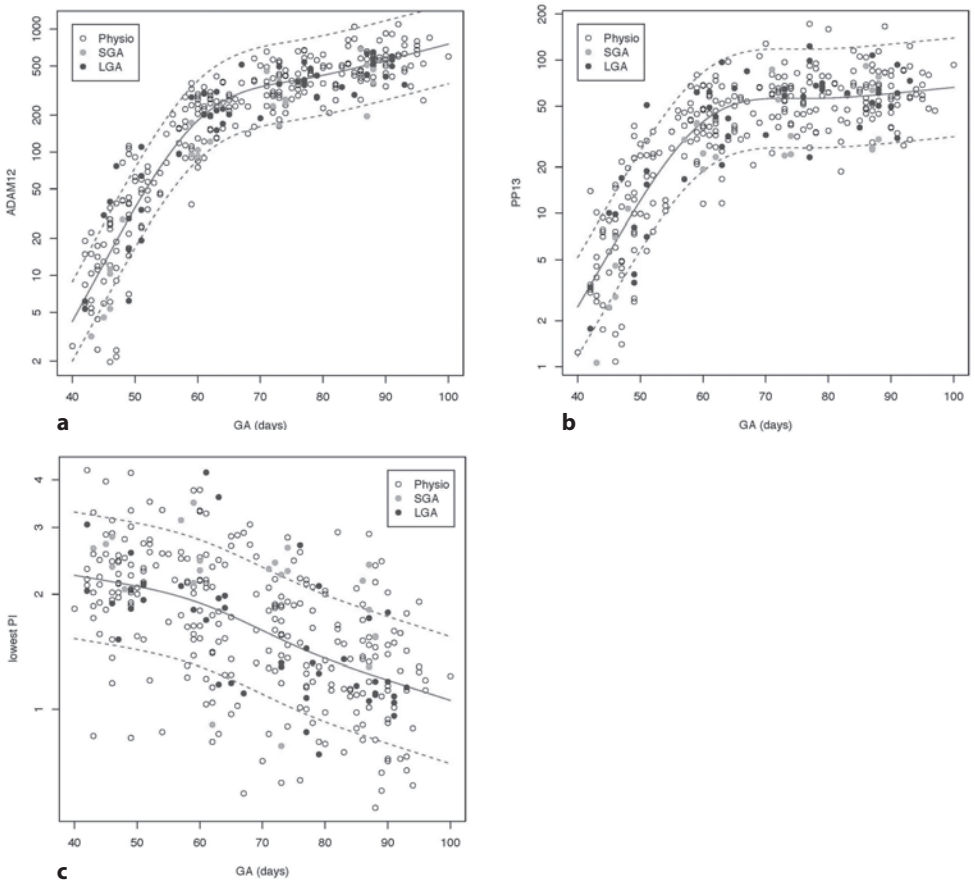

Figure 3: Relationship between ADAM12, PP13 and uterine artery Doppler flow, and SGA and LGA infants, as compared to physiological pregnancies. a) ADAM12, b) PP13 and c) Uterine artery Doppler flow (lowest PI). Black; physiological pregnancies (n=68), green; SGA (n=12) and blue; LGA (n=6)

Discussion

This is the first study that investigates longitudinal changes in concentrations of five serum markers, uterine artery Doppler flow velocities and maternal blood pressure in normal physiological pregnancies during the first-trimester. Collecting serial samples of blood from the same women during early

pregnancy is normally difficult, but we have achieved this in a group of healthy low risk women who became pregnant spontaneously. Of the 108 women included, only 68 had a complete uneventful course and outcome of pregnancy, although it is questionable if SGA and LGA should have been excluded.
The longitudinal serum changes of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF were described using a mixed model (Figure 1). This is a fundamentally different way of calculating the relation between GA and serum concentrations, as compared with the classical one, using hundreds or thousands of unrelated serum concentrations for various GA.

The spread in the range of distribution of the serum markers can be explained by many factors such as BMI: when BMI increased the concentrations of PAPP-A and ADAM12 decreased significantly (P=0.022 and P=0.036, respectively). Furthermore, parity could be an explanatory factor for the range of distribution for PlGF, since in multiparous women a significantly higher PlGF concentration was found (P< 0.0001). Another factor is the assay, for PAPP-A and fβ-hCG assays are known to be very sensitive. In combination with the fact that fβ-hCG and PAPP-A are present in the ng/ml range even early in the first-trimester, they can be measured as early as 6-7 weeks of GA. Performing serial measurements early in the first-trimester also provides an indication as to when a marker can be measured reliably. ADAM12 and PP13 are present in serum in the pg/ml range, but can be measured quite reliably early in the first-trimester, as most concentrations are above the limit of detection or quantification. However, PlGF cannot be measured reliably until at least 10 weeks.

We found that Ut-A PI fell with gestation, which is in agreement with previous research [12-14]. BP is also known to decrease in the first half of gestation[8,15,16], but differences between 7 and 12 weeks were not significant is this population. In our population there was a negative correlation between the biomarkers and the Ut-A Doppler flow velocity waveform patterns, especially after 10 weeks of gestation. PlGF is a member of the angiogenic vascular endothelial growth factor family, and produced predominantly by trophoblast cells it is able to cause endothelial cell proliferation, migration and activation[17,18]. Impaired trophoblast invasion leads to insufficient vascular remodelling of the spiral arteries, therefore, a negative correlation between Ut-A Doppler and this biomarker seems physiologically plausible. The biomarkers ADAM12, PP13 and PlGF are also known to be related to early placentation[2-5]. So remarkably, there is a relation between biochemical markers of early placentation and downstream resistance to flow in the uterine arteries in low risk uncomplicated pregnancies, indicating differences in placentation, even within the "physiological" range. The correlations between the biochemical markers have been described before[5,19-21], but not to this extent and not for separate gestational weeks

The serum markers we investigated are considered to be involved in early placentation and reduced serum concentrations presumably reflect impaired placentation[2-4,18,22,23]. In our study most marker concentrations were significantly reduced among the patients who had a subsequent missed

abortion. Furthermore, we found that ADAM12 was significantly reduced in the SGA group with an almost significant reduction for PP13. ADAM12 is an IGFBP-protease, with specificity for IGFBP3 and 5[23]. It is thought to be of importance in fetal growth, just as PAPP-A[24]. Low levels of these markers may explain the placenta-related complications such as miscarriage and intra-uterine growth restriction[24,25]. PP13 is predominantly produced by the syncytiotrophoblast. It is thought to play a major role in the implantation of the blastocyst. Moreover, it is possibly involved in the remodelling of the common feto-maternal blood-spaces through binding to proteins between placenta and endometrium[26,27]. In PE and pregnancies complicated by intra-uterine growth restriction first-trimester concentrations of PP13 have been found to be significantly reduced[5,28].

