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PO Box 117
221 00 Lund
+46 46-222 00 00

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From the Department of Obstetrics and Gynecology,
University of Lund, Malmö University Hospital

Risk Estimation and Prediction of Preeclampsia, IUGR, and Thrombosis in Pregnancy

Pelle Lindqvist

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Abstract

The aim of this thesis was to improve background knowledge for making a reliable medical evaluation at the first visit of a woman in her 13th gestational week, to the antenatal clinic. We have focused on the prediction and the risk estimation of preeclampsia, intra-uterine growth restriction (IUGR), and thrombosis.

The thesis is based on five studies, in which we have evaluated biochemical analyzes, genetic tests, anamnestic information, and statistics (based on data in the from medical files and at the Swedish Medical Birth and Hospital Discharge Registers).

A high maternal urine chorionic gonadotropin level in early pregnancy was associated with a 3-fold increased risk of preeclampsia, vis-à-vis low values, while low epidermal growth factor levels were associated with IUGR pregnancies.

Maternal smoking was associated with an increased and consumption dependent risk of thrombosis (Odds ratio (OR)=1.24; 95% Confidence interval (CI) 1.02-1.51). Moderate smoking was associated with lower incidence of preeclampsia associated with preterm birth in both study series (OR=0.1; CI 0.01-0.7, and OR=0.6; CI 0.5-0.8, respectively).

Apart from a 1.1% risk of thrombosis, APC resistance was not associated with preeclampsia, IUGR, or spontaneous abortion. However, the carriers of APC resistance had fewer profuse hemorrhages at delivery (3.7% vs. 7.9%), which might have given them an evolutionary advantage, explaining the high prevalence (10.7%). The natural incidence of pregnancy-associated thrombosis was 13/10,000, evenly divided between ante- and postpartum periods. APC resistance was associated with an 8-fold increased risk of thrombosis. Overweight, heredity of thrombosis, and cesarean delivery were all associated with a roughly 5-fold increased risk of thrombosis. Preeclampsia was associated with a 3-fold increased risk of thrombosis, postpartum.

LIST OF PAPERS

This thesis is based on the following papers which will be referred to in the text by their roman numerals:

I. Lindqvist P, Grennert L, Maršál K. Epidermal growth factor in maternal urine – a predictor of intrauterine growth restriction. *Early Human Development* 1999; 56: 127-135.

II. Lindqvist PG, Maršál K. Moderate smoking during pregnancy is associated with reduced occurrence of preeclampsia. *Acta Obstetricia et Gynecologica Scandinavica* 1999; 78: 693-97.

III. Lindqvist PG, Svensson P, Dahlbäck B, Maršál K. Factor V:Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss – a possible evolutionary selection mechanism. *Thrombosis and Haemostasis* 1998;79**:**69-73.

IV. Lindqvist PG, Svensson P, Maršál K, Grennert L, Luterkort M, Dahlbäck B. Activated protein C (FV:Q506) and pregnancy. *Thrombosis and Haemostasis* 1999; 81: 532-7.

V. Lindqvist P, Dahlbäck B, Maršál K. Thrombotic risk during pregnancy, a population study. *Obstetrics and Gynecology* 1999; 94: 595-99.

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ABBREVIATIONS

APC	Activated Protein C
APC resistance	Activated Protein C resistance
ASA	Acetylsalicylic Acid
BMI	Body Mass Index (kg/m^2)
CI	Confidence Interval
EGF	Epidermal Growth Factor
HCG	Human Chorionic Gonadotropin
FV	Coagulation factor V
FVa	Activated coagulation factor V
FV:Q506	Coagulation factor V mutation (Arg 506 Glu)
FVia	Coagulation factor V inactive
FVac	Coagulation factor V anticoagulant
Hb	Hemoglobin value
IUGR	Intra-uterine Growth Restriction
MTHFR TT	Methylenetetrahydrofolate reductase C677T allele in homozygous form
OC	Oral Contraceptives
OR	Odds Ratio
PPV	Positive Predictive Value
RIA	Radio Immuno Assay
SD	Standard Deviation
SGA	Small-for-gestational Age
sicklers	Carriers of sickle cell anemia
TXA_2	Thromboxane A2

Definitions

APC resistance: the presence of the FV:Q506 allele in either heterozygous or homozygous form.

Early spontaneous abortions: abortion before 13 completed weeks of gestation (<13+0). A history of spontaneous abortion was checked for, both at the interview and by scrutiny of the patient´s medical records (Papers III & IV).

Gestational age was estimated by ultrasound measurements of biparietal diameter and femur length, in 98% of the background population (i.e., Paper I, Malmö series of Papers II, and III, and in Paper IV), and from the date of the last menstrual period in the remaining 2%.

Hospitalization time: the number of completed days in hospital care after delivery, at Malmö University Hospital. Among those leaving on the day of delivery, hospitalization time was set to 0.5 day.

Intrapartum blood loss was estimated by the midwife who was attending the delivery, and was entered on the patient´s medical record immediately after delivery. Only women delivered vaginally in Malmö and those not treated with heparin, were included in the analyzes of intrapartum blood loss.

IUGR: a newborn small-for-gestational age at birth, i.e., with a birthweight lower than 2 standard deviations (SD) below the mean for a reference population (Maršál et al. 1996).

Late spontaneous abortion: abortion after 12+6 weeks of gestation. A history of spontaneous abortion was checked for both at the interview and by scrutiny of the patient´s medical records (Papers III & IV).

Low Apgar score: Apgar score less than 7 at 5 minutes after birth.

Maternal death: death of a woman in pregnancy or within 42 days of the termination of pregnancy, irrespective of the duration and the site of the pregnancy, from any cause related to, or aggravated by the pregnancy, or its management, but not from accidental or incidental causes.

Overweight: body mass index (kg/m^2) >1 SD above the mean for maternal weight recorded at the first visit to the antenatal clinics in Paper IV (i.e., body mass index > 27.6).

Parity was classified as nulliparous (no previous birth) or multiparous (at least 1 previous birth) in Papers I, III, IV, and V. In Paper II, parity was classified as: nulliparous, one earlier birth (reference class), two earlier births, and three or more earlier births.

Perinatal death: death in utero during the third trimester, intrapartum, or during the first week of life.

Postpartum anemia: a hemoglobin value <100 g/l on the second day postpartum.

Preeclampsia: pregnancy-induced hypertension and proteinuria ≥0.3 g/1 (Albustix® Boehringer-Mannheim 1+). Pregnancy-induced hypertension was defined as a resting blood pressure ≥140/90 mmHg measured on two occasions with an interval of at least 5 hours, and developing after 20 weeks of gestation in a previously normotensive pregnancy. In Paper I, a diastolic blood pressure ≥90, for pregnancy induced hypertension, and in the Malmö series of Papers II, III, and IV, a diastolic blood pressure >90 mmHg was used.

Preterm delivery: delivery before 37 completed weeks of pregnancy.

Profuse hemorrhage: a blood loss exceeding 600 ml.

Smoking habits were those recorded at the first visit to the antenatal clinic and smokers were classified in terms of daily cigarette consumption, i.e., ≥10 cigarettes (heavy smokers), 1-9 (moderate smokers), or 0 (non-smokers or not regular smokers). For the purpose of analyzis, in Papers I, III, and IV, smoking habits were dichotomized into smokers and non-smokers.

Weight deviation: birthweight or ultrasonically estimated weight, minus expected weight for gestational age of Swedish reference, divided by expected weight, and expressed as a percentage.

Thrombosis: a deep venous thrombosis, pulmonary embolism, or cerebral thromboembolism occurring in pregnancy or during the first 3 months postpartum. In Paper V the International Classification of Diseases 9 definition of thrombosis was used, i.e. including only 42 days in the postpartum period.

Thrombosis heredity: one or more thromboses in first-degree relatives (father, mother, or siblings), occurring before 60 years of age.

OBJECTIVES

The aim of the studies upon which this thesis is based has been to improve our knowledge of individual risk-evaluation of pregnant women during their first visit at the antenatal clinic, and to elucidate the possibility of improving the prediction of pregnancy complications. Specifically, these aims were:

- to establish whether it is possible to predict intra-uterine growth restriction (IUGR) and/or preeclampsia by analyzing the epidermal growth factor (EGF) or human chorionic gonadotropin (HCG) levels in maternal urine (Paper I).

- to assess the impact of smoking on the development of preeclampsia and to discuss possible underlying causes (Paper II).

- to ascertain whether women with activated protein C (APC) resistance (FV:Q506) run an increased risk of preeclampsia, IUGR, or abortion, or a reduced risk of bleeding complications during pregnancy (Paper III).

- to determine the natural course of pregnancy in APC-resistant women (Paper IV).

- to determine the incidence of pregnancy-related thrombosis and its relation to certain risk factors (Paper V).

BACKGROUND

Introduction

The number of routine visits to a doctor during pregnancy in Sweden has gradually decreased from three visits 15 years ago, to one – and the number of routine consultations with a midwife has now been reduced to seven or eight. This change in antenatal health care requires more reliable medical evaluation on the occasion of the first visit in order to maintain, or preferably to improve, medical safety. The purpose of this visit, which usually takes place at the end of the first trimester, or early in the second, is to identify women at risk of pregnancy complications, or those in need of increased support. For these women an individual plan is devised, while others follow the routine consultations with midwife.

The overriding intention is to identify early which pregnancies are likely to have a complicated course or outcome. These pregnancies can then, if feasible, be given intensive surveillance and preventive intervention. Most forms of pregnancy complication are rare; for example, preeclampsia as well as intra-uterine growth restriction (IUGR) occur in 2-4% of gravidae, pregnancy diabetes in 1-2%, and thrombosis and abruptio placentae in less than 0.5%. However, these in all 5-8% of gravidae include the majority of known complications. In our studies we have focused on risk estimation and the prediction of preeclampsia, IUGR, and thrombosis.

Preeclampsia

Preeclampsia complicates 2-3% of all pregnancies and is a leading cause of maternal and perinatal morbidity and mortality, causing about 20% of maternal mortality cases in Western countries (Högberg 1985). Perinatal morbidity and mortality among preeclamptics results mainly from preterm delivery and the presence of IUGR (Montan et al. 1987). Anti-hypertensive treatment is often initiated to reduce the risk of maternal complications such as eclampsia and cerebral hemorrhage. However, no treatment has so far proven to have a beneficial effect on the fetus and the only causal treatment for preeclampsia is delivery.

In normal pregnancy, maternal blood pressure usually decreases until mid -term together with an increase in circulating blood volume. This decrease is thought to be secondary to a reduction in peripheral vascular resistance, due to altered sensitivity to pressor substances. At full-term, there is a slight increase towards the original non-pregnant blood pressure. Still, the true mechanisms of blood pressure and blood volume regulation are unknown.

Pathophysiology

The primary defect in preeclampsia, still under debate, is a partial failure of trophoblastic invasion late in the first, or early in the second trimester. Due to shallow trophoblastic invasion of spiral arteries, these remain narrow, with consequent restricted blood supply to the placenta. This leads to placental ischemia, whose consequence is the release of an unidentified factor(s) into the maternal circulation. This occasions widespread endothelial cell damage, which then leads to the multisystem dysfunction that characterizes preeclampsia. Several other characteristics of preeclampsia seem to be dependent on this endothelial cell damage. There is an impaired endothelial cell prostacycline production leading to an imbalance of the prostacycline and thromboxane A2 (TXA_2) ratio. This has been suggested to cause the characteristic vasoconstriction of preeclampsia (Walsh 1985). Furthermore, the contents of fibronectin, an indicator of endothelial damage, is increased in preeclampsia. The coagulation system and fibrinolysis are activated, giving an increase in coagulation enzymes, increased fibrin degradation products, subendothelial fibrin deposition in the kidney, and at times in the liver (Greer 1994).

Most studies have been made after the development of preeclampsia, and several substances have been shown to be altered in preeclamptic women, e.g. endothelin, atrial natriuretic factor, nitric oxide, uric acid, and various adhesion proteins. These changes might not be involved in the development of preeclampsia, but may rather be a consequence of preeclampsia – so-called markers. Preeclampsia is believed to be a multigenic condition, and since some markers are correlated to the severity of the condition (e.g. uric acid), they might be included among the diagnosis criteria for preeclampsia, in order to get a more homogenous group.

There is a known hereditary factor in preeclampsia with 2–10-fold increased risk in women whose mothers or sisters have had preeclampsia. Various candidate genes have been proposed: endothelial nitric oxide synthetase, angiotensinogen, angiotensin converting enzyme, angiotensin receptor protein, apolipoprotein E, etc. (Higgins and Brennecke 1998). Yet no gene has gained common acceptance as the one that causes the hereditary component of preeclampsia. However, it may be that there is not a single gene and that preeclampsia is just the extreme of normal pregnancy adaption (Redman et al. 1999). If a genetic condition could be linked to the development of preeclampsia, it might be possible to use it for predictive purposes.

Prevention of preeclampsia

The value of prediction lies in the steps that can be taken to reduce the risk. In the case of preeclampsia, prediction and prevention are presumably closely linked. Yet there is no generally accepted treatment or preventive measure other than delivery.

Calcium supplementation during pregnancy has been reported to lower the preeclampsia rate (Lopez Jaramillo et al. 1997; Herrera et al. 1998). However, a recent large prospective randomized placebo controlled study showed no differences (Levine et al. 1997).

After a report from Crandom and Isherwood (1979) of a lower incidence of preeclampsia among nulliparas who took acetylsalicylic acid (ASA) at least once every second week, and Wallenburg et al. (1986) reported a beneficial effect of low-dose ASA on the incidence of preeclampsia. There have been several clinical trials of low-dose ASA for prevention of preeclampsia and IUGR. Low-dose ASA is thought to temporarily inhibit endothelial prostacycline production and permanently inhibit platelet TXA_2 production during the 10-day life span of platelets (i.e. the platelets are incapable of de novo synthesis of TXA_2). This selective effect of low-dose ASA will thus improve the TXA_2 /prostacycline balance. Initially, there were suggestions of a considerable reduction in the prevalence of both preeclampsia and IUGR, though, recent large studies do not support these conclusions (Sibai 1998). There are several, still unanswered, methodological issues regarding low-dose ASA treatment that remain to be resolved, such as: selection of patients, initiation time, dose, and interval of ASA. Therefore, the definitive role of ASA in the prevention of preeclampsia and IUGR is still uncertain.

A randomized placebo controlled study of ketanserin, a specific serotonin-2 - receptor antagonist, showed the incidence of preeclampsia to be reduced in the ketanserin treated group (Steyn and Odendaal 1997). A subgroup reported to run less risk of developing preeclampsia are smokers. If smokers have a true protection against preeclampsia, a preventive might be identified.

Recently, supplementation of the antioxidants vitamin C and vitamin E was shown to be correlated with reduced occurrence of preeclampsia in a high-risk group (Chappell et al. 1999).

Prediction of preeclampsia

Although clinical demographic factors such as nulliparity and preeclampsia among first-degree relatives might help when selecting a group at increased risk, they are neither sensitive enough nor sufficiently specific to be used alone (O'Brien 1990). Gravidae with a known increased risk of preeclampsia include: multigravidae, diabetes, non-smokers, overweight, collagenosis (e.g. SLE), and lupus anticoagulant syndrome. However, since proteinuria is often found in diabetes, hypertension is prevalent among the obese, and multigravidae have a different placentation, one must be careful when including these in studies on the pathogenesis of preeclampsia.

A logical focus of predicting preeclampsia would be to elucidate the mechanisms of trophoblast invasion. Human chorionic gonadotropin (HCG) is produced by the syncytiotrophoblasts and is involved in normal placentation.

When serum screening for Downs syndrome in the second trimester, HCG was found to be higher in women who later developed preeclampsia (Said et al. 1984; Sorensen et al. 1993). However, urinary HCG has not yet been evaluated for the prediction of preeclampsia in the first trimester. A method of measuring the insufficient trophoblastic invasion of uterus might be by doppler evaluation of arteria uterina velocity signals (see below; section Prediction of IUGR)

Both preeclampsia and IUGR are known to have reduced circulatory blood volume, due to defective volume expansion in early pregnancy. Our understanding of the process governing normal maternal circulatory adaptation in pregnancy merits high priority in the research of the pathogenesis of these conditions. Using a doppler method for hemodynamic evaluation of maternal circulation might be an interesting approach.

Investigating the mechanisms regarding the downregulated sensitivity of pressor substances that occur in normal pregnancy would be another approach for the prediction. On the basis of an increased sensitivity to the pressure substance angiotensin II in women with preeclampsia, the angiotensin infusion test and the roll-over test were devised by Gant and co-workers 25 years ago (O'Brien 1990). Both these tests identified women at a 2- to 3-fold increased risk of developing preeclampsia early in the third trimester.

Hypocalciuria has been noted in pregnancies complicated by preeclampsia, and suggested for use as a predictor (Taufield et al. 1987). In addition, recent studies on inactive kallikrein in the urine have suggested it to be a simple and practical indicator of preeclampsia (Kyle et al. 1996; Millar et al. 1996). However, none of these has yet gained common acceptance.

Most studies have attempted to predict, or prevent, preeclampsia in an unselected pregnant population, yielding low positive predictive values (PPV). Improved performance in these methods might well be expected if carried out on a high-risk group of gravidae. Failure to predict preeclampsia accurately has impaired both the study of its pathogenesis and the search for preventive measures. Most researchers have focused on endocrinological and biochemical features in established preeclampsia, rather than on the mechanisms and processes leading to the condition. In addition, both preeclampsia and IUGR are so infrequent (2-3%) that reliable conclusive studies must be very comprehensive, and consequently time-consuming and expensive. A method capable of identifying a high-risk group for preeclampsia, or IUGR, would be of great value, not only for clinical management, but also for research into these conditions.

Intra-uterine growth restriction (IUGR)

IUGR is a pregnancy complication having considerable clinical impact, as it is related to fetal hypoxia, asphyxia, and increased fetal morbidity and mortality.

The perinatal mortality is 10-fold increased, as compared the general population (Wennergren et al. 1988). In addition, nutrition deficiency during fetal life affects fetal brain development and even at school age (7 years old), IUGR children show neurological and psychological impairment (Ley 1997). Furthermore, a 3-fold increase in teen-age schizophrenia among male infants born IUGR, indicates the impact of IUGR on psychological development (Hultman et al. 1999). In addition, a relationship has been found between IUGR and the adult development of hypertension, and non-insulin dependent diabetes mellitus (Barker's theory)(Barker et al. 1993).

Thus, IUGR fetuses are at an enhanced risk of both short- and long-term consequences. Therefore, it is important to identify fetuses at risk of IUGR in order to prevent adverse conditions. It is necessary to supervise these pregnancies so as to stop the pathological process in time, especially at low gestational age.

Definition

A downward deviation from the individual growth curve is fetal growth restriction. In each pregnancy, several measurements would be needed to determine a deviation from the normal, but for practical reasons, this is not possible. Instead, most centres use small-for-gestational age (SGA) as an approximation of IUGR. Intra-uterine fetal weight is estimated by ultrasonographic measurement of biparietal diameter, femur length, and abdominal diameter. This estimated weight is compared with a Swedish reference curve (Maršál et al. 1996), and is expressed as a percentage of expected weight. An 11% weight deviation corresponds to 1 SD, and fetuses with an estimated weight less than 2 SD below the mean of a reference population are classified as SGA (i.e. $\leq$-22% weight deviation). Most SGA fetuses are IUGR, though, some fetuses are genetically small. An advantage of this method is that it could be used for intra-uterine estimated weight as well as for birthweight and it also adjusts for the large number of SGA fetuses among preterm fetuses.

Pathophysiology

IUGR is probably caused by defective placentation in early pregnancy, with consequent limited placental circulation, insufficient fetal growth and impaired oxygen transport. The early pathology presents itself in the second half of the pregnancy as IUGR, placenta infarcts, and fetal hypoxia. The acute atherosis seen in maternal vessels is similar to that of preeclampsia. Maternal factors may contribute to the development of IUGR, such as autoimmune diseases (e.g. SLE), chronic kidney disease, underweight (undernutrition), preeclampsia, thrombophilia, diabetes mellitus, infections, and maternal smoking. Fetal factors such as fetal chromosome aberration, congenital anomalies, and intrauterine

infections, are all associated with an increased risk of IUGR. Furthermore, women with historic factors, such as, preterm delivery, IUGR fetus, or stillbirth, are at an increased risk.

Antenatal identification of IUGR

In order to identify IUGR antenatally, all pregnant women must be screened. Various methods are possible: serial measurements of symphysis–fundal height, preselection by risk factors, routine ultrasound screening in the third trimester. The symphysis–fundal measurement has been routinely used in obstetrics, even though its predictive value is limited (Lindhard et al. 1990). It is easy, cheap, and is available everywhere and is therefore still in use. Secondly, Wennergren and co-workers have constructed a score system for the identification of IUGR which is based on risk factors, and on their relative weight (Wennergren et al. 1982).

A third method, used in Malmö, is routine ultrasound screening by fetometry at 32 weeks. The fetuses with an estimated fetal weight deviation of $\leq$ -22% are diagnosed as SGA, and those $\leq$ -10% weight deviation as 'suspected IUGR' The latter are followed up with a third routine screening at 37 weeks. Those diagnosed as SGA are followed up with a special schedule of doppler velocity measurements of arteria umbilicalis, and uterina, at varying intervals, which have been shown to be effective in a high-risk population (Almström et al. 1992). The PPV and negative predictive value of this ultrasound screening for detecting IUGR antenatally have been shown to be 77% and 99%, respectively (Maršál and Persson 1988).

All three methods discussed above are dependent on a reliable dating of pregnancy, which is preferably done by routine ultrasound fetometry in early pregnancy. In addition, the diagnosis of IUGR, among those found to be at high risk, is finally determined by ultrasound in all three above-mentioned methods. Although antenatal detection of IUGR is known to improve fetal outcome, a lower prevalence of long-term sequelae remains to be proven.

Prediction of IUGR

At present, there is no reliable method for predicting IUGR in the first half of pregnancy. Fifteen years ago, Campbell and co-workers reported doppler velocimetry studies on arteria arcuata to be a prognostic marker for perinatal complications (Irion et al. 1998). Women with signs of increased resistance, increased resistance index, or presence of notches, on the velocity signals recorded from the uterine artery were found to be associated with adverse pregnancy outcome. This has been confirmed in other studies, and attempts have been made to use the method to predict IUGR and preeclampsia by screening the whole pregnant population. Initial reports by Campbell's group reported an odds ratio (OR) of 68 for having preeclampsia, if presenting with a notch in the 24th gestational week. However, most other groups are reporting OR as being

between 2 and 4 for both IUGR and preeclampsia. Thus, reported studies argue that doppler velocimetry measurements cannot be used to predict an unselected low-risk population, but possibly in high-risk pregnancies (Alfirevic and Neilson 1996). Women with a known increased risk are those with a heredity of preeclampsia, those with a lupus anticoagulant syndrome, or those identified as a high-risk by some biochemical test.