The principle aim of this study was to provide a baseline of normal values for future investigation and risk assessment. The search for new potential markers in maternal serum that are capable of detecting the presence of pathologic conditions early in pregnancy with a high sensitivity and specificity is still ongoing. Early screening for fetal and maternal health would provide an early reassurance and a low frequency antenatal visit schedule for women at low-risk, whereas for those at increased risk an intensified antenatal surveillance is advisable. Even in the small number of series of pregnancies resulting in birth of a SGA infant at term we could demonstrate differences from controls, which shows the potential value of these markers for screening. This finding is the more so interesting, since all 6 SGA infants were born at term without serious perinatal complications. Presently, we are expanding the series of pathological pregnancies. Ultimately it has to be established if serial first-trimester measurements constitute a better screening method than one single measurement.

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Chapter 10

Summary and General discussion

Summary and general discussion

The early detection of "hereditary" diseases can reduce and prevent suffering. It can offer people choices in situations where previously their fate was preordained. The aim of early disease detection is simple. Primary prevention seeks to abolish disease by protecting the individual and the population from attack before the challenge has been made. Early detection aims at discovering and curing conditions which have already produced pathological change but which have not so far reached a stage at which medical aid is sought spontaneously. Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment. ***Public health paper nr 34 1968, JMG Wilson, G Jungner principle and practice for disease.***

In this thesis we aimed to answer the following questions:

1. How can we improve the performance of Down syndrome screening in the Netherlands?
2. Can we screen for more than just Down syndrome? Should the screening test for Down syndrome be adapted to detect other pregnancy complications in early first-trimester?
3. And, if so, which biochemical and sonographic markers should be applied and what is their physiology?

To answer these questions we first made an inventory of the past and current status of Down syndrome (DS) screening in the Netherlands.

In **Chapter 2** the life cycle of the triple test is described. The triple test, which was first introduced in the Netherlands in 1990, may be considered a fairly good second trimester screening test. In 1991-1995, 1996-1999 and 2000-2005, the detection rates for DS were 83.3%, 87.9% and 75% for a false positive rate of 11.3%, 14.4% and 13.3%, respectively. Governmental policy has strongly influenced the offer and uptake of prenatal screening in the Netherlands. Between 1977 and 2003 the Dutch government chose not to regulate DS, as it was politically unacceptable to endorse a program using a test with limited performance and with termination of pregnancy as one of the outcomes. The long term experience with a smaller, not governmentally approved program, improvement of the available tests, and valuable advice of the Dutch Health Council, ultimately led to a solid, governmentally approved screening program with special reference to quality assurance and patient information[1]. Health practitioners had gradually gotten acquainted to the triple test and this has facilitated a fluent transition to first-trimester testing. The triple test served as a preparation for the current formal national screening program for DS that started in January 2007. Moving the test to earlier in pregnancy has apparent advantages, e.g. earlier reassurance for the women and the possibility for earlier termination. For late-bookers there still is the possibility for second trimester screening, provided by one dedicated Dutch laboratory. The first-trimester combined test (maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A) and the free β subunit of human chorion gonadotrophin (fβ-hCG) between 8-14 weeks of the pregnancy, first-trimester ultrasound measurement of the fetal nuchal translucency (NT) and maternal age) is currently the test of choice.

Having established the past and current state of DS screening in the Netherlands we tried to answer the first aim of this thesis in **Chapter 3**. In this study (including 20,293 singleton pregnancies), we described changes in the performance of the first-trimester combined test in the Netherlands over the years, and tried to identify factors involved in a possible improvement. The performance of the first-trimester test has improved over the years, which is reflected in an odds of being affected given a positive result (OAPR) of 1:10 in the period 2004-2006, as compared to 1:14 in the previous period[2]. The overall median weight-corrected MoM values of PAPP-A and fβ-hCG were 1.12 and 1.03, respectively. The median MoM value of NT was 0.89 and increased (= improved) from 0.82 to 0.96 over the years. Sixty-six DS cases were detected by the screening test in this period, which resulted in a detection rate of 75.9%, with a false positive rate of 3.3%. Most of the improvements in screening were due to a better NT measurement[3], but there is still room for further improvements, through adequate training and certification of ultrasonographers. A better setting of the medians for the biochemical parameters may also improve the detection rate. Such measures are necessary since the performance is still lower than that in international prospective studies[4,5].

To further elaborate on aims 1) and 2) of this thesis, other potential serum markers to improve the screening, emerging in international scientific literature, were evaluated. Serum levels of biomarkers such as A Desintegrin And Metalloproteinase 12 (ADAM12), Placental Protein 13 (PP13) and Placental Growth Factor (PlGF) have been associated with placental growth and development[6-8]. Altered levels of these markers have been reported to be associated with chromosomal abnormalities, fetal growth restriction and maternal preeclampsia (PE)[6-10]. ADAM12 was introduced as a promising marker for DS screening, but subsequent data were less promising.
In **Chapter 4** we present our own evaluation of ADAM12 in maternal serum of trisomy 21-, 18- and 13-affected pregnancies. The median ADAM12 was 1.00 MoM in controls, and in the DS cases at 8, 9, 10, 11, 12, 13 weeks it was 0.45, 0.73, 0.74, 0.85, 0.92, 1.06 MoM, respectively. The median for trisomy 18 was 0.85 MoM and for trisomy 13 0.63 MoM. Consequently, the ADAM12 MoM values were clearly reduced early in the first-trimester for all trisomies. Routine screening for additional abnormalities in the near future is foreseeable and ADAM12 could be an additional biochemical marker for first-trimester screening for trisomies other than DS. Recently, (May 2010) a license under the Population Screening Act was issued, allowing screening for trisomy 13 and 18 in the Netherlands.

Once again, can we screen for more than just DS? Therefore, we tried to determine if PP13 could be an additional biochemical marker for screening for trisomies other than DS in the first-trimester (**Chapter 5)**. The PP13 MoM in DS cases was 0.91 ($P = 0.06$). PP13 MoMs were significantly decreased in trisomy 18 (median MoM 0.64; $P<0.0001$) and in trisomy 13 cases (median MoM 0.46; $P<0.0001$). In these trisomies serum levels of PAPP-A and fβ-hCG were also largely decreased which is not necessarily the case in pregnancies complicated by PE. Therefore, addition of PP13 to the current screening test could be valuable to make a proper distinction between normal pregnancies, pregnancies complicated by fetal aneuploidies and those complicated by PE.

So far, this thesis has focused on screening for aneuploidy. However, there is growing evidence that the first-trimester test can also be put to use for a broader screening for foetal and maternal health, including trisomy 13 and 18, PE, preterm birth, growth restriction, fetal death and gestational diabetes. Such a screening offers the possibility of early risk selection and therapeutical options to improve maternal and fetal health. In the second part of this thesis we therefore focused on question 2): screening for other pathologies than DS. As mentioned in the introduction, identification of pregnant women at risk for preeclampsia is still one of the most important challenges in prenatal care, since preeclampsia is a serious complication of pregnancy affecting approximately 1-2% of all pregnant women. Identification of women at risk, as early as the first-trimester of pregnancy, would enable intensified antenatal surveillance, timely intervention and hopefully better outcomes in those at high risk, and less intensified antenatal care and additional testing in those at low risk. Current therapeutic possibilities are limited, but low dose aspirin may reduce the incidence of early onset PE.

As a first step, in **Chapter 6** we investigated the predictive value of maternal serum PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF, for first-trimester identification of early-onset PE. PP13 and PlGF were reduced in early-onset PE cases, with medians of 0.68 MoM and 0.73 MoM, respectively (P<0.0001 for both). PAPP-A was reduced (median 0.82 MoM, P<0.02) whilst ADAM12 and fß-hCG did not differ between controls and PE cases. In PE complicated by a small for gestational age fetus, all markers except fß-hCG had lower values, compared to cases with fetuses with normal weight. The model-predicted PE detection rates for a combination of PP13 and PlGF were 44% and 54%, respectively, for a fixed 5% and 10% false-positive rate. These markers should be combined with other variables such as blood pressure, Doppler flow velocity waveforms of the uterine artery and maternal history, to improve the performance of the screening for early PE.

In **Chapter 7** we provide a systematic review of studies on serum markers and uterine artery Doppler velocity waveform patterns (indicative of downstream vascular resistance) for first-trimester prediction of PE, including our data presented in Chapter 6. Reduced first-trimester levels of PP13, PlGF and PAPP-A appeared to be significantly associated with the development of PE later in pregnancy. However, the screening potential of each individual single serum marker is limited, resulting in modest detection rates. The combination of all biochemical markers yields a DR of approximately 55% for a FPR of 10%. For screening of unselected populations high detection rates are needed, in order not to miss a substantial number of high risk cases. Therefore, screening with serum markers only is unsuitable for clinical practice. Combination of serum markers with maternal constitutional characteristics and/or signs of unsuccessful vascular adaptation to pregnancy yield detection rates of approximately 90% for a FPR of 10% and is, therefore, promising to identify patients at high risk of developing PE. However, large scale prospective studies are required to evaluate the power of this integrated approach in clinical practice.

As described previously serum levels of biomarkers such as ADAM12, PP13 and PlGF have been associated with placental growth and development[6-8]. In **Chapter 8** we describe the relationship between biomarkers of early placental function and fetal growth in pregestational type 1 and type 2 diabetes mellitus (PGDM) pregnancies. In this study we investigated the relationship between six first-trimester markers and the occurrence of fetal macrosomia at birth. Marker concentrations were measured in first-trimester maternal serum of 178 PGDM and 186 control pregnancies. In the PGDM group median PAPP-A (0.93; *P*=0.056) and ADAM12 MoMs (0.88; *P*=0.007) were lower than in the controls. Subgroup analyses showed that median MoMs of PAPP-A (0.65), ADAM12 (0.85), PP13 (0.81) and PlGF (0.91) were only reduced in the PGDM non-macrosomic birthweight subgroup (n=93) compared to other weight subgroups. In the PGDM macrosomic birthweight subgroup (n=69) MoMs of all markers were comparable to the control birthweight subgroups. The screening performance for macrosomia at birth in the PGDM group provided a detection rate of 43% for a 10% false positive rate. Although the association between fetal macrosomia and PGDM in pregnancy is widely recognized, very little is known about the role of early placentation in determining macrosomia. The present increase in fetal macrosomia, as has been found in nationwide studies, might well be related to a better glycemic control around conception and during the first-trimester of pregnancy and subsequently to a better early placentation[11]. Macrosomia at birth in PGDM pregnancies may be predicted by normal levels of PAPP-A, ADAM12, PP13 and PlGF already in the first-trimester of pregnancy.

While the relationship between various markers and their associations with different fetal and maternal disease states is clarified more and more, we increasingly experience a lack of information on the normal, physiological behaviour of these markers, and their various interrelationships. That forced us to go back to the very basic work of investigating the physiological concentrations and values of these markers in early pregnancy. Therefore, we tried to gain more insight into the physiology of these markers in healthy pregnancies (**Chapter 9**), providing a baseline of normal values for future investigations and risk assessment. In a longitudinal study during the first trimester, including 108 women, we found that serum concentrations of PAPP-A, fβ-hCG, ADAM12, PP13 and PlGF increased with gestational age. The pulsatility index of the uterine artery Doppler (PI) decreased during the first-trimester. Furthermore, there was a correlation between all serum markers except for fβ-hCG. A negative correlation was found between the biomarkers and the PI. So, even within a low risk pregnancy group differences in Doppler flow exist, which is related to differences in placental markers, suggestive of delayed or impaired placentation. Serum concentrations of ADAM12 and PP13 were reduced in a small subpopulation of infants ultimately born small for gestational age (SGA) (n=6) (*P*=0.031, and *P*=0.054 (NS), respectively). The PI was significantly increased in the SGA group (*P*=0.02). Also these data indicate that in pregnancies considered initially at low risk, differences in early markers may be indicative of impaired placentation, demonstrating its potential value for screening.

A new view on prenatal screening

Screening for DS starts with the pregnant woman, who wishes to make an informed choice about participating in the screening program or not and wants to be served with an adequate test. Nowadays, this test is the first-trimester combined test. Presently, there is a need to investigate whether the first-trimester combined test for the screening for DS, can be expanded into a test that screens for a whole range of fetal and maternal pathologies, e.g. trisomy 13 and 18, preeclampsia, HELLP syndrome, fetal growth restriction, preterm birth, intra-uterine fetal death, and gestational diabetes. Accurate prediction of these pathologies is crucial for prenatal diagnostic testing, for adequate monitoring of pregnancies at risk for hypertensive and other diseases and for the development of preventive treatment strategies to improve maternal and perinatal outcome. The search for new potential markers in maternal serum that are capable of detecting the presence of pathological conditions early in pregnancy with a high sensitivity and specificity is still a major challenge. As yet we are only at the beginning of a whole new development in antenatal care. Identification of women who are at increased risk or otherwise will result in a subsequent individualisation of their antenatal care.

While screening for DS currently has a low uptake, it may be anticipated that a more generic approach to screening as "screening for fetal and maternal health", may be more preferred by the pregnant women. However, this kind of screening, with fetal and maternal health as a leading motivation, will fundamentally change the organization of prenatal screening. For instance, information given at the first outpatient visit will become even more extensive (and complicated) as it currently is, with a focus on maternal and fetal health and less on aneuploidies. This will require better ways of delivering information to the patient. As some interventions, such as low dose aspirin, are especially effective when started early in pregnancy, considering choices by the pregnant women early in pregnancy may even be too late. We may anticipate a more important role for pre-conceptional information and the Dutch screening organization should be adapted to supply that. We will then facilitate 'a la carte' screening, with pregnant women opting in for e.g. screening for preeclampsia or maternal health, trisomy 13 and 18, and may be choosing not to be informed about the DS risk, as a child with DS might be welcome in their family. As maternal history and maternal health before screening is bound to become a powerful tool in risk assessment, it will become more important to obtain full information about general, obstetric and family history.

At the first visit general practitioners, midwives or gynaecologists have to obtain such information and to document this in an electronic data base. Maternal characteristics, e.g. age, body-mass index and blood pressure will be measured and an ultrasound scan will be carried out to determine vitality, number of fetuses, nuchal translucency thickness and uterine artery Doppler flow velocities. In addition maternal blood will be taken for measurement of potential first-trimester serum markers. A prediction model is necessary to calculate the risk for every individual pregnant woman (Figure 1).