Basis of present studies on prediction of IUGR and preeclampsia

Both preeclampsia and IUGR have defective placentation in early pregnancy, manifesting itself in the second half of the pregnancy. Finding the agents or the mechanisms that govern early placentation might give an opportunity for early prediction. Epidermal growth factor (EGF) and HCG are two substances that could be involved in governing placentation. Both preeclampsia and IUGR show signs of activated coagulation, with placental thromboses. Therefore, an association with a prevalent prothrombotic hereditary condition, such as APC resistance, with preeclampsia or/and IUGR, might be useful for predicting these conditions.

Epidermal growth factor (EGF)

EGF, an angiogenic and mitogenic peptide, is essential for normal fetal development in mice. In murine studies, surgically provoked EGF deficiency, by extirpation of the salivary gland, in early (Tsutsumi and Oka 1987), or mid- (Kamei et al. 1993) gestation caused abortions or ‘growth restricted’ pups (i.e. small body, light gut, normal sized head). An EGF replacement improved the outcome. In vitro, EGF has been shown to affect trophoblast differentiation and hormone production from the syncytiotrophoblast, with increased HCG synthesis in early pregnancy (Maruo et al. 1987; Barnea et al. 1990). In normal human pregnancy, the EGF level in maternal urine increases up to 20 weeks of gestation (Hofmann et al. 1988; Kasai et al. 1989; Watanabe 1990), and then decreases toward term (Hofmann and Abramowicz 1990). A reduction of the EGF level has also been reported with increasing maternal age (Stoll et al. 1988; Kasai et al. 1989), and in term pregnancies with IUGR fetuses (Shigeta et al. 1992). Hypothetically, low EGF levels might be involved in the defective placentation occurring in IUGR and preeclampsia.

Human chorionic gonadotropin (HCG)

Soon after the trophoblastic invasion in placentation, the syncytiotrophoblasts start an HCG production. The HCG level increases until 10-12 weeks of gestation and thereafter stabilizes at a lower level. In the middle of the second trimester, and in late pregnancy, higher serum HCG values have been found in

pregnancies complicated by preeclampsia (Said et al. 1984; Sorensen et al. 1993). Thus, HCG is closely involved in early placentation, while increased serum HCG levels in later pregnancy are associated with preeclampsia. This is considered an abnormal trophoblastic secretory response to this disorder.

APC resistance (see Hemostasis during pregnancy)
Successful pregnancy outcome is dependent on adequate uteroplacental circulation, the intervillous flow resembling venous circulation in terms of its low pressure and low flow velocity. The intervillous placental circulation may be particularly susceptible to thrombotic complications in thrombophilic women. Preeclampsia and IUGR are both associated with an activated coagulation system, an acute atherosis in maternal blood vessels, and placental infarcts. Acquired antithrombin deficiency is often evident in women with severe preeclampsia (Greer 1994). The Lupus anticoagulant syndrome, an acquired thrombophilia with functional resistance to APC, is characterized by a high incidence of an early onset of severe preeclampsia, IUGR, spontaneous abortions, and thrombosis (Branch et al. 1985; Jones 1994). It was therefore logical to hypothesize APC resistance as predisposing to the above-mentioned pregnancy complications. If confirmed, this could indicate a viable method for predicting adverse outcome.

Hemostasis during pregnancy

Normal pregnancy is characterized by major changes in the hemostatic system. Since part of this thesis (Papers III and IV) describes a newly discovered hereditary anomaly in the protein C anticoagulant system, a summary of coagulation and anticoagulation is justifiable (see review by Dahlbäck 1999).

Blood coagulation

There is a natural balance between procoagulant and anticoagulant forces. A shift in this balance caused by a genetic or acquired factor may result in a lifelong bleeding or thrombotic disease. In response to trauma to the vascular endothelium, the coagulation system is activated and a platelet plug initiates the first occlusion of the lesion after an activation by thrombin. Contact of tissue factor with blood activates factor VII, which then activates factors IX and X. Activated factor X binds to negatively charged phospholipids of the platelet plug, together with activated coagulation factor V (FVa) to form the prothrombinase complex which generates thrombin from prothrombin (Fig. 1). Thrombin triggers a feedback amplification loop, which activates FV, factors VIII, and XI. The adhesive protein von Willebrand factor, which is essential for the initial platelet plug, circulates bound to factor VIII and the activation of factor VIII separates the two. Activated factors VIII, and IX, and phospholipids

form the 'tenase complex' that activates factor X (Fig. 2).

Finally, a burst of thrombin is generated during the formation of the fibrin clot. This also activates factor XIII, which stabilizes the fibrin clot by cross linking.

All vitamin K-dependent proteins in the 'tenase' and 'prothrombinase' complexes (activated factors IX, and X, factor X and prothrombin) and the cofactors activated factor VIII, and FVa, have an affinity for the negatively charged phospholipid surface of activated platelets or endothelium. The interaction surface of the vitamin K-dependent proteins contains a γ-carboxy-glutamic acid, which is involved in calcium binding for proper 3-dimensional folding. Warfarin inhibits vitamin K, which is the molecular background of an anticoagulant therapy with warfarin.

Anticoagulant system

All steps in the coagulation system are balanced and under the strict control of the anticoagulant system**.** Antithrombin inhibits mainly free enzymes, and less complex-bound enzymes. Heparin and heparin-like molecules stimulate the action of antithrombin, which is for using it as an anticoagulant.

Thus, antithrombin neutralizes and protects the circulation from free active enzymes, but does not inhibit coagulation at the complex-bound active sites.

The protein C anticoagulant system

In addition to their procoagulant properties, both thrombin and FV have anticoagulant functions in the protein C system. These dual functions of thrombin and FV are important for the natural balance between the pro- and anticoagulant systems. Thrombin initiates the protein C system by binding to thrombomodulin present on undamaged endothelium. This activates protein C, forming APC (Fig. 2). APC counteracts the coagulation process by inactivating the two co-factors, FVa and activated factor VIII, in “prothrombinase” and “tenase” complexes. Inactivation of FVa is possible either by a cleavage of FV at position Arg 506 by APC, or at Arg 306 by protein S. APC causes a partial inactivation, and protein S is required for maximum inactivation of FVa. The anticoagulant action of FV is initiated by the cleavage of FV, at the Arg 506 position by APC, forming anticoagulant FV (FVac). FVac functions as a co
-factor to APC together with protein S in the inactivation of activated factor VIII.

Thus, together with its co-factors, APC regulates the membrane-bound generation of thrombin by inactivating activated factor VIII and FVa.

Hereditary thrombophilias in the protein C system

The natural balance between pro- and anticoagulation is normally well established. The clinical impact of this balance is illustrated by the relation between genetic defects of the protein C system and thrombosis (Dahlbäck

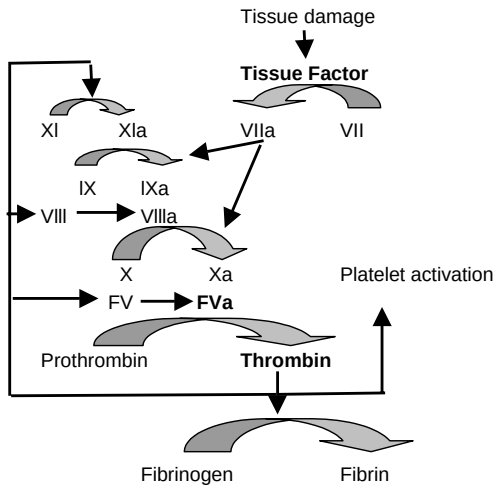
1999). Deficiencies in protein C and protein S, two vitamin K-dependent plasma proteins, are genetic defects of the protein C system occurring in some 2-5% of the thrombosis cases.

APC resistance

Most common among the hereditary thrombophilias is the newly discovered APC resistance (FV:Q506), caused by a single point mutation in the gene for FV, involving the replacement of arginine (R) at position 506 by glutamine (Q) (Dahlbäck et al. 1993; Bertina et al. 1994; Svensson and Dahlbäck 1994). This results in a reduction of the ability of APC to inactivate FVa by cleavage at Arg506. In addition, FV cannot be converted into the anticoagulant FVac, resulting in a less efficient degradation of the activated factor Vlll. Thus, the FV:Q506 mutation causes a lifelong procoagulatory state due to impaired inactivation of FVa and activated factor Vlll. Almost all cases of hereditary APC resistance have the same FV:Q506 mutation. Haplotype analyzis show that the mutation occurred at about 25,000 years ago (Zivelin et al. 1997). At present, the FV:Q506 mutation is highly prevalent among the Caucasian population in Europe, the prevalence ranging between 10 and 15% in Sweden, 4-8% in central Europe, and 2% in the south, with some exceptions. In other countries of mixed but mainly Caucasian origin, such as USA, a prevalence of 5% is reported. This means that around 50 million people of Caucasian origin are carriers of APC resistance in Europe and North America. The mutation is almost non-existent in Asia, Japan, Africa, and South America (Rees et al. 1995).

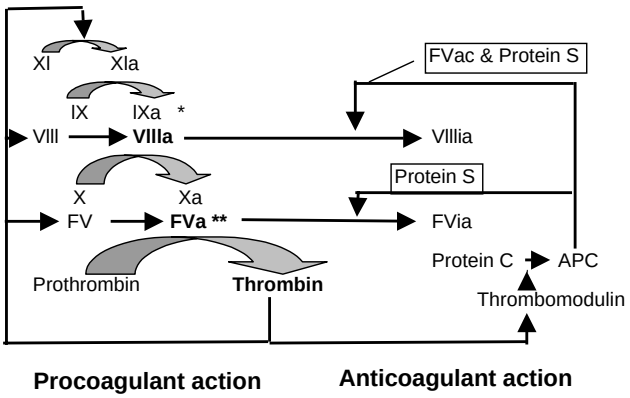
Heterozygous carriers of APC resistance have been estimated to run a 5-fold increased risk of venous thrombosis, and homozygous carriers an 80-fold increased risk (Rosendaal et al. 1995). At the time of the studies described in Papers III and IV, the consequences of APC resistance during pregnancy had not yet been evaluated.

The high prevalence of APC-resistant women among Caucasians suggests that carriers of APC resistance must have an evolutionary advantage in order to be so prevalent (Dahlbäck 1994).



Coagulation factors named by their roman numerals.

Figure 1. Coagulation activation by the tissue factor pathway



* Tenase complex, ** Prothrombinas complex

Figure 2. Dual action of Thrombin

Thrombosis and thrombosis incidence

During pregnancy, the coagulation system is in a procoagulatory state due to increased procoagulatory activity, e.g. an increased Factor VIII coagulant activity, fibrinogen, and von Willebrand factor antigen (Hellgren and Blombäck 1981), and a generally impaired fibrinolytic activity, e.g. increased PAI-1, PAI-2 and plasminogen activity (Greer 1994). These changes are part of a physiological adaptation to pregnancy and childbirth. However, the risk of thrombosis is reported to be 10-fold increased as compared with the non-pregnant state (i.e., one out of 1000 pregnancies). Therefore, a large proportion of thrombosis cases among fertile women will occur in relation to pregnancy. Factors reported to be associated with an increased risk of thrombosis include: high maternal age, high parity, operative delivery, immobilization, obesity, malignancy, previous thrombosis, blood group other than 0, hereditary or acquired thrombophilia (Greer 1994).

In conclusion, the three pregnancy complications that we focused on, – preeclampsia, IUGR, and thrombosis – seem to have common properties. Therefore, it was logical to hypothesize that APC resistance might be linked not only to thrombosis, but also to preeclampsia, IUGR, and spontaneous abortion. If a close association were to be found, the APC resistance test might be used to predict these conditions. An evaluation of the consequences of APC resistance during pregnancy might also answer the question whether reduced profuse blood loss during delivery, or fewer spontaneous abortions may have given APC-resistant individuals an evolutionary advantage. The development of a thrombosis is multigenic, and the consequence of various acquired or hereditary risk factors add up to a thrombosis. In order to make estimates of this acquired risk and to determine the thrombosis incidence in Sweden, a nationwide population study was designed.

SUBJECTS AND METHODS

Subjects

A prospective study on the prediction of preeclampsia and IUGR (Paper I)

A prospective study comprising 1,009 consecutive pregnant women attending the municipal antenatal care unit, Malmö University Hospital, who agreed to participate in this study, was performed between January and November 1993. Their medical history was taken and urine sampled at their first routine visits in pregnancy, usually between 10 and 18 post-menstrual weeks (median 13 weeks). Due to financial constraints, a nested case-control study was done primarily, with an option to enlarge the study to include all women.

Two controls were selected for each case of PE, IUGR, or PE+IUGR, being matched for maternal age ($\pm$2 years), gestational age at urine sampling ($\pm$7 days), and parity (0-para or $\geq$1-para).

As the study was planned for a possible prediction of a dichotomous outcome variables (i.e., the presence of preeclampsia and /or IUGR) and the continuous independent variables, together with dichotomous or categorized variables (EGF, HCG, and smoking), we decided to plan the study for conditional logistic regression analyzis on main outcome variables. The characteristics of the preeclampsia subgroup and their controls are given in Table 1, and those of the IUGR subgroup and their controls in Table 2.

Two retrospective series of women with preeclampsia (Paper II)

The study comprised two populations with singleton pregnancies, one collected from 1990 to 1994 at Malmö University Hospital (Malmö series, *n*= 14,510), and the other from the Swedish Medical Birth Register 1993 (National series, *n*=113,211).

Malmö series

The files of the Department of Obstetrics and Gynecology, University Hospital, Malmö, were scrutinized for cases of women with a diagnosis of preeclampsia. To recruit a large number of women with preeclampsia, a 5-year (1990-94) series of all parturients with preeclampsia (*n*=281) was extracted from the records for comparison with a control group comprising all the 2,811 women without preeclampsia, who gave birth in 1993. The demographic and outcome characteristics of the preeclampsia group, and the control group are given in Table 3. As compared with controls, the preeclampsia group was characterized by a significantly greater proportion of preterm deliveries, IUGR, low Apgar score (Table 3), and lower umbilical venous pH (pH=7.28, SD 0.08 vs. pH=7.30, SD

0.09; p=<0.001).

National series

For validation purposes, a National series was compiled from the Swedish Medical Birth Register. To be able to identify a 50% change in preeclampsia related to preterm delivery among smokers, with a 0.05 two-sided significance and 90% power, in a population of 12% moderate smokers and 80% nonsmokers, a study group of 50,000 women was needed. Therefore, all singleton parturients in 1993 with preeclampsia (n=2,865), and preeclampsia related to preterm birth (n=693), were included as cases, and those without preeclampsia (n=110,346) as controls. The data comprised the same independent variables and the same logistic regression model as the Malmö series. The diagnosis of preeclampsia was that obtained from the Medical Birth Register, which did not necessarily comply with the strict criteria used in the Malmö series.

Bivariate and multiple logistic regression analyzes were used to determine a relationship between the outcome variables (preeclampsia or preeclampsia associated with preterm delivery) *vis-à-vis* combinations of the explanatory variables (smoking, maternal age, parity, and fetal gender).

Table 1. Characteristics of mothers and offspring of the preeclampsia subgroup and controls

	Preeclampsia (n=24)		Controls (n=48)		(p)
Mothers					
Age (years)	26.7	3.7	27.4	3.7	0.7
Nulliparae (n)	17	71%	34	71%	1.0
Smokers (n)	3	13%	9	19%	0.7
Mode of delivery					
Vaginal spontaneous (n)	15	63%	41	85%	0.03
Instrumental vaginal (n)	4	17%	5	10%	0.5
Cesarean section (n)	5	21%	2	4%	0.04
Preterm delivery (n)	6	25%	4	8%	0.07
Offspring					
Male sex (n)	14	58%	25	52%	0.6
Birth weight (g)	3,171	785	3,500	549	0.07
Birth length (cm)	48.8	3.7	50.0	2.6	0.12
pH umbilical artery	7.22 (n=16)	0.09	7.21 (n=35)	0.1	0.74
pH umbilical vein	7.3 (n=23)	0.06	7.29 (n=46)	0.09	0.7
Apgar 5 min <7 (n)	0	0%	2	4%	0.5
Placental weight (g)	580 (n=23)	141	616 (n=46)	118	0.3

Mean and standard deviation or number and percentage are given.

Table 2. Characteristics of mothers and offspring of intra-uterine growth restriction (IUGR) subgroup and their controls

	IUGR (n=30)		Controls (n=60)		(p)
Mothers					
Age (years)	27.9	5.3	27.7	5.3	0.8
Nulliparae (n)	18	60%	36	60%	1.0
Smokers (n)	12	40%	8	13%	0.004
Mode of delivery					
Vaginal spontaneous (n)	20	67%	49	82%	0.1
Instrumental vaginal (n)	1	3%	4	7%	0.7
Cesarean section (n)	9	30%	7	12%	0.03
Preterm delivery (n)	6	20%	1	2%	0.005
Offspring					
Male sex (n)	12	40%	37	62%	0.05
Birth weight (g)	2,436	403	3,528	388	<0.001
Birth length (cm)	45.8	2.8	50.3	2.2	<0.001
pH umbilical artery	7.25 (n=19)	0.09	7.22 (n=38)	0.09	0.3
pH umbilical vein	7.32 (n=26)	0.08	7.31 (n=56)	0.09	0.8
Apgar 5 min <7 (n)	1	3%	0	0%	0.2
Placental weight (g)	445 (n=28)	94	619 (n=60)	138	<0.001

Mean and standard deviation, or number and percentage are given.

A retrospective study of APC resistance in women with a history of preeclampsia and/or IUGR (Paper III)

A retrospective study comprising women with IUGR and /or preeclampsia in their history, compared with a control group. A review of the medical records at the Department of Obstetrics and Gynecology, University Hospital, Malmö, for the 14-month period, January 1993 to February 1994, yielded 197 cases of preeclampsia and/or IUGR occurring in pregnancies resulting in live births. Of the 197 women, 153 accepted an invitation to participate in the study, and underwent blood sampling for APC resistance testing (FV:Q506) and detailed anamnesis (including heredity for venous thrombosis and history of spontaneous abortion). After a scrutiny of the patients' records, 31 cases were excluded. Thus, the study group comprised 122 cases: 47 of preeclampsia, 67 of IUGR, and 8 of both preeclampsia and IUGR. The control group included 465 healthy pregnant women delivered at the University Hospital, Malmö (cases of preeclampsia or IUGR being excluded).

Table 3. Characteristics of controls and preeclamptic subgroups in Malmö series

	Preeclampsia*		Controls**		
	n	%	n	%	(p)
	281		2,811		
Maternal age (years)					
<20	9	3.2	62	2.2	0.3
20-24	68	24.2	510	18.1	0.01
25-29	92	32.7	1,034	36.8	0.2
30-34	72	25.6	831	29.6	0.2
≥35	40	14.2	374	13.3	0.7
Parity					
Nulliparae	204	72.6	1,194	42.5	<0.001
Smoking habits					
Non smokers	243	86.5	2,150	76.5	
All smokers	38	13.5	661	23.5	<0.001
1-9 cigarettes daily	17	6.0	423	15.0	<0.001
≥10 cigarettes daily	21	7.5	238	8.5	0.6
Female fetal gender	157	55.9	1,371	48.8	0.02
Outcome variables					
IUGR	32	11.4	91	3.2	<0.000
Preterm delivery	58	20.6	123	4.4	<0.000
Apgar score <7	9	3.2	35	1.2	0.015

Numbers and percentages are given.

* All women with preeclampsia and singleton pregnancy in Malmö during the 5-year period, 1990-94.

** All women with singleton pregnancy delivered in 1993 in Malmö (women with preeclampsia excluded).

A prospective study of APC resistance and pregnancy (Paper IV)

A prospective study comprising 2,496 pregnant women in Malmö who entered the study at their first routine visit during pregnancy to one of the municipal or private antenatal care clinics in Malmö, was conducted between February 1994 and June 1995.

The women enrolled in the study were interviewed by midwives, and answered a detailed questionnaire (including medical history focused on previous thrombosis, fetal loss, and familial history of thrombosis). At this visit, blood was drawn for an APC resistance testing. Of the 2,496 gravidae, 16 were excluded from the analyzis. Of the remaining 2,480 women, 1,899 gave birth at the University Hospital, Malmö, 485 at other Swedish hospitals; 96 women had an abortion.

Main outcome variables were the presence of pregnancy complications (e.g. preeclampsia, IUGR, spontaneous abortion, thrombosis), and blood loss at delivery. No differences in maternal characteristics, mode of delivery, or outcome variables were found between the APC-resistant and the non-APC resistant subgroups.

All pregnancies proceeded without intervention, and the APC resistance

analyzis was not done until at least 3 months after delivery, after that all the other variables were recorded. All medical files were scrutinized for details of pregnancy and delivery. After this, all women were mailed the answers of their APC status together with general and specific information with our advice to APC-resistant women (see the Swedish and English versions of the written general information and specific information to heterozygous carriers of APC resistance who gave birth Appendixes 1 and 2). In addition to the written information, the 7 homozygous women were all booked for personal information as well. Furthermore, we offered daily telephone consultations to all APC-resistant women who wanted additional information.

Table 4. Clinical characteristics of mothers and offspring

	Study group (n=122)		Control group (n=465)		(p)
Mothers					
Age (years)	30.5	20-42	28.0	16-44	0.001
Nulliparae	75	61.5%	217	46.7%	0.006
Smokers*	43	35.2%	101	21.7%	0.007
Mode of delivery					
Vaginal spontaneous	80	65.6%	375	80.6%	0.001
Vaginal operative	8	6.6%	25	5.4%	0.7
Cesarean section	22	18.0%	54	11.6%	0.06
Cesarean section for imminent asphyxia	12	9.8%	12	2.6%	0.001
Neonates					
Sex (Male/ Female)	56 / 66	46%/54%	245/220	52% /48%	0.2
Gestational age at birth (weeks)	39.0	26-42	40.0	31-43	<0.001
Birthweight (g)	2655	930-5050	3500	1765-5000	<0.001
Birthweight deviation (%)**	-22.5%	-67%-36%	0.0%	-21%-+70%	<0.001
5-min Apgar score <7	4	3.3%	9	1.9%	0.5
pH umbilical artery ***	7.22	6.82-7.40 (n=92)	7.22	6.83-7.43 (n=338)	0.800
pH umbilical vein ***	7.30	6.86-7.46 (n=120)	7.32	6.84-7.50 (n=389)	0.08

Median and ranges, or proportions and percentages are given.

A national retrospective case-control study of pregnant women with thrombosis (Paper V).

A retrospective case-control study comprised women who gave birth in Sweden and are registered at the Swedish Medical Birth Register. To get a large number of thrombosis cases and to keep variables independent, we chose a 4-year interval, 1990-93, of cases (*n*=608 out of 479,422), and one year for controls

(1993; *n*=114,940).

The Medical Birth and Hospital Discharge Registers were used to identify all women with pregnancy-related thromboses during this 4-year period. The diagnosed numbers classified as thrombosis cases were those for deep venous thrombosis, pulmonary embolism, or cerebral thrombosis related to pregnancy or the corresponding non-pregnant diagnosis numbers when occurring in conjunction with pregnancy. Details of maternal age, parity, cesarean delivery, multiple pregnancy, and preeclampsia were obtained from register data (explanatory variables). From the total of 479,422 deliveries, a subgroup of 608 women accounted for 625 thromboses. Of the 608 women with thrombosis (308 antepartum, and 300 postpartum), 90 had pulmonary embolism and 518 had deep vein thrombosis. The thrombosis series (*n*=608) was compared with all thrombosis-free parturients in the country during 1993 (*n*=114,940). Bivariate and multiple logistic regression analyzes were used to determine the relationship between the outcome variable (the occurrence of thrombosis) and the explanatory variables. As risk patterns before vs. after delivery may differ, thromboses were divided into antepartum and postpartum subgroups.

The study was designed to detect a 50% change in thrombosis among smokers vis-à-vis non-smokers, with 0.05 two-sided significance and at least 90% power, in a population comprising 20% smokers.

Methods

APC resistance

To identify the presence of the FV:Q506 mutation (Paper III), blood was sampled in EDTA tubes (Vacutainer, Becton Dickinson, Meylon, France), centrifugated at 2000g for 20 min, and frozen and stored at -70°C. DNA was extracted and analysed for the presence of FV:Q506 as earlier described elsewhere (Zöller and Dahlbäck 1994).

In Paper IV a modified functional APC resistance test using factor V deficient plasma: (Coatest APC-resistance-V; Chromogenix, Mölndal, Sweden) was performed as described elsewhere (Svensson and Dahlbäck 1994). Women with an APC-ratio of ≥1.86 were assumed to be non-carriers, a conclusion supported by the results of parallel analyzis with the APC-resistance-V assay and a polymerase chain reaction analyzis of all with an APC ratio below 1.86. The test distinguished reliable APC-resistant from non-APC-resistant women: All women with normal FV genotype had an APC ratio > 1.67 and all APC-resistant women an APC ratio <1.64. The APC analyzes were not done until at least 3 months after delivery and until all other variables were set.

Epidermal growth factor

EGF concentrations in maternal urine were measured with a double antibody human EGF RIA kit, withsynthetic human EGF as the tracer (Amersham International plc, Bucks, England). To eliminate the effect of variation in water excretion, urinary EGF concentrations were correlated to the corresponding urinary creatinine values, the resulting standardized EGF values being expressed in ng EGF per mg creatinine. Each urine sample was assayed in duplicate and masked as to case or control status. The inter- and intra-assay coefficients of variation were less than 3% and less than 9%, respectively.

Human chorionic gonadotropin

Total β-HCG levels were measured using the Technicon Immuno 1® system (Bayer Corp, Tarrytown, NY, USA). The urinary HCG concentrations were also correlated to the corresponding urinary creatinine values, the resulting standardized values being expressed in IU HCG per mg creatinine.

Data Sources

The Swedish Medical Birth Register

This register contains medical information on most deliveries in Sweden (about 99% (Cnattingius et al. 1990). In the study period, 1990-94, International Classification of Diseases 9 diagnosis codes were used. Information on smoking habits is routinely recorded at the first visit to the antenatal care unit. Information on parity, age, pre-pregnancy weight, maternal length, cesarean delivery, and diagnoses such as preeclampsia is usually included in the register. However, due to a reorganization, information on pre-pregnancy maternal weight and length is missing for 1990-91, and this information was not used.

The Swedish Hospital Discharge Register

This register contains information on diagnoses and operation codes of all in -patients at all Swedish hospitals. The register is compiled from local registers in different Swedish counties and is under the supervision of the National Board of Health and Welfare.

Methodological considerations

Diagnosis of preeclampsia

Diagnosis of preeclampsia can be determined according to different criteria, partly depending on the purpose of the diagnosis. Criteria without proteinuria are often erroneous. In clinical practice the diagnosis criteria of preeclampsia (e.g.,

those of the American College of Obstetrics and Gynecology) are set to group pregnancies with similar outcome. However, they cannot be recommended for research into the etiology of preeclampsia, which is why we used only diastolic blood pressure for the diagnosis of pregnancy hypertension (Perry and Beevers 1994), together with proteinuria for the preeclampsia diagnosis.

Study size

One might ask why we presented both the Malmö and the National series in Paper II, rather than the larger National series only. A very large population series has high power, owing to the large number of cases, but at the same time the precision is lower, i.e., full control of each variable is lacking. In a smaller population study, such as the Malmö series, where each preeclampsia diagnosis was checked by the investigator and virtually all gravidae were dated by ultrasound, precision is higher. Two studies, one with high precision and the other with high power with similar results, will complement each other.

During a scrutiny of the individual medical records in the Malmö series, we found a trend toward using the diagnosis of preeclampsia more liberally in complicated cases, thus not satisfying our preeclampsia definition. In complicated cases in general, smoking is known to be more prevalent. Thus, if the finding of a liberal preeclampsia diagnosis were true for the rest of Sweden, the true incidence of preeclampsia among smokers might be lower, and the protective effect of smoking be underestimated in the National series.

Power estimations

For a study of a rare condition with a 0.05 two-sided significance and 90% power, in order to detect a 50% change in response to smoking, which occurs in 20% of the study population, a large study population is needed. In the case of thrombosis during pregnancy (expected incidence 0.001) a sample size of at least 350,000 is needed. To design a study of preeclampsia (expected incidence 0.025) or preeclampsia associated with preterm birth (expected incidence 0.005), and 14,000 and 68,000 would be needed, respectively. Our use of 4 years of cases only one year of controls (Paper V) fulfils the requirement of independent variables, while having a very little impact on power (0.1%). Among the few women occurring more than once, only the first pregnancy was included for analyzis. Another but easier solution of the issue regarding independent variables would have been to include only nulliparous women. However, since both preeclampsia and thrombosis are dependent on parity, we considered the inclusion of parity to be essential. Our method might introduce a small time-dependent selection bias, though,presumably its influence on the results is small.

Determination of increased or decreased risk

Around 3-4 times more subjects are needed to determine a risk-reduction to half

as compared with doubled risk, given a constant prevalence such as APC resistance. Therefore, it is natural that studies on increased risk will appear in larger numbers, and earlier, than the reduced risk.

Risk estimation and design

Risk estimates of rare multigenic events such as thrombosis and an adverse outcome can differ, depending on study design and background risk in the population. If one compares APC resistance in women with thrombosis to controls, the thrombosis cases will have a higher prevalence of thrombophilia and higher acquired risk than controls. Therefore, the risk tends to be overestimated if no adjustment for the other involved factors is done.

Prediction

We considered a 5-fold increased risk as the smallest to be of interest for use in prediction and a 3-fold increased risk for identification of a high-risk group. We therefore designed Papers III and IV so as to be able to detect a 5-fold increase in risk with reasonable power. A low sensitivity and a low PPV imply that only few of the women who could benefit from preventive intervention would be identified, but most women who proved positive would undergo unnecessary treatment. Thus, since PPV is dependent on the prevalence of a condition, tests will perform better if made on a high risk-group in terms of PPV. The recent treatment study of antioxidants for prophylaxis of preeclampsia used a doppler velocimetry test to identify a homogeneous highrisk group for inclusion in the study (Chappell et al. 1999).

Reliability of smoking information

There are no studies on the reliability of reported smoking habits in the Swedish Medical Birth Register using objective methods such as cotinine in maternal urine when smoking was recorded. However, the birthweight deficit and OR for IUGR for moderate and heavy smokers from the Medical Birth Register are similar (-200 g and OR 2 and 2.5, respectively) to those reported by others (Ericson et al. 1991). This indicates that the figures are valid to some extent.

Non-differential vs. differential bias of smoking information

Since information on smoking habits was gathered prospectively, the putative misclassification of smoking could be expected to be non-differential, i.e., the misclassifications will presumably affect cases and controls similarly – and ORs will not be affected at all. On the other hand, if smoking habits had been collected retrospectively, the results would have been affected by several forms of biases, e.g. recall bias, drop-out bias, and the misclassifications in such cases tend to be differential, i.e. the ORs would be severely affected.

Statistics

Differences between groups

The Students *t*-test was used for normally distributed continuous variables. In studies III and IV, blood loss values were converted into their natural logarithms to normalize a skewed distribution before analyzis. The Mann-Whitney test was used for non-normally distributed variables. Chi -square test or Fisher's exact test was used for categorical variables and the Mantel-Haenszel X^2-test was for degree of linear association. In Paper I, conditioned logistic regression analyzis was used for main dichotomous output variables and in Papers II and V, unconditional logistic regression analyzes were used.

Calculation of risk

Odds ratio (OR) was used as an approximation of the relative risk. An OR approximates how much more likely (or unlikely) the outcome is for those with the variable present (X=1) than among those with it absent (X=0). An OR >1.0 indicates a risk exceeding that of controls, and the inclusion of 1.0 in the 95% confidence interval (CI) indicates a probability (P) of $\geq$ 0.05.

In Papers II, IV, and V cross-tabulations with the chi-square test and a 95% CI were used for bivariate analyzes. In studies II and V, ORs for independent variables were determined with multiple logistic regression analyzes of the respective outcome variables *vis-à-vis* combinations of the independent (explanatory) variables.

Correlation

Spearman's rank correlation was used for a correlation assessment.

Calculation of selection advantage

(Selection advantage) ^{generation} = ratio between carriers and non-carriers as compared with the original relation. This is a simplified model of an idealized population where no other selection mechanisms are involved. Generation is the number of successive generations where the advantage has been present. Selection advantage is the calculated advantage of carriers, compared with non-carriers, per generation.

Statistical package

All calculations were performed with SPSS software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, USA) version 7.5 – except for conditional logistic regression analyzis, where STATA software (Stata Corp, Texas, USA) was used. *p*-values <0.05 were considered statistically significant. All tests presented are two-tailed.

RESULTS

Prediction of preeclampsia and /or IUGR

Association of EGF and HCG in maternal urine with preeclampsia and /or IUGR

Of the 897 pregnancies included in Paper I, 22 were subsequently complicated by PE, 28 by IUGR, and two by both PE and IUGR. For the purpose of analyzis the two pregnancies with both PE and IUGR were included in both PE and IUGR subgroups. The study series as a whole (*n*=156) comprised 52 women and their 104 controls.

Table 5. Epidermal growth factor (EGF) and human chorionic gonadotropin (HCG) in maternal urine

	EGF (ng EGF / mg creatinine)		HCG (IU HCG / mg creatinine)	
IUGR subgroup				
IUGR (n=30)*	26.2 (19.7-33.9)	p=0.031	230.3 (85.6-585.7)	p=0.18
Controls (n=60)*	30.3 (22.1-40.0)		354.8 (107.3-758.6)	
Preeclampsia subgroup				
Preeclampsia (n=24)*	30.7 (19.3-39.7)	p=0.16	416.8 (219.0-723.7)	p=0.22
Controls (n=48)*	27.3 19.7-31.6		206.4 (70.7-532.3)	
Study group as a whole				
Non-smokers (n=126)	30.1 (22.1-38.7)	p=0.028	321 (120.9-660.9)	p=0.026
Smokers (n=30)	25.8 (17.7-30.5)		185.5 (31.0-479.3)	

Median and quartile 1 and 3 are given.
* For the purpose of analyzis, 2 women with both preeclampsia and IUGR and their 4 controls, were included in both IUGR and preeclampsia subgroups.

Urinary EGF levels were significantly lower in the IUGR subgroup than in

their controls, but no such difference was found between the preeclampsia subgroup and their controls (Table 5). HCG values tended to be higher in the preeclampsia subgroup as a whole than in their controls (Table 5). As compared with women whose HCG levels were below the median, women with HCG levels above the median were at 3-fold greater risk of developing preeclampsia (OR 3.0, 95% CI 1.1– 9.2).

In the study group as a whole (n=156), smokers (n=30) were found to have lower EGF and HCG levels than non-smokers (Table 5). In addition, both EGF and HCG levels manifested an inverse correlation to smoking habits (Rho – 0.2, p=0.03, and Rho – 0.2, p=0.03, respectively). A correlation was found to exist between EGF and HCG levels in urine (Rho 0.35, p<0.01), but there was no correlation between placental weight and either EGF or HCG level (Rho 0.06, p=0.05 or Rho 0.07, p=0.39, respectively). The woman with the lowest urinary EGF level had a lupus anticoagulant syndrome.

Association of APC resistance with preeclampsia and IUGR

The frequency of APC resistance (Paper III) was 18% (22/122) in the study group of women as a whole (those with preeclampsia and/or IUGR) and 10% (43/423) in the control group, the difference being significant (p=0.02)(Table 6); one woman in the study group and 2 in the control group carried the FV:Q506 allele in homozygous form. APC resistance was present in 13% (6/47) of the isolated PE subgroup and 18% (12/67) of the IUGR subgroup (neither of them differed significantly from the control group). The frequency of 50% (4/8) in the small PE+IUGR subgroup differed significantly from that in the control group (p=0.008)(Table 6).

In the prospective study (Paper IV) the corresponding proportion of APC resistance was 11% (14/127) in the combined preeclampsia and IUGR subgroup, compared with 11% (243/2022) in the background population, 13% (5/39) in the isolated preeclampsia subgroup, and 10% (9/88) in the isolated IUGR subgroup. No woman with APC resistance had both PE and IUGR (0/8)(Table 6).

Risk of pregnancy complications

Smoking habits and risk of preeclampsia

During the study period of the Malmö series (Paper II), the incidence of preeclampsia in singleton pregnancies was 1.9% (281/14,510) of which 58 ended preterm.

Table 6. Comparison of APC resistance and preeclampsia and/or IUGR subgroups

	APC resistance (n)	(%)	Non-APC resistance (n)	(p)*
Paper III				
Isolated preeclampsia	6	12.8%	41	0.8
Isolated IUGR	12	17.9%	55	0.09
Preeclampsia and IUGR	4	50.0%	4	0.008
Control group	46	9.9%	419	
Paper IV				
Isolated preeclampsia	5	16.1%	26	0.3
Isolated IUGR	9	11.2%	71	0.9
Preeclampsia and IUGR	0	0%	8	0.3
Control group	243	10.7%	2022	

* Compared with respective control group.

Table 7. Effect of smoking on the risk of preeclampsia and preeclampsia associated with preterm birth: Logistic regression analyzis of Malmö series*

	Preeclampsia (n=281)	Controls (n=2811)	OR**	95%CI	(p)
Smoking habits					
Nonsmoker	243	2,150	1.0	reference	
1-9 cigarettes daily	17	423	0.4	0.2-0.6	0.0001
≥10 cigarettes daily	21	238	0.9	0.5-1.4	0.6
	Preeclampsia associated with preterm birth (n=58)	Controls (n=2811)	OR**	95%CI	(p)
Smoking habits					
Nonsmoker	53	2,150	1.0	reference	
1-9 cigarettes daily	1	423	0.1	(0.01-0.7)	0.02
≥10 cigarettes daily	4	238	0.8	(0.3-2.2)	0.6

The table includes odds ratios (ORs) and 95% CI of included variables.
* Malmö series: Women with preeclampsia delivered 1990-94 in Malmö, compared with those without preeclampsia, delivered 1993.
** Adjusted by parity, fetal gender, and maternal age.

Multiple logistic regression analyzis in the Malmö series showed moderate smoking (1-9 cigarettes daily) to be associated with a 3-fold lower risk of preeclampsia, and with a 10-fold lower risk of PE associated with preterm birth (Table 7). The corresponding figures of risk for IUGR among preeclamptic women in Malmö series were 2.3-fold increased risk among moderate smokers and 3-fold increased risk of IUGR among heavy smokers (Table 8).

Table 8. Risk of IUGR related to smoking among women with preeclampsia in Malmö series

	Preeclampsia		
	OR	95%CI	(p)
Smoking habit			
Nonsmoker	1.0*	reference	
1-9 cigarettes	2.34	(1.79-3.09)	<0.0001
>=10 cigarettes	3.02	(2.18-4.19)	<0.0001
All smoking	2.57	(2.03-3.24)	<0.0001

Adjusted by parity, fetal gender, and maternal age.

Table 9. Effect of smoking on the risk of preeclampsia and preeclampsia associated with preterm birth: Logistic regression analyzis of National series*

	Preeclampsia (n=2865)	Controls (n=110,346)	OR**	95%CI
Smoking habit				
Nonsmoker	2,378	83,717	1.0	reference
1-9 cigarettes daily	224	13,878	0.6	0.5-0.7
≥10 cigarettes daily	117	7,929	0.6	0.5-0.7
Missing data	146	4,822		

	Preeclampsia associated with preterm birth (n=693)	Controls (n=110,346)	OR**	95%CI
Smoking habits				
Nonsmoker	566	83,717	1.0	reference
1-9 cigarettes daily	60	13,878	0.6	0.5-0.8
≥10 cigarettes daily	29	7,929	0.6	0.4-0.8
Missing data	38	4,822		

Table includes odds ratios (ORs) and 95% CI of included variables.
* National series: all singleton parturients in Sweden with preeclampsia 1993, compared with those without preeclampsia, and registered at the Swedish Medical Birth Register.
** Adjusted by parity, fetal gender, and maternal age.

In the National series, the overall incidence of preeclampsia was 2.5% (2,865/113,211) of whom 693 were delivered preterm. Both moderate and heavy

smoking were associated with a significantly reduced risk of preeclampsia and preeclampsia associated with preterm birth (Table 9).

Risk of fetal loss

Association of fetal loss and APC resistance

No significant relationship was found between APC resistance and early or late spontaneous abortions in Papers III and IV (Table 10).

Table 10. APC resistance and spontaneous abortions in history (Paper III) and women with fetal loss (Paper IV)

	APC-resistant subgroup		Non-APC-resistant subgroup		(p)
Paper III (n=587)*	(n=68)		(n=519)		
Number of spontaneous abortions in history (n)	19		242		0.1
Paper IV (n=2480)**	(n=270)		(n=2,210)		
Women with fetal loss in present pregnancy (n)					
Spontaneous abortion	9	3.3%	54	2.4%	0.4
Late spontaneous abortion	3	1.1%	16	0.7%	0.5
Women with fetal loss in history (n)					
Early spontaneous abortion	51	18.9%	409	18.5%	0.9
Late spontaneous abortion	3	1.1%	50	2.3%	0.2

* Women with history of preeclampsia and/ or IUGR and controls
** Unselected women

Homozygous individuals

There were 2 women with early spontaneous abortion in their present pregnancy among the 7 homozygous carriers of APC resistance in Paper IV. One of these 2 women, who also had a protein S deficiency, had a history of eight spontaneous abortions and one intra-uterine fetal death. One of the 4 homozygous women included in Paper III had had a spontaneous abortion.

Risk of pregnancy-associated thromboembolism

Incidence of thrombosis

The incidence of pregnancy-related thrombosis in Sweden was found to be 13/10,000 pregnancies (Paper V). The results of bivariate analyzis of Paper V are shown in Table 11.

Table 11. Bivariate analyzis of selected risk factors and thrombosis (Paper V)

	Thrombosis (n=608)(%)	Controls (n=114,940)(%)	OR	95% CI
Maternal age (years)				
<20	26 (4.3)	2,817 (2.5)	1.8	1.2- 2.7
20-34	492 (80.9)	97,904 (85.2)	1.0	reference
35+	90 (14.8)	14,219 (12.4)	1.3	1.0- 1.6
Parity				
Para 0	304 (50.0)	47,425 (41.3)	1.8	1.5- 2.2
Para 1	142 (23.4)	40,734 (35.4)	1.0	reference
Para 2	93 (15.3)	18,113 (15.8)	1.5	1.1- 1.9
≥ Para 3	69 (11.3)	8,429 (7.3)	2.4	1.8- 3.1
missing data	0 (0)	239 (0.2)		
No. of cigarettes daily				
0	423 (69.6)	87,408 (76.0)	1.0	reference
1-9	80 (13.2)	14,295 (12.4)	1.2	0.9- 1.5
≥10	57 (9.4)	8,177 (7.1)	1.4	1.1- 1.9
missing data	48 (7.9)	5,060(4.4)		
Multiple pregnancy				
No	593 (97.5)	113,330 (98.6)	1.0	reference
Yes	15 (2.5)	1,610 (1.4)	1.8	1.1- 3.0
Preeclampsia				
No	562 (92.4)	111,788 (97.3)	1.0	reference
Yes	46 (7.6)	3,152 (2.7)	2.9	2.1- 3.9
Cesarean delivery				
No	420 (69.1)	102,181 (88.9)	1.0	reference
Yes	188 (30.9)	12,759 (11.1)	3.6	3.0- 4.3

Odds ratios (ORs) and 95%CI are given.

The results of logistic regression analyzis of antepartum thrombosis are shown in Table 12. Only parity differed significantly as regards the risk of thrombosis and neither preeclampsia nor advanced age (at least 35 years of age) was associated with an increased risk of antepartum thrombosis.

Table 12. Logistic regression analyzis of selected risk factors and antepartum thrombosis

	Thrombosis (n=308)(%)	Controls (n=114,940)(%)	OR	95% CI
Maternal age (years)				
<20	10 (3.2)	2,817 (2.5)	1.0	0.5- 1.9
20-34	262 (85.1)	97,904 (85.2)	1.0	reference
≥35	36 (11.7)	14,219 (12.4)	1.0	0.7- 1.4
Parity				
Para 0	178 (57.8)	47,425 (41.3)	2.9	2.1- 3.9
Para 1	60 (19.5)	40,734 (35.4)	1.0	reference
Para 2	36 (11.7)	18,113 (15.8)	1.3	0.8- 2.0
≥ Para 3	34 (11.0)	8,429 (7.3)	2.8	1.8- 4.4
missing data	0 (0)	239 (0.2)		
No. of cigarettes daily				
0	221 (71.8)	87,408 (76.0)	1.0	reference
1-9	39 (12.7)	14,295 (12.4)	1.1	0.8- 1.5
≥10	28 (9.1)	8,177 (7.1)	1.3	0.9- 2.0
missing data	20 (6.5)	5,060 (4.4)		
Multiple pregnancy				
No	299 (97.1)	113,330 (98.6)	1.0	reference
Yes	9 (2.9)	1,610 (1.4)	2.1	1.0- 4.6
Preeclampsia				
No	301 (97.7)	111,788 (97.3)	1.0	reference
Yes	7 (2.3)	3,152 (2.7)	0.8	0.4- 1.6

Odds ratios (ORs) and 95%CI are given

* Multivariate analyzis was adjusted for maternal age, parity, multiple pregnancy, smoking, and preeclampsia.

The results of multiple logistic regression analyzis of postpartum thrombosis are shown in Table 13. Cesarean delivery was associated with a five-fold increased risk of postpartum thrombosis. There was an progressive increase in the rate of cesarean delivery with increasing age (Fig. 3). The risk of postpartum thrombosis was twice as great in the para 2 and para 3 subgroups as in the para 1 (reference) subgroup, and three-fold greater in the preeclampsia subgroup and also in the youngest age group (under 20 years old), than in their corresponding reference classes.