This may only be a starting point for various screening tests, both biochemical, sonographical or other. This new approach in prenatal screening fits very well the original idea of Prof. Kypros Nicolaides (Fetal Medicine Foundation, London), who suggested turning around the frequency of antenatal monitoring

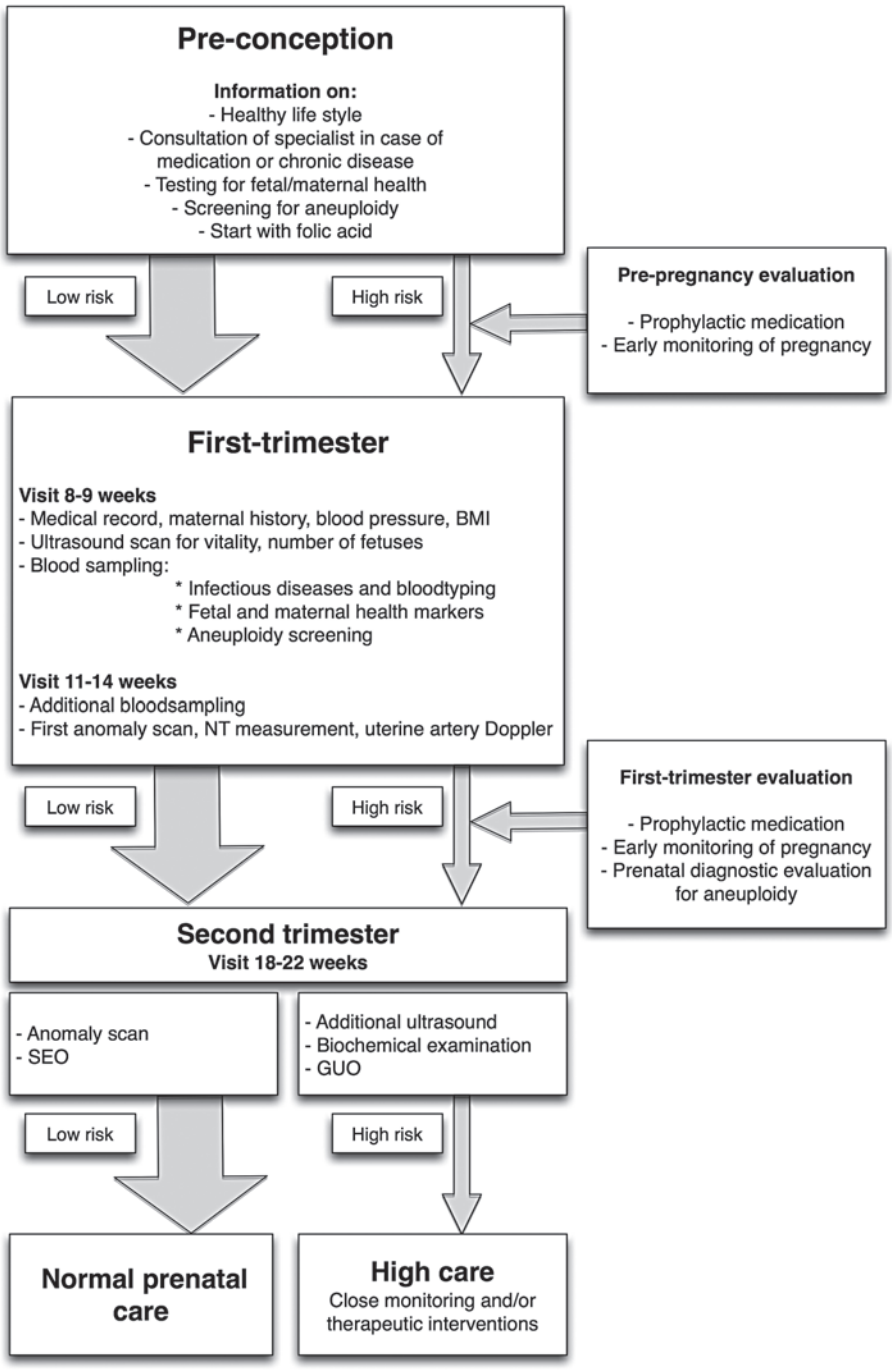

Figure 1: Flowchart prenatal care

('Turning the pyramid upside down'). Instead of more visits towards the end of pregnancy, most of the visits should be carried out before week 12 of pregnancy, with additional tests and monitoring later in pregnancy, only for the population at risk.

This thesis has delivered some of the elements (screening for other pregnancy complications like trisomie 13 and 18, PE, PGDM macrosomia) of such a new screening program. Cost-Effect studies may well show that reduction in the number of visits of the larger -low risk- part of the pregnant population to their health care professional may proof to be quite cost-effective, preserving intensified care to pregnant women who really need this.

In that respect, the words of Wilson and Jungner are still fully applicable;
The early detection of "hereditary" diseases can reduce and prevent suffering. It can offer people choices in situations where previously their fate was preordained. Centralized care of pregnancies at high risk would also lead to better maternal and fetal outcome and is necessary for further prospective longitudinal research.

Such tests will also help in the selection of suitable patients for future Randomized Controlled Trials and investigations for potential preventive medication to be started early in pregnancy. Large prospective longitudinal studies are needed. Further research should focus on combining independent biochemical and sonographic markers for the prediction of these pregnancy complications and to achieve the best predictive models. Further research as to the underlying patho-physiology is required.
In this thesis I have shown that screening in the first-trimester for more than just Down syndrome, will become feasible.

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Chapter 11

Nederlandse samenvatting

Nederlandse samenvatting

De vroegtijdige detectie van "erfelijke" ziekten kan onnodig lijden verminderen en voorkomen. Het biedt mensen keuzes in situaties waar eerder hun lot voorbestemd was. Het doel van de vroegtijdige opsporing van ziekten is eenvoudig. Het doel van primaire preventie is het beschermen van het individu en de populatie voordat een bedreiging heeft plaatsgevonden. Het doel van vroegtijdige opsporing is het ontdekken en behandelen van aandoeningen die al wel aanwezig zijn, maar nog in een zodanig beginstadium dat daarvoor nog geen zorg is gezocht. Patiënten met mogelijke manifestaties van een ziekte moeten verwezen worden naar een arts voor diagnostiek en de nodige behandeling. Public health paper nr 34 1968, JMG Wilson, G Jungner principle and practice for disease.

Wilson en Jungner hebben in 1968 de basisprincipes voor screening opgesteld en ook voor de prenatale screening naar Downsyndroom en andere problematiek in de zwangerschap, waar het in dit proefschrift over gaat, zijn die principes van toepassing.

In dit proefschrift hebben we getracht de volgende vragen te beantwoorden:

1. Hoe kunnen we de prenatale screening van Downsyndroom in Nederland verbeteren?
2. Is het mogelijk prenatale screening naar Downsyndroom uit te breiden met screening naar andere foetale afwijkingen c.q. zwangerschapsziekten?
3. En zo ja, met welke biochemische en echografische merkers kunnen we dat doen en hoe is de fysiologie van deze merkers?