Smoking

In the thrombosis series as a whole (Paper V), i.e. in both ante- and postpartum subgroups, smoking was associated with a significantly increased risk of thrombosis (OR 1.24; 95% CI 1.02, 1.51). There was a statistically significant association between the increased risk of thrombosis and increasing tobacco consumption (p=0.007), but the difference vis-à-vis non-smokers was significant only for heavy smokers (OR 1.41; 95% CI 1.04 – 1.82). For moderate smokers the corresponding figures were OR 1.11; 95% CI 0.87 – 1.41.

Table 13. Logistic regression analysis of selected risk factors and postpartum thrombosis

	Thrombosis (n=300)(%)	Controls (n=114,940)(%)	OR	95% CI
Maternal age (years)				
<20	16 (5.3)	2,817 (2.5)	2.5	1.4- 4.4
20-34	230 (76.7)	97,904 (85.2)	1.0	reference
≥35	54 (18.0)	14,219 (12.4)	1.2	0.9- 1.6
Parity				
Para 0	126 (42.1)	47,425 (41.3)	1.1	0.8- 1.5
Para 1	82 (27.3)	40,734 (35.4)	1.0	reference
Para 2	57 (19.0)	18,113 (15.8)	1.7	1.2- 2.4
≥ Para 3	35 (11.7)	8,429 (7.3)	1.8	1.2- 2.9
missing data	0 (0)	239 (0.2)		
No. of cigarettes daily				
0	202 (67.3)	87,408 (76.0)	1.0	reference
1-9	41 (13.7)	14,295 (12.4)	1.2	0.8- 1.7
≥ 10	29 (9.7)	8,177 (7.1)	1.4	1.0- 2.1
missing data	28 (9.3)	5,060 (4.4)		
Multiple pregnancy				
No	294 (98.0)	113,330 (98.6)	1.0	reference
Yes	6 (2.0)	1,610 (1.4)	0.6	0.2- 1.4
Preeclampsia				
No	261 (87.0)	111,788 (97.3)	1.0	reference
Yes	39 (13.0)	3,152 (2.7)	3.0	2.0- 4.4
Cesarean delivery				
No	177 (59.0)	102,181 (88.9)	1.0	reference
Yes	123 (41.0)	12,759 (11.1)	4.9	3.8- 6.3

Odds ratios (ORs) and 95%CI are given

* Adjusted for age, parity, multiple pregnancy, smoking, cesarean delivery, and preeclampsia

Figure 3

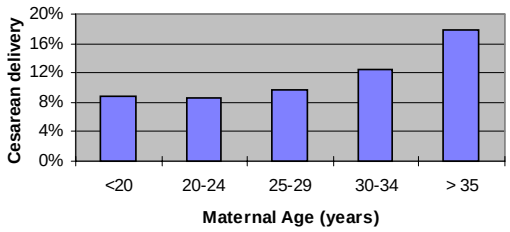
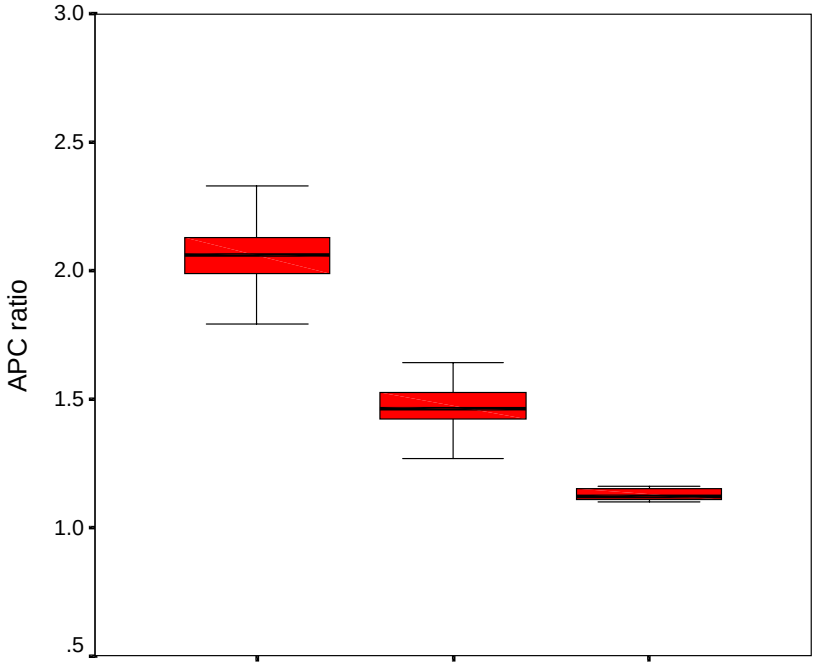


Figure 3. Cesarean delivery rate in different age-groups.

APC resistance

The overall prevalence of APC resistance (in Paper IV) was found to be 10.7% (270/2480) in the Malmö Population, 7 women (0.3%) carrying the FV:Q506 allele in its homozygous form and 263 being heterozygous. All women with normal FV genotype had an APC ratio >1.67, and all those with an FV:Q506 genotype had an APC ratio ≤1.64 (Fig. 4)



Non-APC Resistance (*n*=2044)	Heterozygote APC resistance (*n*=263)	Homozygote APC resistance (*n*=7)

Figure 4. Relationship between FV-genotype and APC ratio in a modified APC resistance test. Boxplot show the interquartile range together with the highest and lowest values, excluding outliers.

The incidence of thrombosis in the present pregnancy was 0.24% (6/2480; 3 antepartum and 3 postpartum thromboses) in the series as a whole, being 8-fold higher in the APC-resistant subgroup than in the non-APC-resistant subgroup (1.11% [3/270] vs. 0.14% [3/2210], respectively) (Table 14). Among the 9 women with a history of thrombosis, four thromboses were associated with pregnancy, four with oral contraceptive usage, two with operations, and one with no known risk factor. One woman in the non-APC-resistant group had experienced two earlier thromboses, and one of the APC-resistant women with an earlier thrombosis suffered a thrombosis in the present pregnancy despite low molecular weight heparin prophylaxis.

Heredity and overweight

Of the 127 women with familial thrombosis, 4 had thromboses in conjunction with pregnancy, one of them in her present pregnancy and four in earlier pregnancies, representing an 8-fold higher incidence than in the subgroup without familial thrombosis (Table 14). The familial thrombosis subgroup did not differ significantly from the non-familial thrombosis subgroup regarding the prevalence of APC resistance (14.2% [18/127] vs. 10.7% [252/2353]; p=0.2). The results of the study also suggest that overweight is a risk factor for thrombosis and 6 of the 319 overweight women had had thromboses, 2 of them in the present pregnancy (Table 14).

Table 14. Risk of thrombosis for women with APC resistance, familial thrombosis,* or overweight

	Present thrombosis (n)		OR	95% CI	Present or historic thrombosis (n)**		OR	95% CI
	Yes	No			Yes	No		
APC resistance								
Yes	3	267	8.3	(1.7-41.2)	5	265	4.6	(1.5-13.9)
No	3	2,207			9	2,201		
Familial thrombosis*								
Yes	1	126	3.7	(0.4-32.1)	4	123	7.6	(2.4-24.6)
No	5	2,348			10	2,343		
Overweight***								
Yes	2	315	3.4	(0.6-18.8)	6	311	5.2	(1.8-15.1)
No	4	2,159			8	2,155		

Odds ratios (OR) and 95%CI are given.

* Familial thrombosis: thrombosis among first-degree relatives (i.e., father, mother or siblings).

** One APC-resistant woman got a thrombosis despite low molecular weight heparin prophylaxis and one of the non-APC-resistant women had two earlier thromboses.

*** Overweight was defined as a body mass index (kg/m^2) > 27.6 (i.e., >1 SD above the mean for the series).

Homozygous individuals

None of the 7 homozygous women in Paper IV had a thrombosis in her present pregnancy or during a previous pregnancy (*n*=18). One of the 7 homozygotes, who also had protein S deficiency, had a first-degree relative with a history of thrombosis. She was now delivered by cesarean delivery, as she had been in three of her four previous term pregnancies. The four homozygous women in Paper III, had had 8 pregnancies.

Risk of bleeding complications associated with delivery

Blood loss and APC resistance

In the series as a whole (Paper III)(*n*=482), the APC-resistant women were characterized by lower intrapartum blood loss (*p*=0.001) and a lower risk of profuse intrapartum bleeding (>600 ml) (*p*=0.01) than non-APC-resistant women (Table 15). Also in the control group, the difference in intrapartum blood loss between those with and those without FV:Q506 mutation was significant (*p*=0.02).

Table 15. Intrapartum blood loss among APC-resistant and non-APC-resistant women (Paper III)

	Blood loss (ml)***				Blood loss >600 ml		
	mean	mean -1 SD	mean +1 SD	(p)	(n)	%	(p)
Study group*							
APC resistance							
Yes	330	245	446	0.34	0	0	0.11
No	370	167	824		11	16	
Control group							
APC resistance							
Yes	318	228	443	0.018	1	3	0.07
No	380	236	614		47	13	
Combined Study and Control group							
APC resistance							
Yes	322	234	443	0.001	1	2	0.01
No	379	220	652		58	14	

Geometrical mean ±1 SD or number and percentage are given
* Pregnancies complicated by preeclampsia and/or IUGR.
** Measured in women delivered vaginally and without heparin treatment.
*** The blood loss values were transformed to natural logarithms, to normalize skewed distribution for the purpose of statistical analyzis.

Pre-post partum hemoglobin (Hb) values for the groups in Paper III are given

in Table 16. It is noteworthy that, as compared with non-APC-resistant women, APC-resistant women were characterized by lower pre-post partum differences in Hb values, and fewer cases of postpartum anemia, although the differences were not significant in all subgroups. A significant correlation was found to exist between the estimated blood loss and pre-post partum difference in Hb values (-0.51, $p<0.001$).

Table 16. Pre-post partum hemoglobin difference and postpartum anemia, among APC-resistant and non-APC-resistant women (Paper III)*

	Hemoglobin difference (g/l)			Postpartum anemia****		
	Mean	SD	(p)	(n)	%	(p)
Study group						
APC resistance						
Yes (n=16)**	4.2	11.8	0.001	0	0	0.007
No (n=60)***	-12.1	18.2		20	33	
Control group						
APC resistance						
Yes (n=23)	-3.0	9.5	0.02	1	4	0.08
No (n=245)	-8.2	17		48	20	
Combined Study and Control group						
APC resistance						
Yes (n=39)	0.0	10.9	0.000	1	3	0.003
No (n=305)	-8.9	17.3		68	22	

Mean and standard deviation (SD) or number and percentage are given.
* vaginally delivered women not treated with heparin and with complete hemoglobin values
** Includes 10 women with IUGR fetuses and 7 women with preeclampsia
*** Includes 38 women with IUGR fetuses and 24 women with preeclampsia
**** Hemoglobin value < 100 g/l on the second day after partus

As compared with the non-APC-resistant subgroup, the APC-resistant subgroup in Paper IV was characterized by a significantly lower risk of profuse intrapartum bleeding (p=0.02) and significantly less intrapartum blood loss (p=0.04)(Table 17). Moreover, among women who delivered in Malmö (n=1,899), the duration of postpartum hospitalization was significantly shorter in the APC-resistant subgroup than in the non-APC-resistant subgroup (2.5 and 2.8 days, respectively; p=0.048).

Table 17. Intrapartum blood loss and postpartum anemia in APC-resistant and non-APC-resistant subgroups (Paper IV)

	APC resistance (n=217)*	%		Non-APC resistance (n=1920)*	%		(p)
Blood loss > 600 ml (n)	8	3.7%		152	7.9%		0.02
Postpartum anemia**(n)	13	6.0%		131	6.8%		0.6
	mean	mean -1 SD	mean +1 SD	mean	mean -1 SD	mean +1 SD	
Blood loss during delivery (ml)***	340	235	494	361	232	562	0.04

Geometrical mean ±1 SD or number and percentage are given

* Only vaginally delivered non-heparin treated women were included.

** Postpartum anemia was defined as Hb <100 g/l on the second day after delivery.

*** Blood loss value was converted to its natural logarithm to normalize a skewed distribution.

DISCUSSION

Prediction of preeclampsia and /or IUGR

Prediction of preeclampsia

Despite decades of research, there is still no reliable predictive test for preeclampsia. Prediction and prevention of preeclampsia are presumably intimately linked, and both problems will only be solved by further research of its pathophysiology and etiology. Although necessary, prospective studies on prediction of preeclampsia tend to be unrewarding high-risk studies. The finding that women with a HCG level in the urine of at least median run a 3-fold increased risk of preeclampsia, compared to those with HCG level below median, could be used to identify a group of women with high risk of preeclampsia (Paper I). However, with our nested case-control design it was not possible to make calculations of predictive measure. Neither EGF values, nor an APC resistance test, was found to be of value for the prediction of preeclampsia.

Our understanding of the mechanisms governing placentation and fetal growth or the finding of a gene predisposing to preeclampsia, would be a breakthrough in the research of preeclampsia. Zhou et al. (1997) have reported most interesting results regarding placentation. They found the normal change in phenotypic appearance of cytotrophoblasts to endothelial cell behavior, to be impaired in preeclampsia.

Prediction and prevention of IUGR

We found the EGF level in maternal urine to be lower in pregnancies that developed IUGR. This may give a clue to the pathophysiological mechanisms underlying IUGR. Our results may indicate that EGF is one of the growth factors that govern fetal and/or placental growth and that EGF deficiency might be a determinant for IUGR. However, the difference between cases and controls was too narrow to be of predictive use.

The maternal causes of IUGR, if identified, offer putative possibilities of prevention. The anti-smoking campaign has been successful over the last 35 years, bringing the prevalence of smokers during pregnancy from 44% down to 20%, which presumably has been a major reason for the decrease in perinatal morbidity and mortality during this period.

There might be several nutritional deficiencies yet to be found, such as the identified risk group of Muslim women with “head cover” who, due to lack of exposure to the sun and fortified provisions, have an impaired vitamin D status (Marya et al. 1988). This causes shorter babies, which could be prevented by giving vitamin D supplements to the gravidae. Other possible causes of IUGR are

folate deficiency, among the 10% of women homozygous to the methylenetetrahydrofolate reductase (MTHFR) TT gene polymorphism, and heritable defects in lipid metabolism.

Adverse outcome and APC resistance

Preeclampsia

One study has reported a significantly increased risk of preeclampsia with preterm delivery among carriers, compared with non-carriers, of APC resistance (8.9% vs. 4.2%)(Dizon Townson et al. 1996), but most studies reported no significant differences: 22% vs.10% (Lindoff et al. 1997), 18.2% vs. 10.2% (Paper III), 5.3 vs. 5.5% (Shaughnessy et al. 1999), and 1.9% vs. 1.5% (Paper IV). There might be a slightly increased risk of preeclampsia among carriers (one- to two-fold). However, none of the studies has been designed to detect such small differences with a reasonable degree of power. We performed our studies with the intention of using APC resistance to predict preeclampsia or IUGR. That is why, they were designed to detect a 5-fold increased risk.

Intra-uterine growth restriction (IUGR)

One case-control study has shown an increased risk of IUGR among carriers of APC resistance (21.3% vs. 10.2%; p=0.006)(Paper III). However, in the isolated IUGR subgroup, no difference was found, but half of the small IUGR and preeclampsia subgroup of eight women were APC-resistant. This finding could not be shown in either of two recent papers (11.4% vs. 6.4%; Kupferminc et al. (1999) (3.3% vs. 3.6%; Paper IV). In the latter, which was of prospective design, neither birthweight nor weight deviation distinguished between carriers and non-carriers of the FV:Q506 mutation. Moreover, none of the 8 women in the preeclampsia and IUGR subgroup was APC-resistant. Therefore, we conclude that the presence of APC resistance is not associated with lower birthweight, nor is it useful for the prediction of IUGR in the general population. Our conclusion was supported by the report by Wisotzkey et al. (1999) showing no difference in the prevalence of APC resistance between placentas from IUGR and control pregnancies.

Spontaneous abortions

In the general population, we found no association between APC resistance and early or late spontaneous abortions in the index pregnancies, nor in their history (Paper IV). This is the only study with a prospective design of an unselected pregnant population and in view of its high power, differences in the incidence of spontaneous abortions depending on APC resistance status are unlikely.

However, this does not exclude the possibility of a higher prevalence of APC resistance in small subgroups of fetal loss.

The results of studies of subgroups of thrombosis cases differ. Preston et al. (1996) reported no significant differences, but Meinardi et al. (1999) found a significantly increased risk of miscarriages among carriers, vis-à-vis non-carriers. Among women with a history of preeclampsia and/or IUGR, no significant differences were found (Paper III).

Women with recurrent miscarriages, a subgroup comprising 1% of our prospective study population (Paper IV), have been of great interest. This subgroup is known to be related to other thrombophilias and an adverse pregnancy outcome (Brenner et al. 1997). Two studies have found APC resistance to be associated with an increased risk among women with recurrent abortions, reporting a 2-4 fold increased risk among carriers, vis-à-vis non-carriers (Grandone et al. 1997; Ridker et al. 1998). Other studies have not confirmed these findings (Balasch et al. 1997; Dizon Townson et al. 1997; Kutteh et al. 1998; Coumans et al. 1999). However, these studies were not designed to detect a doubled risk among APC-resistant women.

Tal et al. (1999) reported women with one spontaneous abortion or more, vis-à-vis no fetal loss, to be characterized by a significantly higher prevalence of APC resistance (14.4% and 5.6%, respectively).

Most interesting results have been reported by Dizon-Townson et al. (1997) who showed FV:Q506 mutation to be more prevalent in placentas with large infarcts ($\geq$10%) than in those with smaller infarcts (<10%) or none at all. Moreover, a significantly higher prevalence of APC resistance was found in aborted than in control fetuses. However, these results might not be generalized as there is a balance in gene frequency according to the Hardy Weinberg equilibrium, which does not support large losses in the APC resistance gene pool (Paper IV; Ehrenforth et al. 1999).

Stillbirth

In a series of women with obstetric complications (n=110) APC resistance was found in 3 of 20 and protein S deficiency in 7 of the 20 women with a history of stillbirth, as compared with a 6% APC resistance among controls (Kupferminc et al. 1999). However, in a large thrombosis cohort, the prevalence of stillbirth did not differ between carriers and non-carriers of APC resistance (Preston et al. 1996). In a group of couples with late fetal loss, APC resistance was found in 6.5% (15/232) of women with stillbirth and 1.5% (7/464) of controls ($p<0.001$)(Gris et al. 1999). However, 11 of the 15 couples with APC resistance were also homozygous for the MTHFR TT gene polymorphism, which makes the results more difficult to interpret. To conclude, it is still too early to decide whether APC resistance is a risk factor for stillbirth, or not.

Abruptio placentae

Wiener-Megnagi et al. (1998) have reported APC resistance in almost 30% (8/27) of women with abruptio placentae vs. 3% (1/29) among controls. This interesting result remains to be confirmed by larger series. In our prospective study, no differences were found (Paper IV).

Adverse outcome

Kupferminc et al. (1999) have grouped pregnancy complications such as adverse outcome including preeclampsia, IUGR, and abruptio placentae. They found a significantly increased prevalence of APC resistance among women with an adverse outcome (20% vs. 6%). When we grouped preeclampsia and /or IUGR subgroups under "adverse pregnancy outcome cases", there was a significant two-fold increased risk for APC-resistant women in Paper III, but not in the prospective study reported in Paper IV.

Bleeding complications

Our studies found smaller blood losses and a lower incidence of profuse blood loss during delivery among carriers of APC resistance (Papers III and IV). It might not seem of interest that the hypercoagulable condition APC resistance is associated with reduced blood loss during delivery, but since pregnancy has been associated with a 10% accumulated mortality rate for the past 200 years, and in view of the historical fact up to half of all maternal deaths were caused by profuse blood loss or anemia, it is certainly of interest. Carriers of APC resistance might have an evolutionary selection advantage reflected by a lower mortality in profuse blood loss, which would explain the present high prevalence of APC resistance (See page 59).

Screening

General screening for APC resistance before prescribing oral contraceptives (OC) has been advocated by some authors (Dahlbäck 1995; Hellgren et al. 1995; Rosendaal et al. 1995; Faioni et al. 1997; Ridker et al. 1997), though others have argued against it (Vandenbroucke et al. 1996; Altes et al. 1997). A positive effect of screening might be that all carriers would know that they have a slightly higher general tendency of developing thrombosis. In addition, screening would identify those with homozygosity who are reported to run an 80-fold increased risk of thrombosis (Rosendaal et al. 1995). Critics maintain that screening is not cost effective (Vandenbroucke et al. 1996; Altes et al. 1997), and that women do not want to know and might be frightened by the information.

Several issues remain to be elucidated before the question of general screening before prescribing OC can be answered. Is screening cost-effective? How do women react to an awareness of being APC-resistant? Does the knowledge affect their choice of anticonceptive? Is it safer for women to know their APC status?

Will APC-resistant women be discriminated by life insurance companies? Some requirements for screening would already be satisfied: APC resistance can be tested easily and cheaply and preventive methods are available, e.g. avoidance of OC or thrombosis prophylaxis in high-risk situations.

General screening for APC resistance before, or in early pregnancy, as screening for increased risk of pregnancy complications has been discussed (De Stefano et al. 1998). Our prospective study (Paper IV) provides basic data which may be of value in the continuing discussion of the pros and cons of APC resistance screening. We conclude that general APC resistance screening before pregnancy could not be justified, since an identification of APC resistance would mean at the most a double risk of adverse outcome and a 1.1% risk of thrombosis among carriers. Moreover, prophylactic treatment might increase other complications such as osteoporotic fractures and bleeding complications. In the future, selective APC resistance testing might be warranted in situations when information of an additional 5-fold risk of thrombosis would alter the clinical management.

Thrombosis and pregnancy

Thrombosis incidence

We estimated the thrombosis incidence related to pregnancy to be 13/10000, which is somewhat higher than generally reported. However, it is not possible to compare incidence figures from different regions without taking into consideration differences in the prevalence of thrombophilia. With some exceptions the APC resistance prevalence in Europe shows a north-south gradient, with 10-15% in Sweden and 2% in south Europe, which may explain some of the differences in thrombosis incidence reported. Therefore, the different reports do not need to stand contradictory to one other. In Figure 4 we show the proportion of thromboses having APC resistance in selected studies . Similarly, differences in the prevalence of APC resistance will be reflected by differences in thrombosis incidence.

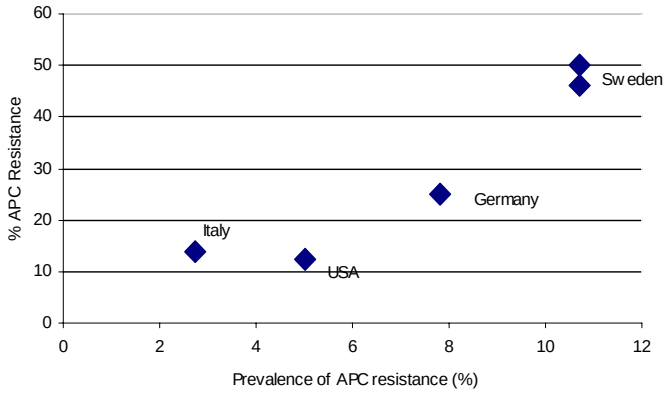


Figure 4. Proportion of women with APC resistance among thrombosis cases Italy: Rodeghiero et al.(1999), USA: Ridker et al. (1997), Germany: Ehrenforth et al. (1999), Sweden: Bokarewa et al. (1996) and Paper IV.