Voorafgaand aan het beantwoorden van deze vragen wordt in dit proefschrift echter eerst een overzicht gegeven van de screening op Downsyndroom (DS) in Nederland in het verleden en de huidige situatie. Gebaseerd op de bevindingen van dit proefschrift wordt tot slot een nieuwe visie op de prenatale screening gegeven, die de zwangere mogelijk beter bedient dan op dit moment.

In **hoofdstuk 2** wordt de "levenscyclus" van de triple test beschreven. De triple test is een voorloper van de combinatietest. Ook deze test is een test die het risico op het krijgen van een kind met het DS berekent, met behulp van een vooraf-kans, gebaseerd op de leeftijd van de moeder en de concentratie van drie eiwitten in het bloed van de moeder, afgenomen in het tweede trimester van de zwangerschap (15-21 weken). De triple test werd in Nederland geïntroduceerd rond 1990, uitgevoerd tot circa 2005 en daarna nog maar sporadisch. In hoofdstuk 2 wordt een overzicht gegeven van de prestaties van deze test door de jaren heen. In 1991-1995, 1996-1999 en 2000-2005 waren de detectiepercentages voor DS 83.3%, 87.9% en 75% bij een fout-positief percentage van respectievelijk 11.3%, 14.4% en 13.3%.
De politieke situatie tussen ca. 1977 en 2003 heeft het aanbod en het gebruik van de prenatale screening in Nederland sterk beïnvloed. Een door de overheid gestuurd landelijk screeningsprogramma kwam toen niet van de grond. De test zou niet goed genoeg zijn en politiek was men niet voor een test waarbij één van de uitkomsten een afbreking van de zwangerschap kon betekenen. De bestaande praktijk (beschreven

in hoofdstuk 2) werd wel gedoogd. Daardoor kregen gynaecologen en verloskundigen in Nederland wel veel ervaring met dit kleinere, niet vanuit de overheid georganiseerde, screeningsprogramma. Dit heeft mogelijk geleid tot een gemakkelijke en vloeiende overgang naar het huidige officiële landelijke programma voor de DS screening. Dat programma kwam er namelijk uiteindelijk wel. Op basis van een advies van de Gezondheidsraad werkte de staatsecretaris van VWS (C. Ross-Van Dorp) in 2004 en 2005 plannen uit voor een dergelijk programma, waarin kwaliteitsborging, voorlichting en geïnformeerde keuze centraal stonden. Als test van keuze werd niet de triple test maar de zgn. eerste trimester combinatietest voorgeschreven. Die is gebaseerd op hetzelfde principe als de triple test. Bij de combinatietest worden de concentraties van twee eiwitten (PAPP-A en fβ-hCG) in het bloed van een zwangere (nu afgenomen tussen 8 en 14 weken zwangerschap) en de echografische meting van de foetale nekplooi (NT) gecombineerd met de leeftijd van de moeder, verwerkt tot een risicoschatting. Een kansbepalende test zo vroeg in de zwangerschap heeft voordelen; zwangeren kunnen eerder gerustgesteld worden en een eventuele zwangerschapsafbreking kan eerder plaatsvinden. Dit officiële programma startte op 1 januari 2007.

Hoofdstuk 3 probeert antwoord te geven op deel 1) van de vraagstelling. We hebben de veranderingen in de prestatie van de eerste trimester combinatietest gedurende een aantal jaren in Nederland bestudeerd en hebben geprobeerd factoren te identificeren die een mogelijke invloed hebben op de prestatie van de test. Tussen 2004 en 2006 bleek de prestatie van de eerste trimester combinatietest beter dan in de jaren daarvoor. Dit kan worden weergegeven als een zogenaamde 'odds' ratio (de kans (odds) op het hebben van de ziekte bij een positieve test uitslag). De odds ratio was 1:10 in de periode 2004-2006, vergeleken met 1:14 in de voorafgaande periode. Hoe komt dat nu? In de periode 2004-2006 waren de concentraties (uitgedrukt in zgn. Multiple of the Median, of MoM), voor PAPP-A en fβ-hCG respectievelijk 1.12 en 1. Die MoM is in het ideale geval 1.0. De mediane MoM voor de NT-meting was 0.89 en verbeterde in de loop van de tijd van 0.82 naar 0.96. De verbeterde prestatie van de combinatietest kan daarom vooral worden toegeschreven aan een verbeterde NT meting. Door adequate training van echografisten en een betere bepaling van de biochemische parameters kan de prestatie nog verder verbeterd worden. Dergelijke maatregelen zijn noodzakelijk omdat de prestatie van onze eerste trimester combinatietest nog steeds lager is dan in andere internationale studies. Om na deze maatregelen de test nog verder te verbeteren moet in een andere richting gedacht worden.

Daarmee komen deelvragen 1 en 2 van dit proefschrift in beeld. Een manier om die prestaties te verbeteren is het gebruik van andere eiwitten in het bloed van de zwangere die voorspellend zijn voor DS. Een aantal kandidaten diende zich in de afgelopen jaren aan in de wetenschappelijke literatuur. Dat zijn bijvoorbeeld A Desintegrin And Metalloproteinase 12 (ADAM12), Placental Protein 13 (PP13) en Placental Growth Factor (PlGF). Deze eiwitten zijn geassocieerd met de ontwikkeling en groei van de placenta. Afwijkende concentraties van deze eiwitten lijken geassocieerd met het voorkomen van chromosomale afwijkingen, foetale groeirestrictie en pre-eclampsie (PE).

ADAM12 werd aanvankelijk geïntroduceerd als een veelbelovende merker voor de DS screening, al waren latere publicaties minder rooskleurig. In **hoofdstuk 4** presenteren wij onze eigen evaluatie van ADAM12 voor het voorspellen van DS (drie kopieën van chromosoom 21 in iedere lichaamscel), het syndroom van Patau (drie kopieën van chromosoom 13) en het syndroom van Edwards (drie kopieën van chromosoom 18). In de groep van controle-zwangerschappen was de mediane MoM van ADAM12 1.00 en bij DS-zwangerschappen bij respectievelijk 8, 9, 10, 11, 12 en 13 weken was deze 0.45, 0.73, 0.74, 0.85, 0.92 en 1.06. De mediane MoM bij Edwards-syndroomzwangerschappen was 0.85 en bij Patau-syndroomzwangerschappen 0.63. De ADAM12 MoM waren dus lager bij DS (vooral bij bloedafname voor 11 weken), Edwards en Patau-zwangerschappen. ADAM12 zou dus best een goede aanvullende merker kunnen zijn bij de DS screening, vooral vroeg in het eerste timester en bij uitbreiding van het screeningsaanbod naar trisomie 13 en 18. In **hoofdstuk 5** hebben we onderzocht of ook PP13 een aanvullende merker zou kunnen zijn om te screenen naar andere trisomiëen dan DS in het eerste trimester. De PP13 MoM bleek inderdaad verlaagd in geval van DS, maar ook bij trisomie 13 en 18. In geval van trisomie 13 en 18 waren ook de serumwaarden van PAPP-A en fβ-hCG verlaagd. PP13 zou dus ook voor de screening naar trisomie 13 en 18 belangrijk kunnen zijn. In Mei 2010 werd in het kader van de Wet op het bevolkingsonderzoek (WBO) een vergunning afgegeven om de DS screening uit te breiden met de screening op trisomie 13 en 18.