The even distribution between ante- and postpartum thromboses that we found and the preponderance of antepartum thromboses reported by Macklon and Greer (1996), is probably part of a change occurring in the past decade. Earlier postpartum thrombosis was more prevalent, presumably due to a strict regime of bed rest in the postpartum period.

Risk estimation of thrombosis during pregnancy

Heredity and overweight

Heredity of thrombosis and overweight are known risk factors for thrombosis and our calculated risk estimates of a 5-fold increased risk might be useful for individual risk estimation.

Cesarean delivery

Since cesarean deliveries are so prevalent (11%), the 5-fold increased risk makes cesarean delivery related to almost half of all cases of postpartum thrombosis (43%). The increase in cesarean deliveries with increasing age tallies with earlier reports and is suggested to reflect a progressive, age-related deterioration in myometrial function (Rosenthal and Paterson Brown 1998).

Preeclampsia

Surprisingly, women with preeclampsia, who are usually reported as a hypercoagulable group at increased risk of thrombosis, were found not to be at an increased risk of thrombosis in the antepartum period. This might be explained by the more pronounced fibrinolysis than fibrin formation in women with preeclampsia (Borok et al. 1984). In bivariate analyzis we found the thrombosis risk to be increased 5-fold during the postpartum period, but after adjustment for other variables, it was only 3-fold increased. The reason for this adjustment was mainly the greater prevalence of cesarean deliveries among women with preeclampsia.

Age

Our finding of a significantly increased risk of thrombosis among women with advanced age in bivariate analyzis agrees with earlier reports (Macklon and Greer 1996). However, after an adjustment for other variables, the significance of this risk disappeared. This was mainly attributable to an increased prevalence of cesarean delivery with increasing age. Thus, we did not find a statistically significant association between advanced age and thrombosis in our large series. Our figures are similar to those reported by Macklon and Greer (1996), though we draw different conclusions regarding age as a risk factor (Paper V).

Parity

Our findings agree with an increased risk among multiparous gravidae, when compared with those with one previous delivery, but we also found a 3-fold increased risk among primiparas in the antepartum period, with 58% of thrombosis cases being primiparas.

Hereditary thrombophilias

As large prospective studies are not available, it is more difficult to estimate the risk of thrombosis in cases of certain heritable thrombophilias (protein C, protein S, prothrombin A20210 mutation, MTHFR TT gene polymorphism, and antithrombin deficiencies). However, risk estimates have been made, in which individuals with heterozygous deficiency of protein S and protein C appear to run a risk similar to that in those with heterozygous APC resistance (Zöller et al. 1999). The risk of prothrombin A20210 mutation has been estimated to be 3–4-fold increased (Zöller et al. 1999) and the risk to carriers of MTHFR TT gene polymorphism has been reported to be twice that of non-carriers. The risk among individuals with antithrombin deficiency appears to be somewhat higher than APC resistance (10–20-fold increased risk) and in symptomatic families, a high prevalence of thrombosis related to pregnancy has been reported (Zöller et al. 1999).

APC resistance

Our finding of an 8-fold increased risk of thrombosis among heterozygous women agrees with earlier reports. The high prevalence (10.7%) of APC resistance (FV:Q506) among gravidae in Sweden makes it a major risk factor for thrombosis during pregnancy, present in some 30-60% of women with pregnancy-related thrombosis (Paper IV). The distribution of APC resistance among mainly Caucasians explains at least part of the regional difference and the heredity of thrombosis, although we did not find any significant difference in the prevalence of APC resistance between women with vs. without thrombosis heredity (14.2% vs. 10.7%; p=0.2)(Paper IV).

We found that APC-resistant gravidae ran a 1.1% risk of thrombosis, which differs from the 0.2–0.25% risk reported by McColl et al. (1997). However, the numbers on which the estimates were based were low in both studies, with a high degree of uncertainty. Moreover, the estimate of a cumulative 5.7% thrombosis risk of APC resistance at the age of 65 tallies with our figures (Rodeghiero and Tosetto 1999).

The risk of thrombosis among homozygous carriers of the FV:Q506 alleles has been estimated to be 80-fold (Rosendaal et al. 1995). When using our figures of thrombosis incidence, this would mean that 11% of homozygous women are liable to develop thrombosis during each pregnancy. However, since none of the 7 homozygotes had thrombosis in their current (n=7) or previous pregnancies (n=18), the suggestion of an 80-fold increased risk among homozygotes is not supported by our present results.

Prophylaxis during pregnancy

Hirsch et al. (1996) suggested that APC-resistant women should be offered prophylactic treatment from early pregnancy, based on the assumption that 60% of women with pregnancy-associated thrombosis were APC-resistant. The results of our Paper IV argue against this recommendation, as the 1.1% risk of developing thrombosis for APC-resistant women can hardly warrant prophylactic treatment. The risks of such treatment would probably outweigh the possible beneficial effects. However, prophylaxis may be justifiable if additional risk factors for thrombosis are present, such as a heredity of thrombosis, cesarean delivery, overweight, etc., though the level of risk at which prophylaxis is indicated remains to be determined.

In order to elucidate the question whether or not to initiate prophylaxis, a thorough investigation of the risks and benefits of prophylaxis is mandatory. Dahlman (1993) reported 2% osteoporotic fractures and 1% major bleeding complications, when standard unfractionated heparin treatment was given during pregnancy. Low molecular weight heparin treatment is thought to be associated with fewer complications, though, no proof has yet been presented for pregnancy treatment. Furthermore, the protective effect of low molecular weight heparin

treatment against thrombosis during pregnancy remains to be established. Presently, only women at high risk, mainly women with earlier thrombosis, are given prophylactic heparin treatment during pregnancy. Treatment of this high-risk group may reduce the risk of re-thrombosis during pregnancy, but does not prevent "de novo" thrombosis. We have to decide at what level of risk the beneficial effect outweighs the risk for both the ante- and postpartum period. Since the latter is shorter, and only one individual is treated, it would be logical to set a lower risk level for an initiation of postpartum prophylaxis. The duration of treatment is shorter, too.

Including our studies, there is now sufficient information to make an adequate individual estimation of the thrombosis risk based on the presence or absence of risk factors such as APC resistance, heredity of thrombosis, cesarean delivery, overweight, etc. By using individual risk estimation we can determine set the level of risk when we recommend thrombosis prophylaxis, instead of trying to make a crude classification of risk, as high or low.

Information to APC-resistant women (see Appendix 1 and 2)
We have generally recommended that APC-resistant women should inform their physician of their APC resistance in any case of surgery, pregnancy, or contraceptive counselling. By informing in this way we let their physician decide whether or not prophylaxis is indicated, depending on the nature of surgery and other factors. We have recommended heterozygote carriers preferably to use contraceptives other than OC, and we recommended homozygous carriers not to use OC at all due to the very high risk. Our extra service with telephone consultations was only used by a few, but all homozygous carriers accepted additional information.

The reason why we chose to give more detailed information regarding OC is that the prescriptions are more spread, and previously all women with thrombophilia were advised not to use OCs. In addition, almost all women with previously known thrombophilia have additional risk factors, such as a history or a heredity of thrombosis.

Smoking during pregnancy

Cigarette smoking is presumably the largest preventable risk factor for adverse pregnancy outcome. Therefore, the aim of all who are interested in perinatal morbidity and mortality must be to reduce the number of smokers during pregnancy. It is important to elucidate both the short-term and long-term effects of cigarette smoking.

Since most gravidae are young, smoking will not usually yet have had any deleterious effects. However, the dread of adverse effects on their pregnancy, fetus, or their child´s future lives might be the first time they seriously consider

stopping smoking. To present adequate information is therefore of crucial interest in order to lower the number of smokers during pregnancy.

In Sweden the reported prevalence of cigarette smoking during pregnancy has been slowly decreasing, from around 44% in the 1960s (Kullander and Källén 1971), to around 30% in the 1980s, and 22% in early 1990s (Cnattingius et al. 1997)(Table 18).

Table 18. Change in smoking habit among pregnant women in Malmö.

	Non-smokers (%)	Moderate smokers (%)	Heavy smokers (%)
1963*	56.0	35.2	8.8
1985**	64.2	19.0	16.4
1994**	78.2	14.7	6.6

* From study by Kullander and Källén (1971)

** From Swedish Medical Birth Registry

Among pregnant women in general, smokers run an increased risk of perinatal morbidity and mortality (Ericson et al. 1991; Cnattingius et al. 1997). Smokers have been shown to run a 40% increased risk of preterm delivery (Wisborg et al. 1996). Thus, the high prevalence of smoking in pregnancy (20%), makes it a major determinant of prematurity. Smoking is also known to be a major causative consumption-dependent factor associated with the development of IUGR, with a 2–3-fold increased risk. There have been reports of an average reduction of 200 g in birthweight of babies of mothers who smoke (Ericson et al. 1991). Yet the mechanisms by which smoking causes IUGR are still not known.

There is an increased risk of a sudden infant death syndrome among children whose parents smoked during pregnancy and in the postpartum period (Blair et al. 1996; Cnattingius and Nordström 1996). Both the number of cigarettes and the number of persons smoking in the household increase the risk in a consumption-dependent manner. Blair et al. (1996) conclude that sudden infant death syndrome can be reduced by almost two-thirds if parents refrain from smoking.

Smoking and preeclampsia

The finding that moderate smoking was associated with a lower risk of developing preeclampsia was in agreement with earlier reports (Cnattingius et al. 1997). However, moderate smokers were also found to be associated with a lower incidence of preterm delivery among women with preeclampsia makes the suggestion that some factor related to smoking protects against preeclampsia

more convincing (Paper II). The way in which this protection works is not known, but several mechanisms are conceivable. For instance, Davis and colleagues (1987) found an increase in prostacycline metabolites (6-keto PGF1 alpha) in smokers as pregnancy advanced, but noted a decrease in non-smokers. This will in turn lower the thromboxane A2/prostacycline ratio, and presumably facilitate vasodilation (Walsh 1985). Nicotine too induces relaxation of smooth muscle by increasing the release of nitric oxide, a potent vasodilator (Toda et al. 1994). It is known that in women with preeclampsia the normal increase in plasma volume, is inhibited but there is a compensatory increase in Hb in the second trimester. The decrease in Hb values found in smokers might reflect an increased plasma volume (Huisman and Aarnoudse 1986).

Preeclampsia is associated with widespread endothelial damage and increased serotonin levels (Middelkoop et al. 1993). The vascular balance mediated by serotonin shifts towards vasoconstriction (Steyn and Odendaal 1997). Smoking alters the serotonin metabolism probably via nicotine receptors (Racke et al. 1992), and might thereby affect the development of preeclampsia.

In the general population, smokers tend to have lower blood pressure than non-smokers (Green et al. 1986). Thus, smoking might mask the development of preeclampsia when using diagnosis criteria with a fixed blood pressure limit rather than an increase in blood pressure.

Preeclampsia is a hypocalciuric state (Taufield et al. 1987), and it has been suggested that calcium supplementation during pregnancy lowers the incidence of preeclampsia (Lopez Jaramillo et al. 1997). Smokers have lower parathyroid hormone levels than non-smokers (Landin-Wilhelmsen et al. 1995), whereas women with preeclampsia tend to have increased levels (August et al. 1992). Thus, a restricted supply of calcium might be a characteristic of preeclampsia, and the lower parathyroid hormone levels in smokers may reflect an increased availability of calcium. Furthermore, smoking alters adrenal cortex steroid production by lowering the activity of 11β and 21β hydroxylase (Barbieri et al. 1989; Hautanen et al. 1993), resulting in a more androgenic steroid balance, which in turn contributes to an altered calcium metabolism.

In view of the above-mentioned mechanisms, we suggest that nicotine might be the agent protecting against preeclampsia.

To present some 'beneficial' effects of smoking during pregnancy might seem irresponsible by an obstetrician. However, the value of this information lies in the search for a possible agent or mechanism that will reduce the incidence of preeclampsia. If nicotine is a protective agent it could be administered in some other form, rather than cigarette smoke and could in the future, be used to prevent or to treat preeclampsia. However, this does not necessarily establish that it is the smoke per se that gives this protection. Smokers differ in several ways from non-smokers, which we were unable to adjust for. Morbidity and mortality rates are reported to be higher among smokers who develop preeclampsia (Duffus and

MacGillivray 1968; Cnattingius et al. 1997). Therefore, with present knowledge, it cannot be justified to recommend women to smoke during pregnancy in order to lower the risk of preeclampsia.

The finding that 25% of parturients with preeclampsia in the National series gave birth preterm, vis-à-vis only 20% in the Malmö series, was paradoxical as we applied stricter criteria of preeclampsia in the Malmö series, and would have expected the converse relation. We could only speculate as to the reasons for this. The diagnosis criteria in the National series included systolic blood pressure and there might be a relation between systolic blood pressure and preterm delivery. There might be a more 'benign' preeclampsia in the Malmö area, or there might have been a 'liberal' application of the preeclampsia diagnosis in complicated cases in the National series, a subgroup with a known preponderance of smokers.

Smoking and IUGR

Our finding that smokers had significantly lower EGF levels and that women with anticipated IUGR fetuses were associated with lower urinary EGF levels, is interesting (Paper I). We know that smoking increases frequency of IUGR, but we do not yet have proof of the mechanism. Our results suggest that smoking might interfere with local growth factors such as EGF, which govern fetal and placental growth and would thereby restrict growth. In vitro studies of human placental tissue have shown that maternal cigarette smoking is associated with selective alterations in two major EGF-associated receptor-mediated pathways thought to be involved in cell growth and differentiation in the human placenta.

Smoking and thrombosis

We found that smokers run an increased risk of pregnancy-associated thrombosis. This increase was consumption dependent. The reason for this increased risk of thrombosis is not known, but inhibited or defective fibrinolysis in smokers might be one explanation (Mercelina-Roumans et al. 1996).

Although smoking has not previously been associated with thrombosis in fertile women, it has been accepted as a fact in Sweden and it has been customary to inform women that smoking increases the risk of thrombosis related to pregnancy. To the best of my knowledge, the basis of this was a finding of altered fibrinolytic activity in female smokers above 35 years of age (Kjaeldgaard and Larsson 1983).

Blood loss and evolutionary selection advantage

The clinical impact of anemia and profuse blood loss during delivery in ancient times - a historical background

Our suggestion of a possible evolutionary selection advantage of APC resistance conferred by decreased number of profuse hemorrhages is based on an assumption of the historical importance of profuse blood loss as a cause of maternal mortality. Since the FV:Q506 mutation in APC resistance is thought to have occurred in one individual some 21,000 to 34,000 years ago (Zivelin et al. 1997), a brief background description on anemia and the clinical significance of profuse blood loss during childbirth in ancient times is motivated in order to understand the discussion regarding the evolutionary selection mechanism. To obtain reliable information on human life at the time of the FV:Q506 mutation is impossible, but we might use the earliest sources available.

Before the discovery of antibiotics and the perfection of such techniques as cesarean section and blood transfusion, rich and poor were equally affected by maternal mortality. A study of the ruling families of Europe showed a maternal mortality rate of 2% per birth between A.D. 1500 and 1850, the cumulative mortality risk during pregnancy being approximated to 10% (Dobbie 1982). This is close to the 2.3% mortality rate in the 16th century London (Forbes 1971). These figures might well be an adequate estimate of the population as a whole and presumably, mortality rates had not been lower prior to that. Sir Percivall Willughby (1596-1685), a male midwife in London, wrote on profuse blood loss as a cause of maternal mortality in the 17th century, and expressed his despair at being powerless against exsanguination death (Note 1).

In 17th- and 18th-century England, excess female mortality was reported during the first year after marriage (Dobbie 1982). This finding has been attributed to the very high mortality rate during first pregnancy that occurred soon after marriage. In 18th century rural France, the maternal mortality rate was well over 1%, as it also was in Sweden. In late 19th and early 20th century Sweden, 40% of women dying between 20 and 34 years of age died in childbirth (Högberg and Broström 1985). Puerperal sepsis caused 54% of maternal deaths among hospitalized women in the late 19th century (Högberg and Wall 1986), but in Swedish parishes, the main causes were hemorrhage, eclampsia and obstructed labor (Högberg and Broström 1985).

Note 1.
"I hold the flux of blood deadly, if it be great, I never heard of any woman that escaped, but that they all perished.... Many have perished through this sac accident and usually it proves fatal to all women. If possible, I heartily could wish, that some worthy practicer would be pleased to direct some powerful ways, or medicines, to bridle this raging destroying evil. Women would have cause to acknowledge his worth, and all succeeding ages would give him thanks...I confess my ignorance, and I believe, that there is no other, but God alone, that can do this work, to help the woman. "

Sir Percivall Willughby (1596-1685)

The rarity of maternal death in the modern, industrially developed world is a relatively new phenomenon. In the USA as late as 1920, the maternal mortality rate was 0.8%, and until 1935 the registered mortality rate for England and Wales remained constant at about 0.4% (WHO 1991). Anemic women have a highly increased case fatality rate from sepsis, eclampsia, and profuse blood loss and the combination of anemia and obstetric hemorrhage has been estimated to be responsible for up to 50% of maternal deaths (Högberg and Broström 1985). Thus, a condition that conferred on the carrier a lower incidence of anemia or profuse blood loss during pregnancy presumably reduced the maternal fatality rate. The reduction of anemia by adequate antenatal care, combined with the development of blood transfusion technique are thought to be two of the most significant explanations for the decline in maternal deaths caused by antepartum and postpartum hemorrhage (Högberg 1985). The present maternal mortality rate in Sweden has been approximated to be 0.007% per delivery (Högberg et al. 1994).

Selection advantage from sickle cell anemia

If we have reason to assume that homozygous carriers of a gene are less physically fit, the theory of natural selection then requires that heterozygous carriers have a selective advantage, i.e., the contribution of such individuals to the gene pool of succeeding generations is above the average.

These specific mechanisms have been extremely difficult to identify in Man. It has been reasonably certain that the heterozygous carriers have a selective advantage only in carriers of sickle cell anemia. In 1954 Allison reported a higher incidence of carriers of sickle cell anemia (sicklers) in malaria-prevalent regions. Moreover, compared with non-sicklers, sicklers were less likely to have a palpable spleen, indicating malaria. Thus, since malaria is associated with high mortality and sicklers suffered less often and less severely from malaria, they ought more often to survive. These findings were taken as a proof of an

evolutionary selection advantage for sicklers in malaria endemic regions. If this were not so, the sickler gene pool would be slowly depleted due to the inability of homozygote carriers of the sickler trait to reproduce themselves. Thus, in every homozygous individual in a population two sickle-cell genes are lost in every generation. In 1967 Wiesenfeld (1967) constructed a mathematical model of the evolutionary advantage, based on Allison's proposal (Allison and Phil 1954). Subsequently it has been shown that heterozygote carriers of the sickle-cell trait have a 2–8-fold increased rate of sickling malaria parasitized cells, and that these cells would be more effectively removed from circulation by phagocytosis (Luzzatto et al. 1970).

APC resistance and evolutionary selection advantage

Carriers of APC resistance have an increased incidence of thrombosis and for every carrier succumbing to a lethal thrombosis, one or two APC resistance genes will be lost. Since APC resistance arouse from a single mutation (FV:Q506), there are no 'de novo' mutations to fill up the APC resistance gene reservoir. Thus, APC resistance must confer on the carriers an evolutionary advantage in order to be so prevalent.

What conceivable mechanisms could be involved in an evolutionary selection mechanism of APC resistance? Any quality that increases the number of carriers of APC resistance, as compared with non-carriers, in a given population over generations. The advantage could be male or female, could affect by one, or by several means. A slight increase per generation, as compared with non-carriers, will generate an appreciable difference in hundreds of generations (see below). Events after or at the end of the fertile period will have little or no impact on the reproductive capacity in a population.

Since pregnancy is the most hazardous event in a woman's fertile life and APC resistance is involved in the anticoagulant system, it was logical to focus on factors related to blood loss during pregnancy that could improve the maternal mortality rate, e.g. a lower prevalence of anemia, lower incidence of profuse blood loss, and fewer spontaneous abortions. If APC-resistant women possess any of these properties, this would have been an advantage. However, these factors could have interfered with each other, and secondary anemia due to a profuse hemorrhage during delivery or during a spontaneous abortion might not have been compensated before the next pregnancy, resulting in a high fatality rate. Moreover, orphans had a very short life expectancy after the death of their mother (Högberg and Broström 1985). A hereditary condition reducing the risk of blood loss during delivery would have been selected in such an environment. Even though thrombosis during pregnancy is a clinically serious problem, as many as 15% of maternal deaths in Sweden are due to pulmonary embolism (Högberg 1986). The 5–10-fold increased risk of thrombosis in APC-resistant women might not have been a particularly potent adverse survival factor during the evolution.

Fictitious example of selection advantage of APC resistance

We suggested that, owing to fewer cases of profuse hemorrhage, more APC-resistant women might avoid anemia, would be in better health, and fewer would die from exsanguination during delivery. Accordingly, it is tempting to speculate that, over time, the reduced incidence of profuse blood loss during delivery among APC-resistant women might have provided them with a survival advantage. The following theoretical example illustrates the potency of the gene mutation on reproductive capacity. Although this example is fictitious, calculation of the effect is based on assumptions that are in no way inconsistent with reality.

(1) Carriership of a FV:Q506 is associated with a 50% reduction in pregnancy mortality due to profuse hemorrhage, as in 17th century London, 10% of women died of pregnancy complications and we assume that 20% of these were bleeding complications - yielding a survival advantage of 1.01;

(2) The annual incidence of thromboembolic complications in female non-carriers of FV:Q506 genotype is 1/10,000, women with the mutation run a 7-fold increased risk of thromboembolic complications, and the span of reproductive life is 30 years;

(3) Of the thrombotic episodes, 20% are complicated by pulmonary embolism which proved fatal in 15% of cases. From assumptions 2 and 3, the mortality risk associated with thromboembolic complications is estimated to be 0.00063 (i.e., 0.0001*7*30*0.2*0.15)(Sipes and Weiner 1990).

Based on the above figures, the following calculation can be made of the magnitude of a selection effect. After one generation, 1.0094 times more survivors will carry the mutation (i.e., 1.01*(1–0.00063)= 1.0094). The FV:Q506 mutation is estimated to have occurred 25,000 years ago, i.e. 1,000–1,400 generations ago. Groube estimated the world population to have been at least 2 million individuals 20,000 years ago (Groube 1996). Thus, assuming that the FV:Q506 mutation occurred in a Caucasian population of 200,000 individuals (and disregarding other selection mechanisms), after 1,000 generations, the prevalence of the mutation would be 11,500 times the original prevalence (1.0094^{1000}) and the prevalence of the mutation would be 6% (11,500/200,000).