Tot nu toe hebben we ons in dit proefschrift gericht op screening naar DS, Edwards en Patau syndroom. Er zijn steeds meer aanwijzingen dat de combinatietest ook gebruikt kan worden voor een veel uitgebreidere screening naar veel meer aspecten van de foetale en maternale gezondheid, bijvoorbeeld: vroeggeboorte, foetale groeirestrictie, foetale sterfte, suikerziekte in de zwangerschap en PE. Een dergelijke screening biedt de mogelijkheid van vroege risicoselectie en therapeutische mogelijkheden ter verbetering van de gezondheid van moeder en kind en daarmee zouden potentieel veel meer zwangeren geholpen kunnen worden dan alleen die, die zwanger zijn van een kind met een chromosomale afwijking. In het tweede deel van dit proefschrift hebben we ons dan ook gericht op de vragen 2 en 3: screening naar andere ziekten dan DS.
Zoals vermeld in de inleiding van dit proefschrift, is identificatie van zwangere vrouwen met een risico voor PE nog steeds een van de belangrijkste uitdagingen in de prenatale zorg. PE is immers een ernstige complicatie in de zwangerschap en komt bij 1-2% van alle zwangere vrouwen voor. Door het vroegtijdig identificeren van vrouwen met een hoog risico op PE, kan er eerder in de zwangerschap intensieve prenatale zorg aangeboden worden. De huidige therapeutische mogelijkheden zijn beperkt, maar een lage dosis aspirine vroeg in de zwangerschap zou de incidentie van vroege PE aanzienlijk kunnen verlagen. Het onderscheid tussen hoog - en laagrisico zwangerschappen op een eerder tijdstip zou er ook toe kunnen leiden dat laatstgenoemde categorie minder intensief begeleid hoeft te worden.

In **hoofdstuk 6** beschrijven we een onderzoek naar de voorspellende waarde van PAPP-A, fβ-hCG, ADAM12, PP13 en PlGF, met betrekking tot vroege screening naar een te ontwikkelen PE. Voor dit

onderzoek werden serummonsters gebruikt van 88 zwangeren die een vroege PE (bij de bevalling was de zwangerschapsduur minder dan 34 weken) ontwikkelden. Vergeleken met een controlegroep van normale zwangerschappen waren de MoM PAPP-A, PP13 en PlGF aanzienlijk lager (respectievelijk 0.82, 0.68 en 0.73). Er werd geen verschil gevonden voor ADAM12 en fβ-hCG. Bij zwangerschappen met PE èn een foetale groeirestrictie waren de concentraties van alle biochemische merkers (behalve fß-hCG) lager dan bij controlezwangerschappen. Deze biochemische merkers zijn dus voorspellend voor zwangerschappen met PE, maar in welke mate? Daarvoor is een voorspellend predictiemodel opgesteld. Statistische analyse met behulp van zo'n model leerde dat door het meten van PP13 en PlGF 54% van alle vroege PE-gevallen kan worden voorspeld. Bij 10% van de zwangerschappen die als hoog-risico zwangerschap worden aangemerkt blijkt achteraf niets aan de hand (het zgn fout positief-percentage).

In de literatuur zijn nog veel meer parameters beschreven die voorspellend kunnen zijn voor PE. In **hoofdstuk 7** beschrijven we de resultaten van een literatuurstudie naar de voorspellende waarde voor PE van serummerkers en echoscopisch doppleronderzoek van de arterie uterina in het eerste trimester van de zwangerschap. Onze eigen gegevens uit hoofdstuk 6 werden daarin meegenomen. Ook uit het literatuuronderzoek bleek dat lage concentraties in moederlijk bloed van PP13, PlGF en PAPP-A geassocieerd zijn met het voorkomen van PE. De voorspellende waarde van de merkers afzonderlijk was echter bescheiden. De combinatie van alle biochemische merkers leverde een statistisch gemodelleerd detectiepercentage op van ongeveer 55% en een fout-positief percentage van 10%. Een screeningstest met zo een laag detectiepercentage is in de praktijk niet bruikbaar. Nu zijn ook veel eigenschappen van de zwangere en de zwangerschap voorspellend voor PE. Denk daarbij vooral aan de ziektegeschiedenis van de moeder (ook bij eerdere zwangerschappen), de bloeddruk in de vroege zwangerschap en de doorbloeding van de baarmoeder (die wordt gemeten met het eerder genoemde doppleronderzoek van de arterie uterina). Zou je die ook meenemen in de risicoschattende test voor PE, dan zou een detectiepercentage van ongeveer 90% haalbaar kunnen zijn, bij een fout-positief percentage van 10%, zo blijkt uit de literatuur. Dat is veelbelovend en een dergelijke test zou wèl klinisch bruikbaar zijn. Er zijn echter wel grootschalige prospectieve studies nodig om de betekenis van deze geïntegreerde aanpak in de klinische praktijk te evalueren en om het effect van vroege medicamenteuze interventie te bestuderen.

Zoals eerder beschreven zijn serumconcentraties van merkers, zoals ADAM12, PP13 en PlGF geassocieerd met de groei en ontwikkeling van de placenta. In **hoofdstuk 8** beschrijven we de relatie tussen zes eerste trimester biochemische merkers voor vroege placentafunctie en het uiteindelijk geboortegewicht bij vrouwen met suikerziekte. De concentraties van de merkers werden gemeten in serum, afgenomen in het het eerste trimester van de zwangerschap, van 178 zwangeren met suikerziekte en 186 zwangeren zonder suikerziekte. In de suikerziekte groep waren de PAPP-A en ADAM12 concentraties lager dan in de controle zwangerschappen. Het detectiepercentage voor foetaal overgewicht (macrosomie) bij de geboorte in de suikerziekte groep was 43% bij een fout-positief percentage van 10%. De relatie tussen

macrosomie en suikerziekte in de zwangerschap is al veel langer bekend maar we weten heel weinig over de rol van de vroege placentatie en macrosomie bij de geboorte. De huidige toename van foetale macrosomie zou te maken kunnen hebben met een betere glucoseregulatie rond de conceptie en tijdens het eerste trimester van de zwangerschap. Dit zou kunnen resulteren in een betere placentatie. Samengevat: de biochemische parameters van de combinatietest zouden mogelijk ook gebruikt kunnen worden bij het voorspellen van macrosomie bij de geboorte bij zwangere vrouwen met suikerziekte; zijn concentraties van PAPP-A, ADAM12, PP13 en PlGF bij die zwangerschappen normaal, dan is de kans op een macrosoom kind vergroot.