Present situation

We do not know if carriership of APC resistance is an advantage or a disadvantage in present-day society. A study by Mari et al. (1996) found a similar prevalence of APC resistance among centenarians (individuals above hundred years of age) as in the general population, which indicates risk similar

to others. The survival advantage of APC resistance probably changes with time. The better we prevent and treat anemia and bleeding complications, the smaller will be the benefits of APC resistance.

The change from an advantage to the present risk might well have occurred in the time period after 1935, a period of dramatic change in maternal mortality. Until this period, 60% of gravidae had a Hb value below 70% (Törner 1933). Initiation of iron therapy was shown to be effective in increasing Hb values and lowering of the incidence of postpartum anemia (from 41% to 18%)(Törner 1933). The prevention of anemia is believed to be a major reason for the decline of maternal mortality and presumably this prevention also decreased the fatality rate of other pregnancy complications. The paper by Törner was one of the studies on which the Swedish antenatal health care system was based. Due to the never ending search of reducing risk in pregnancy and an excellent maternal antenatal health care, Sweden has today one of the lowest rates of maternal mortality.

We are trying to identify factors, in early pregnancy, that affect the risk of pregnancy complications, and, hopefully, our work will help to identify the right individuals for preventive measures, or the right preventives for individuals at risk.

> "When blood issueth forth in a large quantity, it is good to deliver the woman speedily... Otherwise, the woman is likely to perish"
>
> Sir Percivall Willughby (1596-1685)

SUMMARY and CONCLUSIONS

Summary

The purpose of this thesis was to improve our background knowledge for making a reliable medical evaluation at the first visit to the antenatal health care unit, already when the woman is in her 13th gestational week. In early pregnancy, there is no reliable way to predict which pregnancies will turn out to be complicated. The five studies were focused on the prediction and the risk estimation of preeclampsia, IUGR, and thrombosis.

To attain this goal we have used biochemical analyzes, genetic tests, anamnestic information, and statistical information (based on the data from medical files and from the Swedish Medical Birth and Hospital Discharge Registers).

The biochemical tests that have been evaluated are: epidermal growth factor (EGF) and human chorionic gonadotropin (HCG) levels in maternal urine. EGF is known to be essential for normal fetal development in mice and deficiency in mid-gestation is associated with growth-restricted pups. The exact function of HCG during pregnancy is not known, but studies have shown HCG levels in plasma to be higher in women who later developed preeclampsia. Our hypothesis was that a relative deficiency of EGF, or high HCG values, would predict the development of IUGR and/or preeclampsia.

The newly discovered coagulation anomaly, APC resistance (FV:Q506), caused by a single point mutation in the gene coding for coagulation factor V (FV) was evaluated. This causes a lifelong procoagulatory state due to impaired inactivation of activated FV and activated factor Vlll, resulting in a 5-fold increased risk of venous thrombosis. It is believed that the mutation occurred in one person 25,000 years ago; today some 10-15% of Swedes are now carriers of the mutation. A potentially dangerous mutation, such as APC resistance, ought to be rare, and should be reduced – if not eradicated by selection during the course of human evolution. Thus, APC resistance must confer the carriers with an advantage in evolution in order to be so prevalent.

Both preeclampsia and IUGR are associated with an activated coagulation system, which leads to increased numbers of placental infarcts. The intervillous placental flow resembles venous circulation in terms of its low pressure and low flow velocity, and may be particularly susceptible to thrombotic complications in thrombophilic women. Lupus anticoagulant syndrome, an acquired thrombophilia with functional resistance to APC, is characterized by a high incidence of early onset severe preeclampsia, IUGR, spontaneous abortion, and thrombosis. It was therefore logical to hypothesize APC resistance to predispose to the above-mentioned pregnancy complications.

Paper I

In the first study, urine was sampled prospectively from 1,009 consecutive pregnant women at their first routine visit at 13 weeks of gestation in order to find a test that would predict preeclampsia or IUGR.

The study showed that a high maternal urine HCG level in early pregnancy was associated with a 3-fold increased risk of preeclampsia, vis-à-vis low values, while low EGF levels were associated with IUGR pregnancies. Maternal smoking was related to a lower level of both EGF and HCG.

Paper II

The second paper comprised two series of pregnant women, one Malmö series comprising all women with preeclampsia during a 5-year period, who were compared with healthy gravidae, and a nationwide validating series. The objective was to ascertain whether smokers had any protection against preeclampsia. Since perinatal morbidity and mortality among women with preeclampsia is due mainly to preterm delivery, we concentrated on this subgroup.

Moderate maternal smoking (1 to 9 cigarettes /day) was associated with a lower incidence of preeclampsia, and preeclampsia associated with preterm birth both in the local Malmö series (OR=0.4, CI 0.2-0.6 and OR=0.1, CI 0.01-0.7, respectively) and in the validating series from the Swedish Medical Birth Register (OR=0.6, CI 0.5-0.7 and OR=0.6, CI 0.5-0.8, respectively).

Paper III

A retrospective study comparing 122 women with IUGR and /or preeclampsia in their history with 465 healthy controls. Blood was sampled for APC resistance testing (FV:Q506) and the women were interviewed. Blood loss during delivery and Hb measurements were monitored.

APC resistance was more prevalent in the study group as a whole, compared with controls. However, half of the small subgroup of 8 women with both preeclampsia and IUGR were APC-resistant; neither the subgroup of women with preeclampsia nor those with IUGR differed significantly from the controls. The mean number of spontaneous abortions in history did not distinguish between APC-resistant and non-APC-resistant subgroups. However, the former lost less blood, and fewer of them had a profuse hemorrhage associated with delivery (322ml vs. 379ml, and 2% vs.14%, respectively).

Paper IV

A prospective study of 2,480 women was performed to evaluate the natural course of pregnancy in APC-resistant women. The gravidae were interviewed and blood was drawn for APC resistance testing at 13 weeks of gestation.

The prevalence of APC resistance among women was 10.7%. Apart from an 8-fold increase in thrombosis, APC resistance was not associated with other

pregnancy complications, such as, preeclampsia, IUGR, abruptio placentae, early or late miscarriage, when compared with non-APC-resistant women. The lower blood loss and fewer cases of profuse hemorrhage among APC-resistant women found in paper III were confirmed (340ml vs. 361ml, and 3.7% vs. 7.9%). Among other risks, both overweight women, and those with first-degree relatives with thrombosis, ran an about 5-fold increased risk of thrombosis.

Paper V

Out of 479,422 deliveries recorded at the Swedish Medical Birth and Hospital Discharge Registers, we have identified 608 women with 625 thromboses. To determine the incidence of pregnancy-related thrombosis and its relation to selected risk factors, the women with thrombosis were compared with those without thrombosis delivered in Sweden 1993.

The incidence of pregnancy-associated thrombosis in Sweden was 13/10,000, evenly distributed in the ante- and postpartum periods. Of the factors evaluated, only parity was found to be discriminative in the antepartum period and both primi- and multi-paras ran an increased risk of thrombosis, as compared to those with one earlier birth (OR=2.9, CI 2.1-3.9 and OR=2.8, CI 1.8-4.4, respectively). In the postpartum period, however, both cesarean delivery and preeclampsia were associated with an increased risk of postpartum thrombosis (5-, and 3-fold increased risk, respectively). Maternal smoking was associated with a consumption dependent increased risk of thrombosis related to pregnancy.

Conclusions

It might be possible to identify gravidae with a high risk of developing preeclampsia, by analyzis of HCG in maternal urine. The difference in EGF levels found between IUGR and control pregnancies was too small to be of use in predicting IUGR. However, the finding might give a clue as to the pathophysiological mechanisms involved in governing fetal and/or placental growth. The finding that both EGF and HCG levels were lower among smokers than in non-smokers indicates that smoking might cause IUGR by influencing local growth factors.

The finding that moderate smoking was associated with a lower incidence of preeclampsia associated with preterm birth supports the suggestion that some factor related to smoking protects against preeclampsia. We suggested that nicotine might be the agent causing this “protection”. If true, it could be administered in preparation other than cigarettes in order to prevent the development of preeclampsia in the future.

Apart from an 1.1% risk of thrombosis, APC-resistant women were not associated with preeclampsia, IUGR, or spontaneous abortion. Thus, our

results do not support the idea of general screening for APC resistance in early pregnancy.

We suggest that APC resistance might have given the carriers an evolutionary selection advantage. The procoagulant effect of APC resistance may have constituted a survival advantage, reflected in a lower historic mortality rate of severe bleeding episodes during delivery, resulting in the high prevalence of APC resistance among Caucasians.

The incidence of pregnancy-associated thrombosis in Sweden was 13/10,000. The finding of a consumption-dependent increased risk of thrombosis among smokers might be used in information to pregnant women, with the intention of reducing the number of smokers.

The risk estimates of thrombosis reported in the present studies might be used to identify those women running a high risk of thrombosis, in order to plan preventive measures.

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SWEDISH SUMMARY

Svensk sammanfattning

Inledning

För att kunna sätta in resurser där de behövs bäst, finns det en önskan att kunna förutsäga vilka graviditeter som kommer att bli komplicerade. Vid preeklampsi (havandeskapsförgiftning), tillväxthämning hos fostret samt blodproppssjukdom finns det idag inget säkert sätt att tidigt i graviditeten veta vilka som kommer att drabbas. Denna oförmåga att kunna förutse vilka som kommer att drabbas har försvårat studier på bakomliggande mekanismer samt sökandet efter en behandling.

Syftet med dessa studier är att öka kunskapen om risken för gravida kvinnor att drabbas av ovan nämnda komplikationer. Utgångspunkten har varit att förbättra underlaget till den bedömning som görs vid första besöket, när kvinnan är i 13:e graviditetsveckan. För att uppnå detta mål har vi i fem arbeten studerat och analyserat kemiska tester, genetiska tester, anamnestiska uppgifter samt relevanta statistiska uppgifter.

Graviditetshormon och 'epidermal growth factor'

De kemiska tester som vi har studerat är 'epidermal growth factor' (EGF), en tillväxtfaktor för bland annat huden och magtarmkanalen. Djurförsök har visat att brist på EGF gav tillväxthämmade foster. Hypotesen var att EGF var med och styrde moderkakans funktion och fostrets tillväxt samt att brist kunde ge tillväxthämning eller preeklampsi. Graviditetshormonet HCG finns normalt bara i blodet hos gravida och används därför som graviditetstest. Funktionen av HCG är ej klarlagd, men man har sett att halten HCG i blodet hos kvinnor som får preeklampsi är högre än hos dem som inte drabbas av detta tillstånd.

APC-resistens

Vi undersökte det nyupptäckta ärftliga tillståndet, APC-resistens, som ger blodet en ökad tendens att levra sig (koagulera) samt en livslång ökad risk att drabbas av venös blodpropp. APC-resistens uppstod för cirka 25 000 år sedan genom att en aminosyra i vår genetiska uppsättning förändrades till en annan, en punktmutation. Denna förändring finns nu hos cirka 1 miljon svenskar och cirka 50 miljoner Kaukasier, men den finns nästan inte alls i t.ex Afrika och Asien. Både vid preeklampsi och tillväxthämning utvecklas moderkakan på ett felaktigt sätt i den tidiga graviditeten, men tillstånden visar sig först under graviditetens andra hälft. Vid båda tillstånden finns det en aktiverad koagulation, vilket ofta leder till ökat antal blodproppar (infarkter) i moderkakan. Eftersom cirkulationen i moderkakan liknar den venösa, med lågt tryck och flöde, var det logiskt att anta att APC-resistens kunde predisponera till den bakomliggande sjukligheten i

moderkakan vid ovan nämnda tillstånd. Om ett starkt samband hittades, skulle det kunna användas för att förutsäga vilka graviditeter som skulle kunna drabbas. Ett potentiellt farligt tillstånd som APC-resistens skall normalt vara sällsynt, det skall selekteras bort i människans evolution. Den höga förekomsten tyder på att APC-resistenta individer måste ha en evolutionär fördel, annars hade inte tillståndet varit så vanligt.

Publikation I

I det första arbetet följe vi 1009 kvinnor som lämnat urinprov i tidig graviditet för att hitta en analys som kunde förutse utvecklingen av preeklampsi och / eller tillväxthämning. Kvinnor som födde tillväxthämmade barn hade lägre EGF-halt i urinen i den tidiga graviditeten. Fyndet indikerar att EGF kan vara ett av de tillväxthormon som styr moderkakans funktion eller fostrets tillväxt. Kvinnor med höga värden av HCG i urinen hade en trefaldigt ökad risk att få preeklampsi än kvinnor med lägre halt. Detta skulle kunna användas för att identifiera den grupp gravida som har en ökad risk för preeklampsi. Även rökare hade lägre nivå av både EGF och HCG i urinen. Vi spekulerade i möjligheten att rökning kunde ge tillväxthämmning genom att påverka tillväxtfaktorer, såsom EGF. Detta är intressant, då vi sen länge vet att rökning orsakar tillväxthämning, men utan att känna till de exakta mekanismerna.

Publikation II

Det andra arbetet bestod av två delar, ett Malmö material bestående av alla preeklamptiker under 5 år och ett landsomfattande material. Båda dessa jämfördes med friska gravida kvinnor. Målet var att undersöka om rökare hade ett skydd mot att utveckla preeklampsi samt att diskutera den eventuella orsaken. Eftersom tillståndet framförallt påverkar sjuklighet och dödlighet hos spädbarn genom för tidig (preterm) födsel, var vi särskilt intresserade av denna delgrupp. Måttlighetsrökare (1-9 cigaretter dagligen) hade lägre förekomst av både preeklampsi och av den tidiga födsel som ofta hänger ihop med svår preeklampsi. Vi framförde flera tänkbara förklaringar och spekulerade i att detta “skydd” kunde vara en effekt av nikotin. Om detta visar sig vara sant, skulle man i framtiden kunna använda nikotin – i annan form än cigaretter – mot preeklampsi. Men det krävs ytterligare studier innan det kan bli aktuellt.

Publikation III

I det tredje arbetet har vi studerat om kvinnor med tidigare preeklampsi eller tillväxthämning (IUGR), hade en ökad förekomst av APC-resistens samt om de APC-resistenta kvinnorna blödde mindre vid förlossningen. Studiegruppen som helhet hade högre förekomst av APC-resistens (18% mot 10%). Denna skillnad var koncentrerad till den lilla grupp kvinnor som hade både IUGR och preeklampsi, och det fanns ingen skillnad hos de kvarvarande i studiegruppen (de med bara preeklampsi eller IUGR). Det fanns ingen skillnad i antalet missfall per

kvinna mellan APC-resistenta och icke APC-resistenta kvinnor. Däremot hade de förra mindre blödningsmängd samt färre stora blödningar.

Publikation IV

I det fjärde arbetet studerades graviditetens naturalförlopp hos APC-resistenta kvinnor. Vi följde 2480 kvinnor, som lämnade blod och intervjuades i början av sin graviditet. Förekomsten av APC-resistens var 10.7% hos de gravida kvinnorna i Malmö. Förutom en 8-faldigt förhöjd risk att få blodpropp, fann vi ingen ökad risk för graviditetskomplikationer såsom; preeklampsi, tillväxthämning och sen- eller tidigt missfall hos de APC-resistenta kvinnorna. Däremot hade de mindre blödningsmängd och färre stora blödningar än andra kvinnor. Vid jämförelse av andra parametrar fann vi att överviktiga kvinnor samt kvinnor med blodpropp hos nära släktingar hade cirka 5 gånger ökad risk att få blodpropp, jämfört med övriga.

Publikation V

I det femte arbetet gjorde vi en statistisk studie från Medicinska Födelseregistret och Patientregistret, omfattande alla kvinnor med graviditetsrelaterad blodproppsdiagnos. Av de 479 422 kvinnor som födde barn under denna tid fann vi 608 kvinnor med 625 blodproppstillbud i samband med graviditet. Dessa 608 kvinnor jämfördes med alla kvinnor som var förlösta 1993 (utan blodpropp). Vi fann att förekomsten av blodpropp var 13/10 000 graviditeter, jämnt fördelade före och efter förlossningen. Kejsarsnitt var förknippat med en 5-faldigt ökad risk och preeklampsi med en 3-faldigt ökad risk för blodpropp i efterbördsskedet. Men det finns inget samband mellan preeklampsi och blodpropp före förlossningen. Det var förvånande att ett tillstånd som preeklampsi, som har aktiverad koagulation, inte ledde till en ökad risk för blodpropp under graviditeten. Hos rökare fann vi en ökad risk för blodpropp, en risk som steg med antalet cigarretter per dag. Tidigare har man ej funnit något samband mellan venös blodpropp och rökning.

Evolutionär fördel

Fyndet att APC-resistens (en genetisk förändring som ökar blodets förmåga att koagulera) medförde färre stora blödningar vid förlossningen verkar kanske inte så intressant. Men det har uppskattats att var tionde kvinna avslutade sitt liv i graviditetskomplikationer för två hundra år sedan, samt att uppemot hälften av dessa dödsfall hade ett samband med blodbrist eller stora blödningar. Det minskade antalet stora blödningar i samband med förlossningen hos APC-resistenta, kan ha medfört en historisk minskad risk att dö vid förlossning. Detta kan ha gett kvinnorna med APC-resistens en evolutionär fördel gentemot de icke APC-resistenta och att familjer med APC-resistens därigenom blivit större än andra, vilket skulle kunna förklara den höga förekomsten av APC-resistens idag. Detta är inte den första gången en ärftlig avvikelse har visat sig ha en evolutionär

fördel. Tidigare har individer med sickelcellsanemi (en typ av ärftlig blodbrist) visats ha en fördel i områden där malaria är vanligt. Malaria har en hög dödlighet, men dessa bärare får malaria mindre ofta och mildare och överlever på så vis.

Slutsats

Vi har försökt förutse vilka patienter som kommer att utveckla preeklampsi och tillväxthämning och vi har funnit att ett HCG-prov av urinen skulle kunna användas för att identifiera kvinnor med högre risk att utveckla dessa symptom. Att kvinnor som fick tillväxthämmade barn hade lägre EGF-nivå i urinen kan indikera att EGF deltar i styrningen av moderkakan och fostrets tillväxt. Fyndet att måttlighetsrökarna med preeklampsi mer sällan födde för tidigt kan vara en effekt av nikotin. Våra studier har förbättrat kännedomen om hur vissa faktorer (APC-resistens, rökning, kejsarsnitt, preeklampsi, paritet, ålder, övervikt, samt blodpropps förekomst i nära släkten) påverkar risken att få blodpropp. APC-resistens förekom hos cirka 11% av gravida kvinnor och förutom en ökad risk för blodpropp fann vi ingen ökad risk för graviditetskomplikationer. Det lägre antalet stora blödningar vid förlossningen kan ha gett APC-resistenta kvinnor en evolutionär fördel i form av färre dödsfall vid förlossningen. Detta kan vara orsaken till den höga förekomsten av APC-resistens.

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APPENDIX

Appendix 1. Swedish original information to heterozygote carriers of APC resistance who gave birth.

Angående det forskningsprojekt som du deltog i under din graviditet;

Under tiden juni 1993 t.o.m mars 1995 genom förde vi en undersökning angående sambandet mellan blodpropp och APC-resistens (ett vanligt ärftligt tillstånd som ger bäraren ökad risk för blodproppar). Tidigt under Din graviditet lämnade Du ett blodprov, samt fyllde i ett formulär angående blodproppar hos Dig och Din nära släkt. Ungefär 2500 kvinnor deltog i studien och cirka 11% hade APC-resistens. **Provet Du lämnade visade att Du har APC-resistens** (av heterozygot typ)

APC-resistens är ett nyligen beskrivet tillstånd som innebär att blodet har något ökad förmåga att levra sig (koagulera). Blodets ökade förmåga att koagulera innebär en lätt förhöjd risk att få blodpropp. Det kan finnas flera olika orsaker till varför en kvinna får blodpropp. Graviditet utgör i sig en riskfaktor. Andra riskfaktorer är övervikt, p-piller, operation samt ärftliga faktorer varav APC-resistens är den vanligaste. APC-resistens i sig är en lindrig riskfaktor och majoriteten av de 11% av befolkningen som bär på anlaget får aldrig någon blodpropp.

Vi fann inget samband mellan APC-resistens och graviditets komplikationer såsom: havandeskaps förgiftning, tillväxthämmning av fostret, eller missfall.

Vi anser inte att Du i samband med kommande graviditeter behöver någon blodpropps-förebyggande behandling bara med tanke på APC-resistensen. Om du har flera riskfaktorer, diskutera med din doktor eller med oss. Vid val av preventivmetod är annat preventivmedel att föredra framför p-piller, diskutera detta med din gynekolog. Om du skall genomgå en operation eller blir gipsad bör du upplysa behandlande läkare att du har APC-resistens.

Appendix 2. Information to heterozygote carriers who gave birth.

Regarding the research study that you participated in your pregnancy:

Between June 1993 and Mars 1995, we carried out a study concerning the relationship between thrombosis and APC resistance (a recently discovered inherited condition that gives the carrier an increased risk of thrombosis). Early in your pregnancy, a blood sample was drawn, and you answered a questionnaire including thrombosis in your history and among your close relatives. About 2,500 women entered the study and 11% had APC resistance. **The blood sample showed that you have APC resistance (of heterozygous type)**

APC resistance is a recently described condition, which slightly increases the ability of blood clotting (coagulation). This increased coagulability implies a slightly increased risk of thrombosis. There are different causes why a woman gets a thrombosis: Pregnancy in itself is a risk factor; other risk factors are obesity, the use of oral contraceptives, surgery, and heritable factors of which APC resistance is the commonest. APC resistance by itself is a mild risk factor and the majority of the 11% of the population that is carrying APC resistance will never have a thrombosis.

We found no association between APC resistance and pregnancy complications like: preeclampsia, growth restriction of the fetus, or spontaneous abortions.

We do not consider that you need thrombosis prophylaxis during your next pregnancy just because of the APC resistance. If you have other risk factors, discuss them with your doctor or with us. In the choice of preventive are other preventive measures preferable to oral contraceptives, discuss this with your gynecologist. If you are going to have an operation, or be in plaster, inform your doctor that you have APC resistance.

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Epidermal growth factor in maternal urine — a predictor of intrauterine growth restriction?