Terwijl de relatie tussen verschillende merkers en de associatie met foetale aandoeningen en aandoeningen van de zwangere steeds duidelijker wordt, weten we eigenlijk nog maar weinig over het gedrag van deze merkers bij normale zwangerschappen. We hebben daarom geprobeerd meer inzicht te krijgen in de fysiologie van deze merkers in gezonde zwangerschappen (**hoofdstuk 9**) en referentiewaarden voor deze merkers bepaald. Dit longitudinale onderzoek (opeenvolgende metingen bij dezelfde zwangere) tijdens het eerste trimester van de zwangerschap liet zien dat serumconcentraties van PAPP-A, fβ-hCG, ADAM12, PP13 en PlGF stijgen met de zwangerschapsduur. Echoscopisch doppleronderzoek van de arterie uterina, uitgedrukt in de zgn pulsatiliteitsindex (PI), liet zien dat de PI daalde tijdens het eerste trimester. Bovendien was er een correlatie tussen alle serummerkers, met uitzondering van fβ-hCG, en een negatieve correlatie tussen de serummerkers en de bloedstroomprofielen in de arterie uterina (dus; hoe hoger de concentraties van de merkers, hoe lager de PI). De serum concentraties van ADAM12 en PP13 waren verlaagd in een kleine subgroep van zwangeren die een kind kregen met een laag geboortegewicht. De PI in deze groep was aanzienlijk verhoogd. Laatstgenoemde negatieve correlatie suggereert dat er zelfs binnen de categorie van zogenaamd normale zwangerschappen sprake kan zijn van een vertraagde of verminderde placentatie.

Een nieuwe kijk op prenatale screening

Dit proefschrift beschrijft de historische ontwikkeling van de screeningstest voor DS. In het tweede gedeelte wordt beschreven welke nieuwe mogelijkheden er voor deze screeningstest zijn.
Het is van groot belang te onderzoeken of de eerste trimester combinatietest voor de screening naar DS kan worden uitgebreid tot een test die screent naar een hele reeks foetale en maternale aandoeningen, zoals trisomie 13 en 18, PE, HELLP-syndroom, foetale groeirestrictie, vroeggeboorte, sterfte en macrosomie. Een nauwkeurige voorspelling van ziekten en aandoeningen is van cruciaal belang voor een prenatale screeningstest, voor adequate begeleiding van de hoogrisico zwangerschappen en voor de ontwikkeling van preventieve behandelingsmogelijkheden ter verbetering van de maternale en perinatale uitkomst. De zoektocht naar nieuwe potentiële merkers in het maternale serum die ziekten vroeg in het eerste trimester van de zwangerschap kunnen detecteren met een hoge sensitiviteit en specificiteit, is een grote uitdaging. Vooralsnog zijn we pas aan het begin van een geheel nieuwe ontwikkeling in de prenatale zorg, namelijk de identificatie van vrouwen die een verhoogd risico

hebben of die later intensief gebruik gaan maken van de prenatale zorg. Daarmee kunnen uiteindelijk zwangerschappen beter begeleid worden met potentieel gezondheidswinst voor moeder en kind.

Deelnemen aan een screeningsprogramma begint bij de zwangere vrouw zelf. Zij maakt een keuze om wel of niet deel te nemen aan de screening naar het DS. Momenteel wordt het screeningsprogramma uitgevoerd met de eerste trimester combinatietest.
Slechts ca. 25% van de zwangere vrouwen maken momenteel gebruik van de screening naar DS. Uitbreiding van screeningsmogelijkheden zou echter weleens tot een veel groter gebruik kunnen leiden, waarbij de zwangere zelf dient aan te geven naar welke foetale afwijkingen c.q. potentiële zwangerschapscomplicaties gescreend zou moeten worden. Dit zal de gehele prenatale screeningsorganisatie veranderen. De informatievoorziening bij het eerste poliklinische bezoek zal uitgebreider en ingewikkelder worden, omdat de nadruk zal komen te liggen op de gezondheid van de moeder en het kind en minder op chromosomale afwijkingen. Om deze toegenomen en grotere hoeveelheid informatie duidelijk over te brengen zijn nieuwe manieren nodig om de zwangere te informeren. Dit kan bijvoorbeeld door al voor de zwangerschap mensen met een zwangerschapswens gestructureerde en volledige informatie aan te bieden.
Misschien ontstaat er zo wel een soort "à la carte" screeningsprogramma, waarbij de zwangere er bij voorbeeld wel voor kiest om deel te nemen aan screening naar PE of maternale gezondheid, trisomie 13 en 18. Daarbij zou ze er voor kunnen kiezen om juist niet geïnformeerd te willen worden over het risico op het krijgen van een kind met DS, aangezien een kind met DS mogelijk welkom zou kunnen zijn in het gezin.
Bij het eerste bezoek dient een uitgebreide anamnese te worden afgenomen door de arts, verloskundige of gynaecoloog, om informatie over familie en voorgeschiedenis van de zwangere te verkrijgen en vast te leggen in een elektronisch patiënten dossier. Ook de leeftijd van moeder, body mass index en bloeddruk worden gemeten en gedocumenteerd. Echografisch onderzoek wordt uitgevoerd voor het vaststellen van een vitale één - of meerling zwangerschap, eventueel wordt de dikte van de nekplooi en de PI van de arterie uterina gemeten. Bovendien zal bloed worden afgenomen voor het meten van potentiële eerste trimester serummerkers. Deze nieuwe aanpak in prenatale screening past heel goed bij een oorspronkelijk idee van Prof. Dr. Kypros Nicolaides (Fetal Medicine Foundation, Londen). Hij is van mening dat de frequentie van prenatale zorg omgedraaid dient te worden ('Turning the pyramid upside down'). In plaats van meer controles aan het eind van de zwangerschap, zouden de meeste controles vóór 12 weken moeten plaatsvinden, eventueel met aanvullende screeningstesten, om zo vroeg mogelijk inzicht te krijgen in het individuele risicoprofiel. Controles later in de zwangerschap zijn voornamelijk bestemd voor die groep zwangere vrouwen die een verhoogd risicoprofiel hebben.

In dit opzicht zijn de woorden van Wilson en Jungner nog volledig van toepassing;
De vroegtijdige detectie van "erfelijke" ziekten kan lijden verminderen en voorkomen. Het biedt mensen keuzes in situaties waar eerder hun lot voorbestemd was. Gecentraliseerde zorg van zwangerschappen met een hoog

risico zou kunnen leiden tot een betere uitkomst voor moeder en kind en is nodig voor verdere prospectieve longitudinale onderzoeken.

In dit proefschrift zijn enkele elementen beschreven die kunnen leiden tot een nieuw soort screeningsprogramma. Kosten-effectivteitsanalyses moeten uitgevoerd worden om te onderzoeken of reductie van het aantal controles van de grote - laag risico - groep zwangere vrouwen kosteneffectief is, in relatie tot de toegenomen intensieve zorg aan zwangere vrouwen die het echt nodig hebben.
Een nieuw screeningsprogramma waaraan veel zwangeren deelnemen, zal ook helpen bij een selectie van geschikte patiënten voor toekomstig gerandomiseerd onderzoek naar mogelijke preventieve behandelingen. Verder onderzoek moet zich vooral richten op het combineren van onafhankelijke biochemische en echoscopische merkers voor de voorspelling van deze zwangerschapcomplicaties en om predictiemodellen te optimaliseren. In dit proefschrift is getracht aan te tonen dat in de nabije toekomst screening in het eerste trimester van de zwangerschap uitgebreider zal zijn dan alleen screening naar het syndroom van Down.

Dankwoord

Dankwoord

Het zit erop, voor u ligt mijn proefschrift. Een proefschrift schrijf je niet alleen, dankzij de hulp en steun van anderen heeft dit onderzoek een stevige basis gekregen. Ik wil iedereen bedanken die direct of indirect heeft bijgedragen aan dit proefschrift en een aantal van hen graag met naam noemen.

Allereerst alle zwangere vrouwen die hebben meegedaan aan het onderzoek. Zonder jullie was dit boekje niet tot stand gekomen.

Mijn promotor, prof. dr. G.H.A. Visser, beste Gerard, hartelijk dank voor de mogelijkheid om bij jou te promoveren. Het begon met onderzoek naar nieuwe merkers voor de Downsyndroom screening, maar uiteindelijk is het veel breder geworden. Jij bent altijd de rode draad blijven zien waar ik deze al lang kwijt was. Gelukkig had jij er altijd alle vertrouwen in. Iedere bespreking zat je vol nieuwe ideeën en liep ik naar buiten met nog een hoofdstukje! Wat een enthousiasme heb jij voor de verloskunde en de wetenschap, dit werkt behoorlijk aanstekelijk. Je bent een geweldige promotor en altijd zeer betrokken.

Dr. P.C.J.I. Schielen, beste Peter, als Gerard niet met een nieuw idee kwam, was jij het wel! Ik kan me ons eerste gesprek nog goed herinneren. Ik zou onderzoek gaan doen naar nieuwe merkers, ADAM12 en PP13 werden genoemd. Ik probeerde mee te schrijven zodat ik het nog even na kon zoeken…… waar was ik aan begonnen dacht ik toen, maar gelukkig met jouw hulp en steun is het goed gekomen. Hartelijk dank voor alles wat je me geleerd hebt, je enthousiasme en geduld!

Dr. Ph. Stoutenbeek, beste Philip, dank je wel voor deze mooie combinatie plek, arts-onderzoeker en arts-echoscopist, ik vond het een super combinatie. Vanaf het begin was je betrokken bij mijn onderzoek. Ik heb veel van je geleerd de afgelopen 4 jaar, met name op het gebied van echoscopie.

De leden van de commissie, Prof dr. H.A. Smit, Prof. dr. D. Linthout, Prof. dr. J.M.G. van Vugt, Prof. dr. A. Franx en Dr. C.M. Bilardo, wil ik bedanken voor hun bereidheid om het manuscript te lezen en beoordelen.

Lieve secretaresses van het WKZ en UVC, dank voor het denken aan mijn studie en het aanmelden van alle patiënten. Zonder jullie was de MUPPIT-studie nooit zo'n succes geworden.

Graag wil ik alle medewerkers van de poli verloskunde van het AMC bedanken voor de hulp en bijdrage aan mijn onderzoek. Dr. C.M. Bilardo, lieve Katia, je hebt mij niet alleen bij mijn onderzoek maar ook persoonlijk zeer gesteund, dankjewel. Ik hoop dat er ook in de toekomst nog ruimte is voor samenwerking.

Lieve collega-echoscopisten, heel veel dank voor het overnemen van mijn MUPPIT-echo's tijdens mijn zwangerschapsverlof en vakanties en natuurlijk voor de interesse in mijn onderzoek. Ik heb 4 jaar met veel plezier met jullie mogen samenwerken. Beste Rita, hartelijk bedankt dat ik naast mijn echo vaak

even de tijd kreeg om iets te doen aan mijn onderzoek en de laatste maanden me vooral kon richten op schrijven.

De staf en arts-assistenten van het UMCU, dank voor alle hulp, begeleiding, gezelligheid en samenwerking. Lieve onderzoekers, het is fijn om steun te krijgen in de wetenschap. Met name door jullie steun, enthousiasme en gezelligheid is het allemaal helemaal goed gekomen. DANK! Lieve collega's in Apeldoorn ik ben blij dat ik het eerste deel van mijn opleiding met jullie mag samenwerken.

Lieve dames van de 4e, dank voor al jullie hulp en de gezellig koffietjes!

Lieve Wendy, wat ben je toch handig met Excel! Wat was het een leuke tijd ook al zat jij op het RIVM en ik op het UMC. Je had altijd wel even de tijd om snel te antwoorden of om gewoon even te kletsen. Als ik op het RIVM was, hadden we veel te bespreken! Ons onderzoek had ook veel overeenkomsten. Ik heb veel aan je gehad en ook veel van je geleerd de afgelopen jaren, wat heerlijk dat we nu allebei ons boekje af hebben.

Lieve collega's van het RIVM, heel veel dank voor al jullie tijd en energie die jullie besteed hebben aan mijn onderzoek, alle dopjes erop en eraf draaien, alle analyses, de samples opzoeken uit de -30 graden! Wat was het daar koud! Naar Arnhem! Etiketten opnieuw plakken… en ga zo maar door! Ik vond het gezellig om op de vrijdagen bij jullie op het RIVM te zijn. Beste Sylwia, je kwam als enthousiaste en zeer gemotiveerde student een review schrijven. Het is gelukt en niet alleen een review! Nu begin jij aan je eigen promotie-traject. Veel dank voor je hulp bij mijn onderzoek en veel plezier bij het voorzetten van de MUPPIT-studie.

Lieve paranimfen, Annemiek en Go, wat ben ik blij dat ik bij jullie op de kamer heb gezeten de afgelopen jaren! Ik heb veel van jullie geleerd en veel met jullie gelachen! Wat is er een mooie vriendschap ontstaan. Trouwen, kinderen, vakanties, noem maar op! Ben zo blij dat jullie de 26ste naast me willen staan. Gaat een mooi feestje worden.

Lieve vrienden, in het bijzonder, Fleur, Juud, Klaar en Xan al ruim 15 jaar kennen wij elkaar, dank voor het luisteren naar mijn verhalen, voor het delen van jullie ervaring of het meedoen aan de MUPPIT-studie! Maar vooral ook het gewoon gezellig borrelen in de kroeg!

Lieve familie; broer, zus, aanhang, kinderen en 'schoonfamilie', dank voor al jullie steun en vertrouwen. Ik hou van jullie. Papa en mama, zonder jullie liefde en onvoorwaardelijke steun bij al mijn keuzes in mijn leven was ik hier niet geweest. Dank voor jullie hulp en niet alleen die tijdens de afgelopen vier jaar. Hoewel het natuurlijk fijn is als er iemand op Anne kan passen als ik weer voor een spoed afspraak naar het UMC moest. Lieve Mary, 3 dagen in de week, dank voor de liefdevolle zorg voor Anne.

Lieve Inne en Anne, zonder jullie was het nooit gelukt, wat hou ik ontzettend veel van jullie. Inne, wat is ze lief hè!

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