Pelle Lindqvist[a,*], Lars Grennert[b], Karel Maršál[c]

[a]*Department of Obstetrics and Gynaecology, Sundsvall Central Hospital, Sundsvall, Sweden*
[b]*Department of Obstetrics and Gynaecology, Lund University, University Hospital Malmö, SE-20502 Malmö, Sweden*
[c]*Department of Obstetrics and Gynaecology, Lund University, Lund, Sweden*

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Abstract

Epidermal growth factor (EGF), an angiogenic and mitogenic peptide, is known to be essential for normal fetal development in mice. Hypothetically, low maternal urine EGF levels might be associated with intrauterine growth restriction (IUGR) or pre-eclampsia (PE). We carried out a prospective study of 1009 consecutive women whose urine was sampled in early pregnancy (at a median of 13 weeks of gestation) between January and November 1993. Thirty women gave birth to IUGR babies and 24 developed PE. The study was designed as a nested case-control study with two matched controls for each case. EGF and human chorionic gonadotrophin (HCG) levels were measured and expressed in ng EGF/mg creatinine and IU HCG/mg creatinine. Logistic regression analysis was made with EGF or HCG levels as explanatory variables. Urinary EGF levels were significantly lower in the IUGR subgroup than in their controls, but no such difference was found between the PE subgroup and their controls. In the series as a whole, smokers were found to have lower EGF and HCG levels than non-smokers. In addition, correlation was found to exist between EGF and HCG levels (Spearman's rho 0.35; $P < 0.001$). We conclude that a relative deficiency of EGF in early pregnancy might be one of the pathophysiological mechanisms of IUGR. However, the EGF level was an insufficiently discriminative variable to be of use for screening purposes.
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Keywords: Epidermal growth factor; Fetal development; Urine; Screening

*Corresponding author. Department of Obstetrics and Gynaecology, University Hospital, Malmö, SE-20502 Malmö, Sweden. Tel.: +46-40-332-166; fax: +46-40-158-910.
E-mail address: pelle.lindqvist@obst.mas.lu.se (P. Lindqvist)

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1. Introduction

Pre-eclampsia (PE) is a complication occurring in the second half of pregnancy with an incidence of 2–3% among Swedish women. Despite advances in antenatal care, PE remains a major cause of maternal and perinatal mortality and morbidity. Intrauterine growth restriction (IUGR) is another complication of pregnancy, which is associated with an increased risk of fetal hypoxia, neonatal morbidity and mortality. Although the pathogenesis of PE is unclear, most authors consider it to be due to uteroplacental under-perfusion, associated with structural and occlusive changes in the spiral arteries [1]. Cases of fetal growth restriction in the absence of maternal hypertension are characterised by the same type of spiral artery lesions, which might be interpreted as suggesting the pathogenesis to be similar to that of PE, and to differ only in the maternal response to placental pathology [2].

In the 1960s, epidermal growth factor (EGF) was found to induce earlier opening of the eyelids in newborn mice. EGF has since been found to be a potent stimulator of various tissues. In normal human pregnancy, the EGF levels in maternal urine increase up to 20 weeks of gestation [3–5], and then decrease toward term [6]. Urinary excretion of EGF has been reported to be reduced with increasing maternal age [5,7] and in term pregnancies with IUGR fetuses [8]. Mouse studies using surgically provoked EGF-deficiency (by extirpation of the salivary gland) in early [9] or mid [10] gestation caused abortions or 'growth retarded' pups (i.e. small bodies, light guts, but normal sized head). In vitro, EGF has been shown to affect the trophoblast differentiation and hormone production from the syncytiotrophoblast with increased human chorionic gonadotrophin (HCG) synthesis in early pregnancy [11,12]. In the middle of the second trimester and in late pregnancy, elevated serum HCG values have been found in pregnancies complicated by PE [13,14].

The purpose of this prospective study was to determine whether it would be possible to identify gravidae at higher risk of developing IUGR or PE, by means of EGF, or HCG analysis in an untimed urinary sample in the late first or early second trimester. The hypothesis was that a relative deficiency of maternal EGF may be involved in the defective placentation known to occur in both PE and IUGR.

2. Materials and methods

Between January and November 1993, 1009 consecutive women attending the municipal antenatal care unit, Malmö University Hospital, agreed to participate in this study. Their medical histories were taken and urine sampled during their first routine visits during pregnancy, usually between 10 and 18 post-menstrual weeks (median 13 weeks). The urine samples were frozen at below −20°C, until analysed. The pregnancies continued without interference, and after delivery all women were classified as having developed IUGR and/or PE, or not.

PE was defined as pregnancy-induced hypertension and proteinuria ≥ 0.3 g/l ($\geq 1+$ Albustix©Boehringer Mannheim). Pregnancy-induced hypertension was defined as a resting diastolic blood pressure ≥ 90 mmHg on two occasions with an interval of at least 5 h, developing after 20 weeks of gestation in a previously normotensive woman. IUGR was defined as birthweight below the mean -2 standard deviations (S.D.) of a Swedish reference curve [15]. After scrutiny of patients' records and ultrasonographic evaluation, 112 women were excluded: 37 women who had undergone abortion (33 spontaneous abortions, four legal abortions of which three were made on fetal indications (two trisomy 18, one anencephaly)), 10 women with multiple pregnancies, 64 women whose urine was sampled at either less than 10 or more than 18 weeks of gestation, and one illegal refugee was lost for follow up. Of the remaining 897 women in the study, 92 were delivered at other hospitals. Gestational age was estimated by ultrasonographic measurements of biparietal diameter and femur length in 98% (881/897) of the background population, and in all of the study group as a whole ($n = 156$). Preterm delivery was defined as delivery before 37 completed weeks of pregnancy.

Two matched controls were selected for each case of PE, IUGR, or PE + IUGR. They were selected in a computer-randomised manner from a background population of all women delivered in Malmö, being matched for maternal age (± 2 years), gestational age at urine sampling (± 7 days), and parity (0-para or ≥ 1-para). In matching for IUGR, the birthweight criterion for controls was a birthweight > 1 S.D. below the mean of the reference curve. According to smoking habits routinely recorded in early pregnancy, the women of the study series were classified in terms of daily cigarette consumption: ≥ 10 cigarettes, 1–9, or 0 (non-smoker or non-regular smokers). For the purposes of the study, they were classified as smokers or non-smokers.

EGF concentrations in maternal urine were measured with a double antibody human EGF RIA kit using synthetic human EGF as the tracer (Amersham International plc, Bucks, UK). Freezing and thawing has been shown not to affect the stability of EGF [3]. To eliminate the effect of variation in water excretion, urinary EGF concentrations were related to the respective urinary creatinine values, the resulting standardised EGF values being expressed in ng EGF per mg creatinine. Each urine sample was assayed in duplicate and masked as to case or control status. The inter- and intra-assay coefficients of variation were less than 3% and 9%, respectively. Total β-HCG levels were measured using Technicon Immuno 1® system (Bayer Corp, Tarrytown, NY, USA). The urinary HCG concentrations were also related to the respective urinary creatinine values, the resulting standardised values being expressed in IU HCG per mg creatinine.

The study was designed for analysis with matched logistic regression analysis with two controls for each case, the development of IUGR or PE being the main outcome variables, and urinary EGF or HCG levels the independent variables. For the purpose of analysis the cases with both PE and IUGR were included in both PE and IUGR subgroups. Spearman's rank correlation was used for correlation assessment. Student's *t*-test, or the Mann–Whitney *U*-test, was used for the analysis of continuous data, with or without a normal distribution, and the chi-squared test or Fisher's exact

test for categorical data, as appropriate. P-values < 0.05 were considered significant. STATA software (Stata corp, Texas, USA) was used for conditional logistic analysis, and SPSS software (SPSS Inc, Chicago, USA) for other statistics.

3. Results

Of the 897 pregnancies originally included in the study, 22 were subsequently complicated by PE, 28 by IUGR, and two by both PE and IUGR. The study series as a whole ($n = 156$) whose data were subjected to statistical analysis comprised these 52 women and their 104 controls. The characteristics of the IUGR subgroup and their controls are shown in Table 1, and those of the PE subgroup and their controls in Table 2.

Urinary EGF levels were significantly lower in the IUGR subgroup than in their controls, but no such difference was found between the PE subgroup and their controls (Table 3). HCG values tended to be higher in the PE subgroup as a whole than in their controls (Table 3). As compared with women whose HCG levels were below the median, women with HCG levels above the median were at threefold greater risk of developing PE (odds ratio 3.0, 95% confidence interval 1.1–9.2).

Table 1
Characteristics of mothers and offspring of intrauterine growth restriction (IUGR) and control groups[a]

	IUGR ($n = 30$)		Controls ($n = 60$)		Significance of difference (P)
Mothers					
Age (years)	27.9	5.3	27.7	5.3	0.8
Nulliparae (n)	18	60%	36	60%	1.0
Smokers (n)	12	40%	8	13%	0.004
Mode of delivery					
Vaginal spontaneous (n)	20	67%	49	82%	0.1
Instrumental vaginal (n)	1	3%	4	7%	0.7
Cesarean section (n)	9	30%	7	12%	0.03
Preterm delivery[b] (n)	6	20%	1	2%	0.005
Offspring					
Male sex (n)	12	40%	37	62%	0.05
Birth weight (g)	2436	403	3528	388	< 0.001
Birth length (cm)	45.8	2.8	50.3	2.2	< 0.001
Ponderal index[c]	25.2	2.7	27.8	2.2	< 0.001
pH umbilical artery[d]	7.25	0.09	7.22	0.09	0.3
	($n = 19$)		($n = 38$)		
pH umbilical vein[d]	7.32	0.08	7.31	0.09	0.8
	($n = 26$)		($n = 56$)		
Apgar 5 min < 7 (n)	1	3%	0	0%	0.2
Placental weight (g)[d]	445	94	619	138	< 0.001
	($n = 28$)		($n = 60$)		

[a] Mean and standard deviation or number and percentage are given.
[b] Delivery before 37 completed weeks.
[c] Ponderal index {weight (kg)/length (m)^3}.
[d] Not measured in all cases.

Table 2
Characteristics of mothers and offspring of the pre-eclampsia and control groups[a]

	Pre-eclampsia ($n = 24$)		Controls ($n = 48$)		Significance of difference (P)
Mothers					
Age (years)	26.7	3.7	27	3.7	0.7
Nulliparae (n)	17	71%	34	71%	1.0
Smokers (n)	3	13%	9	19%	0.7
Mode of delivery					
Vaginal spontaneous (n)	15	63%	41	85%	0.03
Instrumental vaginal (n)	4	17%	5	10%	0.5
Cesarean section (n)	5	21%	2	4%	0.04
Preterm delivery[b] (n)	6	25%	4	8%	0.07
Offspring					
Male sex (n)	14	58%	25	52%	0.6
Birth weight (g)	3171	785	3500	549	0.07
Birth length (cm)	48.8	3.7	50.0	2.6	0.12
Ponderal index[c]	26.7	2.7	27.9	3.2	0.1
pH umbilical artery[d]	7.22	0.09	7.21	0.1	0.74
	($n = 16$)		($n = 35$)		
pH umbilical vein[d]	7.3	0.06	7.29	0.09	0.7
	($n = 23$)		($n = 46$)		
Apgar 5 min < 7 (n)	0	0%	2	4%	0.5
Placental weight (g)	580	141	616	118	0.3
	($n = 23$)		($n = 46$)		

[a] Mean and standard deviation or number and percentage are given.
[b] Delivery before 37 completed weeks.
[c] Ponderal index {weight (kg)/length (m)^3}.
[d] Not measured in all cases.

In the study group as a whole ($n = 156$), smokers ($n = 30$) were found to have lower EGF and HCG levels than non-smokers (Table 3). When comparing the control group separately ($n = 104$), HCG levels differed significantly between smokers and non-smokers ($P = 0.03$). In addition, both EGF and HCG levels manifested inverse correlation to smoking habits ($\rho = -0.2$, $P = 0.03$, and $\rho = -0.2$, $P = 0.03$, respectively). Correlation was found to exist between EGF and HCG levels in urine ($\rho = 0.35$, $P < 0.01$), but there was no relationship between placental weight and either EGF or HCG levels ($\rho = 0.06$, $P = 0.05$ or $\rho = 0.07$, $P = 0.39$, respectively). When comparing smokers and non-smokers in the control group separately ($n = 104$), HCG levels differed significantly ($P = 0.03$). In PE subgroup analysis smoking was not found to be significant ($P = 0.7$) when included together with HCG in the logistic regression model. The woman with the lowest urinary EGF level had a lupus anticoagulant syndrome.

4. Discussion

To the best of our knowledge this is the first prospective study of the relationship between urinary EGF concentrations in early pregnancy and the risk of IUGR and PE.

Table 3
Epidermal growth factor (EGF) and human chorionic gonadotrophin (HCG) in maternal urine[a]

	EGF (ng EGF/mg creatinine)		HCG (IU HCG/mg creatinine)	
IUGR subgroup				
IUGR ($n = 30$)[b]	26.2 (19.7–33.9)	$P = 0.031$	230.3 (85.6–585.7)	$P = 0.18$
Controls ($n = 60$)[b]	30.3 (22.1–40.0)		354.8 (107.3–758.6)	
Pre-eclampsia subgroup				
Preeclampsia ($n = 24$)[b]	30.7 (19.3–39.7)	$P = 0.16$	416.8 (219.0–723.7)	$P = 0.22$
Controls ($n = 48$)[b]	27.3 19.7–31.6		206.4 (70.7–532.3)	
Study group as a whole				
Non-smokers ($n = 126$)	30.1 (22.1–38.7)	$P = 0.028$	321 (120.9–660.9)	$P = 0.026$
Smokers ($n = 30$)	25.8 (17.7–30.5)		185.5 (31.0–479.3)	

[a] Median and quartiles 1 and 3 are given.
[b] For the purpose of analysis, two women with both PE and IUGR and their four controls, were included in both IUGR and pre-eclampsia subgroups.

We found a low maternal urinary EGF level to be associated with the occurrence of IUGR, but not with the development of PE.

The fact that women with early pregnancy urine HCG levels above the median were found to be at threefold increased risk of developing PE might be used in clinical studies in order to select a subgroup at risk. Since we found lower HCG levels among smokers, which are known to have lower prevalence of PE [16], it is tempting to use the combination of HCG and smoking habits in selecting a high risk group. However the correlation between the two might make it less relevant as indicated by no significant improvement by adding smoking in the PE subgroup model. In several studies, the serum HCG level in the second trimester has been found to be increased in pregnancies complicated by PE [17,18], and also among those with other adverse outcome [19–22]. In several studies, attempts have been made to identify women at risk of PE, by using various predictors such as hypocalciuri, fibronectin, and kallikrein [23,24]. However, the positive predictive values tend to be low. The predictive performance of these methods would presumably be improved if they were used in a selected subgroup of gravidae. However, since both PE and IUGR are characterised by multifactorial etiology, perfect discrimination in early pregnancy is not to be expected. The value of risk prediction lies in the steps which can be taken to reduce the risk. Investigation of possible ways of predicting PE and IUGR would also increase our knowledge of early pathophysiological mechanisms and contribute to developing new ways of preventing PE and IUGR in the future. Since women with a lupus anticoagulant syndrome are characterised by late spontaneous abortion and IUGR, the finding of the lowest EGF value in a woman with this syndrome merits further evaluation.

A drawback of the present study was that we measured EGF in maternal urine in early pregnancy without knowing whether EGF passes the placenta at this stage of gestation. Findings later in gestation indicate no passage to occur, and amniotic EGF to be of fetal origin [4]. In earlier studies, EGF in maternal urine was measured either in 24-h samples, or in spot samples. We chose the latter alternative for its simplicity. Since the study was prospective and community based, there was little risk of selection bias.

Although maternal smoking is known to be a major causative factor vis-à-vis development of IUGR, mechanisms whereby smoking causes growth restriction remain to be elucidated. Our findings that smokers had significantly lower EGF levels than non-smokers, and that women with IUGR fetuses had lower urine EGF levels are noteworthy. Smoking might interfere with growth factors, such as EGF, which govern fetal and placental growth, thereby causing growth restriction. Our results showing that the EGF level was correlated to IUGR but not to placental weight, is reminiscent of the high placenta-to-birth weight ratio among smokers reported by Zarén et al. [25]. This might indicate smoking and lack of EGF to be major determinants of impaired fetal growth. However, the finding of correlation between EGF and HCG levels among our gravidae suggests an alteration in placental function. Furthermore, an in-vitro study of human placental tissue has shown maternal cigarette smoking to be associated with alterations in two major EGF-associated receptor-mediated pathways presumably involved in placental cell growth and differentiation [26]. Further investigation is needed to elucidate the function of EGF in human feto-placental development. Our study suggests EGF to be involved in both fetal and placental development in early human pregnancy.

The main objective of this study was to select, in early pregnancy, a group of gravidae at high risk of PE or IUGR. Low maternal urine EGF values were associated with IUGR which might provide a clue as to the mechanisms of fetal growth restriction, even though the differences in urinary EGF levels were insufficiently discriminative for the variable to be used for screening purposes. However, increased urinary HCG levels might possibly be used to select a high-risk population of gravidae.

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ORIGINAL ARTICLE

II

Moderate smoking during pregnancy is associated with a reduced risk of preeclampsia

PELLE G. LINDQVIST[1] AND KAREL MARŠÁL[2]

From the Department of Obstetrics and Gynecology, University of Lund, University Hospitals of [1]Malmö and [2]Lund, Sweden

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Background. To investigate the effects of maternal smoking during pregnancy on the development of preeclampsia (PE).
Methods. The study comprised two populations with singleton pregnancies, one collected from 1990 to 1994 at the Malmö University Hospital (Malmö series, n=14,510) and the other from the National Birth Registry of Sweden 1993 (National series, n=113,211). Women with PE (n=281 and n=2,865, respectively) were compared to those without PE, delivered in 1993 (n=2,811 and n=110,346, respectively). The subgroups of women who had PE associated with preterm birth (n=58 and n=693, respectively) were compared with the same control groups in both series.
Results. Multiple logistic regression analysis showed that, in comparison with non-smokers, moderate smokers (1–9 cigarettes per day) were characterized by a lower incidence of PE (odds ratio (OR) 0.4; 95% confidence interval (CI) 0.22–0.60), and PE associated with preterm birth (OR 0.1; 95% CI 0.01–0.67). The corresponding figures in the validating National series were also significantly lower for moderate smokers (OR 0.6; 95% CI 0.5–0.7 and OR 0.6; 95% CI 0.5–0.8, respectively).
Conclusion. Moderate smoking during pregnancy seems to protect against the development of PE and PE associated with preterm birth. Nicotine might be the agent responsible for this protective effect.

Key words: incidence; nicotine; preeclampsia; premature birth; smoking

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Prematurity is a major determinant of fetal morbidity and mortality. Both preeclampsia (PE) and smoking are major causes of preterm delivery. Several studies have reported cigarette smoking to be associated with a reduced risk of PE (1–6). Whether smoking reduces the incidence of PE associated with preterm birth is not known, however. And the reason for a lower incidence of PE among smokers is still obscure. A reduction of the thromboxane A2 /prostacyclin ratio and higher serum levels of thiocyanate, a substance known to lower blood pressure, have been suggested (4, 6). There might be a PE protective agent related to cigarette smoking, and if this agent could be identified, it might be used for the prevention or treatment of PE in the future.

The study was performed to reevaluate the risk reduction of PE by smoking, with special attention to PE associated to preterm birth and to elucidate possible underlying causes.

Materials and methods

The study comprised two populations with singleton pregnancies, one collected from 1990 to 1994 at the Malmö University Hospital (Malmö series, n=14,510) and the other from the National Birth Registry of Sweden 1993 (National series, n= 113,211).

Abbreviations:
PE: preeclampsia; OR: odds ratio; CI: confidence interval.

The files of the Department of Obstetrics and Gynecology, at the University Hospital, Malmö, were scrutinized for cases of women with the diagnosis of PE. To acquire a large number of women with PE, and to satisfy the criteria of independent variables for logistic regression analysis, a five-year (1990–1994) series of all parturients with PE (n= 281) was obtained from the records for comparison with a control group of which comprised all the 2,811 women without PE, who gave birth in 1993. To avoid the same patient being included twice, only data regarding their first pregnancies were included in an analysis of the 24 women who delivered twice during the study period.

For validation purposes (see discussion), a National series was compiled from the Swedish National Birth Registry in 1993 (n=113,211). All singleton parturients with PE were included as cases, and those without PE as controls. For both secondary groups, data comprised the same independent variables as in the Malmö series. The diagnosis of PE was that obtained from the birth registry, which did not necessarily comply with the strict criteria used in the Malmö series (see below).

PE was defined as pregnancy-induced hypertension and proteinuria ≥0.3 g/l (Albustix© ≥1+ Boehringer Mannheim). Pregnancy-induced hypertension was defined as a resting diastolic blood pressure of above 90 mmHg on two occasions with at least a 5-hour interval in a woman with normal blood pressure before 20 postmenstrual weeks of gestation. Intrauterine growth restriction was defined as a newborn with a birth weight ≥2 standard deviations below the mean of a Swedish reference population (7). Preterm delivery was defined as delivery before 37 completed weeks. Gestational age was estimated by ultrasonographic measurements of the fetal biparietal diameter and femur length before 20 postmenstrual weeks. Low Apgar score was defined as Apgar less than 7 after 5 minutes. Parity was classified as nulliparous (no previous birth) and multiparous (at least one previous birth).

As smoking habits are routinely recorded during a gravida's initial appointment at the antenatal care unit (usually between 10 and 15 postmenstrual weeks), baseline data for this item were available in all but ten cases (0.36%) in the control group. These ten women were excluded. Smokers were classified in terms of daily cigarette consumption i.e. ≥10 cigarettes (heavy smokers), 1–9 (moderate smokers), or 0 (non-smokers or not regular smokers).

Relative risk was estimated in terms of odds ratios (ORs) determined with multiple logistic regression analysis of the respective outcome variables (PE or PE associated with preterm birth) *vis-à-vis* combinations of the independent (explanatory) variables (smoking, maternal age, parity, and fetal gender). Age was classified as below or at least 35 years, since only the at least 35-years-old age group differed significantly in multivariate analysis from the other age- groups. An OR >1.0 indicates a risk exceeding that of controls, and the inclusion of 1.0 in the 95% confidence interval indicates a probability (p) of ≥0.05. No significant interactions were found between any of the independent variables. Cross-tabulations with the chi-

Table I. Characteristics of controls and pre-eclamptic subgroups in Malmö series

	Pre-eclampsia*		Controls**		
	n 281	%	*n* 2,811	%	Significance of difference (p)
Maternal age (years)					
<20	9	3.2	62	2.2	0.3
20–24	68	24.2	510	18.1	0.01
25–29	92	32.7	1,034	36.8	0.2
30–34	72	25.6	831	29.6	0.2
≥35	40	14.2	374	13.3	0.7
Parity					
Nulliparae	204	72.6	1,194	42.5	<0.001
Smoking habits					
Non smokers	243	86.5	2,150	76.5	
All smokers	38	13.5	661	23.5	<0.001
1–9 cigarettes daily	17	6.0	423	15.0	<0.001
≥10 cigarettes daily	21	7.5	238	8.5	0.6
Female fetal gender	157	55.9	1,371	48.8	0.02

Numbers and percentages are given.

* All women with pre-eclampsia and singleton pregnancy in Malmö during the 5-year period, 1990–94.

** All women with singleton pregnancy delivered in 1993 in Malmö (women with pre-eclampsia excluded).

Table II. Effect of smoking on the risk of pre-clampsia (PE) and PE associated with preterm birth: Logistic regression analysis of Malmö series*

	PE	Controls			PE associated with preterm birth	Controls		
n	281	2,811	OR**	95% CI	58	2,811	OR**	95% CI
Smoking habits								
Nonsmoker	243	2,150	1.0	reference	53	2,150	1.0	reference
1–9 cigarettes daily	17	423	0.4	0.2–0.6	1	423	0.1	(0.01–0.7)
≥10 cigarettes daily	21	238	0.9	0.5–1.4	4	238	0.8	(0.3–2.2)
Nulliparae	204	1,194	3.7	2.8–4.9	42	1,194	3.5	(1.9–6.3)
Female fetus	157	1,371	1.3	1.0–1.6	36	1,371	1.7	(1.0–2.8)
Maternal age ≥35 years	40	374	1.5	1.0–2.2	6	374	1.0	(0.4–2.4)

The table includes odds ratios (ORs) and 95% confidence intervals (CIs) of included variables.
* Malmö series: Women with PE delivered 1990–94 in Malmö, as compared to those without PE, delivered 1993.
** Adjusted by parity, fetal gender, and maternal age.

Table III. Effect of smoking on the risk of pre-eclampsia (PE) and PE associated with preterm birth: Logistic regression analysis of National series*

	PE	Controls			PE associated with preterm birth	Controls		
n	2,865	110,346	OR**	95% CI	58	110,346	OR**	95% CI
Smoking habits								
Nonsmoker	2,378	83,717	1.0	reference	566	83,717	1.0	reference
1–9 cigarettes daily	224	13,878	0.6	0.5–0.7	60	13,878	0.6	0.5–0.8
≥10 cigarettes daily	117	7,929	0.6	0.5–0.7	29	7,929	0.6	0.4–0.8
Missing data	146	4,822			38	4,822		
Nulliparae	1,954	45,521	3.2	3.0–3.5	475	45,521	3.4	2.0–4.0
Female fetus	1,394	53,809	1.0	0.9–1.1	351	53,809	1.1	0.9–1.3
Missing data	5	155			3	155		
Maternal age ≥35 years	402	13,499	1.6	1.4–1.8	121	13	2.1	1.7–2.6

The table includes odds ratios (ORs) and 95% confidence intervals (CIs) of included variables.
* National series: all singleton parturients in Sweden with PE 1993, as compared to those without PE and registered in the national birth registry.
** Adjusted by parity, fetal gender, and maternal age.

squared test were used for bivariate analysis. The same logistic regression model was used in the National series as in the Malmö series. The SPSS (Statistical Package for the Social Sciences (SPSS inc., Chicago, USA)) statistical package was used. *p*-values <0.05 were considered statistically significant.

Results

During the study period of the Malmö series the incidence of PE in singleton pregnancies was 1.9% (281/14,510) of which 58 delivered preterm. The demographic and outcome characteristics of the PE group and the control group are given in Table I. As compared to controls, the PE group was characterized by a significantly higher percentage of preterm deliveries, intrauterine growth restriction, and low Apgar score (20.6% *vs.* 4.4%; $p<0.001$, 11.4% *vs.* 3.2%; $p<0.001$, and 3.2% *vs.* 1.2%; $p=0.015$, respectively).

Multiple logistic regression analysis in the Malmö study showed moderate smoking (1–9 cigarettes daily) to be associated with a 3-fold lower risk of PE, and with a 10-fold lower risk of PE associated with preterm birth (Table II).

In the National series, the overall incidence of PE was 2.5% (2,865/113,211) of which 693 delivered preterm. Both moderate and heavy smoking were associated with a significantly diminished risk of PE (OR=0.6 95% CI 0.5–0.7 and OR=0.6 95% CI 0.5–0.7, respectively) and PE associated with preterm birth (OR=0.6 95% CI 0.5–0.8 and OR=0.6 95% CI 0.4–0.8, respectively) (Table III).

Discussion

In Malmö as well as in the National series, moderate smokers were found to be associated with a lower incidence of PE associated with preterm birth. These results support the theory that some agent related to smoking protects against PE. Furthermore, the findings were present without taking into consideration the added risk of prematurity

among smokers, which would have further increased the differences. The overall reduction in the risk of PE among smokers in our series is in line with previous reports (3, 4, 6).

Earlier studies have reported that smokers who developed PE have increased perinatal morbidity and mortality (2, 6). In order to exclude the possibility that only 'harmless' cases of PE were reduced by smoking, we focused on women with PE that delivered preterm, a subgroup of PE with high fetal morbidity and mortality (8). Since parity is a major determinant of PE, we considered the inclusion of parity as an independent variable essential for a generalization of the results.

Information on smoking habits was confined to data collected at the end of the first or early second trimester. Moreover, we had no objective evidence of exposure to tobacco smoke. On the other hand, information on smoking habits was collected prospectively which precludes recall bias. Confounding factors, such as socio-economic status, malnutrition or drug abuse, were not taken into account. The design of the Malmö series, i.e. including five years of cases and one year of controls, may have introduced a time dependent bias. In addition, the inclusion of only the first delivery of those who gave birth more than once during the study period might have introduced a selection bias. Moreover, the number of smokers among women with PE who delivered preterm were low. For the purpose of validating our findings, we therefore performed the National series based on a large number of cases, to reduce uncertainty and possible bias. We were able to confirm our findings in the Malmö series with the same variables in the National series. The National series differed from the Malmö series with regard to the degree of PE reduction, but was consistent with our conclusion that moderate smokers had a lower incidence of PE and PE associated with preterm birth.

There are several possible mechanisms whereby moderate smoking might protect against PE. Davis and coworkers' (9) finding of an increase in prostacyclin metabolites (6-keto PGF1 alpha) among smokers as pregnancy advanced, but a decrease among non-smokers, is in agreement with an improved thromboxane A2/prostacyclin ratio previously suggested.

Nicotine induces relaxation of smooth muscle by an increased release of nitric oxide, a potent vasodilator (10). In addition, the decrease of hemoglobin concentration seen among smokers in the second trimester might reflect an increased plasma volume (11). This is in contrast to the increased hemoglobin concentration in women with PE. Furthermore, in the general population, smokers tend to have lower blood pressure than non-smokers (12). Thus, smoking might mask the development of PE.

PE is associated with endothelial damage and increased serotonin levels (13). The balance of vascular response to serotonin is shifted towards vasoconstriction (14). A recent randomized placebo controlled study of ketanserin, a specific serotonin-2-receptor antagonist, showed incidence of PE to be reduced in the ketanserin treated group (15). Smoking is associated with a decrease in platelet serotonin content, a change probably mediated via nicotine receptors (16). Thus, nicotine might alter the serotonin metabolism.

PE is a hypocalciuric state (17) and it has been suggested that calcium supplementation lowers the incidence of PE (18). Smokers have lower parathyroid hormone levels than non-smokers (19), whereas women with PE tend to have increased levels (20). Thus, a decreased supply of calcium might be a characteristic of PE, and the lower parathyroid hormone levels in smokers may reflect an increased availability of calcium. Furthermore, smoking alters adrenal cortex steroid production by lowering the activity of 11 and 21 hydroxylase (21, 22), resulting in a more androgenic steroid balance which also might contribute to an altered calcium metabolism.

Moderate smoking during pregnancy seems to protect against the development of PE and PE associated with preterm birth. Nicotine might be the agent responsible for this protective effect.

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Address for correspondence:
Pelle Lindqvist, M.D.
Department of Obstetrics and Gynecology
University of Lund
University Hospital, Malmö
SE-20502 Malmö, Sweden

Errata

Paper III

Two lines from the bottom on page 70 where 43/423 should read 46/465

In the last paragraph on the result section on page 71; 23% (15/66) should read 19/68 and 43% (208/480) should read 229/519. In the same paragraph, 6.3% (3/48) and 0.4% (2/465) should be corrected to 4.4% (3/68) and 0.4% (2/519).

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Factor V Q^{506} Mutation (Activated Protein C Resistance) Associated with Reduced Intrapartum Blood Loss – a Possible Evolutionary Selection Mechanism

Pelle G. Lindqvist[1], Peter J. Svensson[2], Björn Dahlbäck[2], Karel Maršál[1]

From the Department of Obstetrics and Gynaecology[1], and Department of Clinical Chemistry and Coagulation Research[2], University of Lund, University Hospital, Malmö, Sweden

III

Summary

Objectives. To ascertain whether relationship exists between the presence of APC resistance [a hypercoagulable state due to a mutation (R^{506}Q) in the factor V gene] and the occurrence of pre-eclampsia (PE), intrauterine growth retardation (IUGR), and pregnancy bleeding complications. *Design.* A retrospective study. *Subjects.* A study group of 122 women with PE and/or IUGR during a recent pregnancy and a control group of 465 healthy pregnant women. *Results.* A significantly reduced risk of intrapartum bleeding complications in the APC-resistant subgroup as compared to the non-APC-resistant subgroup was suggested by reduced intrapartum blood loss, and pre- and postpartum haemoglobin measurements. The prevalence of APC resistance in the PE and IUGR subgroups did not differ significantly from that in the control group. *Conclusion.* The remarkably high prevalence of the potentially harmful factor V gene mutation in the general population may be the result of an evolutionary selection mechanism conferring such survival advantages as reduction in the risk of intrapartum bleeding on carriers of the FV:Q^{506} allele.

Introduction

Normal pregnancy is characterized by changes in the plasma concentrations of several proteins involved in blood coagulation, and shifts in the protein C and fibrinolytic systems toward decreased anticoagulation and inhibited fibrinolysis (1, 2). Although these changes are of physiological importance in minimizing the risk of blood loss, they may also increase the risk of thromboembolic complications. Reported figures for the incidence of such complications during pregnancy have varied from 0.018% to 0.29% (3).

Activated protein C resistance (APC resistance), is the most common of the known inherited risk factors for venous thrombosis (4, 5). In a majority of cases, APC resistance is caused by a point mutation in the gene for coagulation factor V (FV), involving the replacement of arginine (R) at position 506 by glutamine (Q) (6). The FV:Q^{506} allele is not only common among thrombosis patients, but is also highly prevalent (1%-11%) in Western societies (7-12). Women carrying the FV:Q^{506} allele have been shown to be at increased risk of developing venous thrombosis during oral contraceptive usage, pregnancy, and the puerperium (13, 14). Two pregnancy conditions which further aggravate the shift in the haemostatic system towards hypercoagulation are the presence of pre-eclampsia (PE) and intrauterine growth retardation (IUGR). Both in PE and IUGR, spiral artery lesions with atherosclerosis and thrombosis are often to be seen in the placenta (15). The placenta circulation, which resembles venous circulation with low pressure and flow velocity, may be particularly susceptible to thrombotic complications in APC-resistant women. Recently, Decker and co-workers (16), using a functional APC resistance test, found the prevalence of the APC resistance phenotype to be 16% in a series of gravidae with early onset PE, though the FV genotype was not determined in this study.

The present study was designed to investigate the possible relationship between APC resistance and the development of IUGR, PE, and bleeding complications during pregnancy. The hypothesis was that there would be fewer cases of bleeding complications and more of PE and IUGR among the APC-resistant women.

Patients and Methods

The Study was approved by the Ethics Committee of Lund University, and informed consent was obtained from all participants. A review of the medical records at the Department of Obstetrics and Gynaecology, University Hospital, Malmö, for the 14-month period, January 1993 to February 1994, yielded 197 cases of PE and/or IUGR occurring in pregnancies resulting in live births. Of the 197 women, 153 accepted an invitation to participate in the study, and underwent blood sampling and detailed history taking (including heredity for venous thrombosis and history of spontaneous abortion).

After scrutiny of the patients' records, 31 cases were excluded (17 did not fulfil the PE criteria, 10 did not fulfil the IUGR criteria, and in 4 cases the fetus had serious malformations). Thus, a total of 122 cases were included in the study group, 47 of PE, 67 of IUGR, and 8 of both PE and IUGR. The patients' clinical characteristics are given in Table 1. The control group included 465 healthy pregnant women delivered at University Hospital, Malmö (cases of PE or IUGR being excluded).

The presence of APC resistance was defined as carriership of the FV:Q^{506} allele either in heterozygous or homozygous form. The FV genotype was determined with a PCR-based assay, as previously described (17).

The delivering midwife estimated intrapartum blood loss by measuring the volume of free blood, and in addition, approximating the amount of blood in swabs and cloths, and subtracting the amount of any amniotic fluid mixed with it. The midwife filled in the estimated blood loss in the patient's medical record immediately after delivery. Since the blood loss was estimated and recorded directly postpartum by the delivering midwife, and the results of the APC analysis and interview were not known until all measurements had been recorded, the study was blind. The estimation was made in the same manner and by the same personnel at all times. A postpartum haemoglobin (Hb) value was routinely obtained in cases where intrapartum blood loss exceeded 600 ml, in high-risk patients, or on other general indications. Pre-post partum Hb difference was defined as the difference between the Hb value (g/l) on the second day after delivery and the last value before delivery. Postpartum anaemia was de-

Correspondence to: Dr. Pelle Lindqvist, Department of Obstetrics and Gynaecology, University Hospital, Malmö, S-20502 Malmö, Sweden – Tel.: +46 40 332168; FAX Number: +46 40 979846; E-mail Pelle.Lindqvist@obst.mas.lu.se

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Table 1 Clinical characteristics of mothers and offspring (Median and ranges, or proportions and percentages are given)

	Study group		Control group		Significance of difference
	122		465		(p)
Mothers					
Age (years)	30.5	20–42	28.0	16–44	0.001
Nulliparae	75	61.5%	217	46.7%	0.006
Smokers*	43	35.2%	101	21.7%	0.007
Mode of delivery					
Vaginal spontaneous	80	65.6%	375	80.6%	0.001
Vaginal operative	8	6.6%	25	5.4%	0.7
Caesarean section	22	18.0%	54	11.6%	0.06
Caesarean section for imminent asphyxia	12	9.8%	12	2.6%	0.001
Neonates					
Sex (Male/Female)	56/66	46%/54%	245/220	52%/48%	0.2
Gestational age at birth (weeks)	39.0	26–42	40.0	31–43	<0.001
Birthweight (g)	2655	930–5050	3500	1765–5000	<0.001
Birthweight deviation (%)**	–22.5%	–67% - +36%	0.0%	–21% - +70%	<0.001
5-min Apgar score <7	4	3.3%	9	1.9%	0.5
pH umbilical artery***	7.22	6.82–7.40 (n = 92)	7.22	6.83–7.43 (n = 338)	0.800
pH umbilical vein***	7.30	6.86–7.46 (n = 120)	7.32	6.84–7.50 (n = 389)	0.08

* Smokers defined as daily smokers of at least 1 cigarette, non-smokers as not smoking or not smoking regularly at the first visit to the antenatal health care centre.
** Birthweight deviation from the expected mean of reference population (19). *** Cord blood samples not taken in all cases.

Table 2 Intrapartum bleeding among APC-resistant and non-APC-resistant women

	Study group*			Control group			Combined Study and Control group		
	APC resistance	Non-APC resistance	Significance of difference (p)	APC resistance	Non-APC resistance	Significance of difference (p)	APC resistance	Non-APC resistance	Significance of difference (p)
n**	18	69		38	357		56	426	
Blood loss (ml)***									
geometric mean	330	370	0.34	318	380	0.018	322	379	0.001
±1SD	245–446	167–824		228–443	236–614		234–443	220–652	
Blood loss > 600 ml									
number	0	11	0.11	1	47	0.07	1	58	0.01
proportion	0%	16%		3%	13%		2%	14%	

* Pregnancies complicated by pre-eclampsia and/or intrauterine growth retardation. ** Measured in women delivered vaginally and without heparin treatment.
*** The blood loss values were transformed to natural logarithms, to normalize skewed distribution for the purpose of statistical analysis.

fined as an Hb value <100 g/l on the second day after delivery. Profuse haemorrhage during delivery was defined as a blood loss >600 ml, in accordance with ICD-9 (18). Only patients who were delivered vaginally and who were not treated with heparin were included in comparisons of Hb values and intrapartum bleeding. There were no differences between the APC-resistant and the non-APC-resistant group regarding age, parity or smoking habits.

PE was defined as pregnancy-induced hypertension and proteinuria >0.3 g/l (Albustix® ≥1+). Pregnancy-induced hypertension was defined as a resting diastolic blood pressure >90 mm Hg on two occasions at an interval of at least 5 h, developing after 20 weeks of gestation in a previously normotensive pregnancy.

In singleton pregnancies, IUGR was defined as a newborn small for gestational age at birth, i.e., with a birthweight ≤ mean –2 SD of a reference population (19), and in twin pregnancies as a birthweight ≤ mean –2 SD in both newborns.

The Mann-Whitney U test or Student's t-test was used for the analysis of continuous data and the Chi-squared test or Fisher's exact test for categorical data, as appropriate. Spearman's rank correlation was used for correlation assessment. For the purposes of statistical analysis, the intrapartum blood loss values were transformed to natural logarithms to normalize a skewed distribution. P-values <0.05 were considered significant.

Results

The characteristics of the study and the control group are given in Table 1.

The frequency of APC resistance was 18% (22/122) in the study group as a whole (PE and/or IUGR) and 10% (43/423) in the control group, the difference being significant (p = 0.02), one woman in the

Table 3 Pre-postpartum haemoglobin difference, postpartum haemoglobin and postpartum anaemia, among APC-resistant and non-APC-resistant women in the study-, control- and combined groups. Mean and standard deviation (SD) or number and percentage are given

	Study group*			Control group			Combined Study and Control group		
	APC resistance	Non-APC resistance	Significance of difference (p)	APC resistance	Non-APC resistance	Significance of difference (p)	APC resistance	Non-APC resistance	Significance of difference (p)
n*	16**	60***		23	245		39	305	
Haemoglobin difference (g/l)									
mean	4.2	–12.1	0.001	–3.0	–8.2	0.02	0.0	–8.9	0.000
SD	11.8	18.2		9.5	17.0		10.9	17.3	
Postpartum haemoglobin (g/l)									
mean	121	110	0.002	116	113.0	0.28	118	113	0.008
SD	10.2	18		12.0	16.7		11.4	16.9	
Postpartum anaemia****									
number	0	20	0.007	1	48	0.08	1	68	0.003
proportion	0%	33%		4%	20%		3%	22%	

* Vaginally delivered and non-heparin treated women with complete haemoglobin values were available.; ** Includes 10 women with IUGR foetuses and 7 women with PE; *** Includes 38 women with IUGR foetuses and 24 women with PE; **** Haemoglobin value < 100 g/l on the second day after partus.

study group and two women in the control group carried the FV:Q^{506} allele in homozygous form. APC resistance was present in 13% (6/47) of the isolated PE subgroup and 18% (12/67) of the IUGR subgroup (neither of which differed significantly from the control group in this respect). However, the frequency of 50% (4/8) in the small PE + IUGR subgroup differed significantly from that in the control group ($p = <0.001$).

In the series as a whole (n = 482), the APC-resistant women were characterized by less intrapartum blood loss ($p = 0.001$) and a lower risk of profuse intrapartum bleeding (>600 ml) ($p = 0.01$) than non-APC-resistant women (Table 2). Also in the control group, the difference in intrapartum blood loss between those with and those without FV:Q^{506} mutation reached significance ($p = 0.02$).

Pre- and postpartum Hb values for the groups are given in Table 3. It is noteworthy that, as compared with non-APC-resistant women, APC-resistant women were characterized by higher postpartum Hb values, lower pre-postpartum differences in Hb values, and fewer cases of postpartum anaemia, although the differences were not significant in all subgroups. Significant correlation was found to exist between the estimated blood loss and both pre-postpartum difference in Hb values (–0.51, $p <0.001$) and the postpartum Hb value itself (–0.51, $p <0.001$).

There was a non-significant tendency towards fewer cases of spontaneous abortion in previous pregnancies in the APC-resistant subgroup than in the non-APC-resistant subgroup [23% (15/66) vs. 43% (208/480), respectively: $p = 0.11$]. The respective frequencies of a history of venous thrombosis were 6.3% (3/48) and 0.4% (2/465). Two non-APC-resistant women developed postpartum thromboembolic events, one woman with lupus anticoagulants developed a pulmonary embolism and the other had a cerebral thrombosis/infarct. She had a low postpartum Hb (65 g/l) and a pre-postpartum Hb-difference of 61 g/l, but refused blood transfusion.

Discussion

The 18% prevalence of APC resistance found among pregnant women with PE and/or IUGR differed significantly from the figure of 10% for the control group. The 10% prevalence of APC resistence in the control group is high, but not different from the 11% reported from another study performed in the same region as ours (12). A noteworthy finding in our study was that in the series as a whole, figures both for intrapartum bleeding and for pre-postpartum difference in Hb measurements were consistent with the reduced intrapartum blood loss among APC-resistant women, as compared to non-APC-resistant woman, though in some of the subgroup comparisons, the difference was non-significant. However, a prospective study in a large series would be necessary before any general conclusions can be drawn as to a link between APC resistance and a lower incidence of bleeding complications in pregnancy.

For obvious reasons, assessing intrapartum blood loss is problematic, as at best it is inevitably an estimate. Even the more objective Hb measurement also represents a crude approximation of blood loss. In the present study, however, the validity of assessment was attested by the fact that both types of assessment were used and found to yield similar patterns of results manifesting correlation with each other (Spearman's Rho).

Although APC resistance is a potentially dangerous hereditary defect, its high prevalence in the population raises the question of whether carriership of the FV:Q^{506} allele may confer a survival advantage (11, 20-22). The most hazardous event of a woman's reproductive life is pregnancy. In 17th century London, pregnancy was associated with an approximately 2% risk of death and the cumulative mortality risk during pregnancy was 10% (23), profuse haemorrhage during labour and delivery being a major cause of death. A hereditary condition that reduces the risk of bleeding complications would be selected in such an environment. Even though thrombosis during pregnancy is a clinically important problem (as many as 15% of maternal deaths in Sweden being due to pulmonary embolism) (24), the 5-10-fold increase in the risk of thrombosis that is associated with APC resistance may not have been a particularly strong adverse survival factor during evolution.

If the prevalence of intrapartum bleeding complications were lower in carriers of the FV:Q^{506} allele, such women would be in better health and avoid anaemia. Accordingly, it is tempting to speculate that over time the reduced incidence of profuse blood loss during delivery among APC-resistant women might have provided them with a survival advan-

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tage. The following theoretical example illustrates the potency of the possible effect of such a gene mutation on reproductive capacity. Although this example is fictitious, calculation of the effect is based on assumptions that are in no way inconsistent with reality.

1. Carriership of a certain mutation in the fictitious δ gene is associated with a 50% reduction in pregnancy mortality due to profuse haemorrhage; as in London during the 17th century, 10% of women died of pregnancy complications and we assume that 20% of these were bleeding complications – yielding a survival advantage of 1.01;

2. The annual incidence of thromboembolic complications in women with the normal δ genotype is 1/10,000, women with the mutation are at 7-fold increased risk of thromboembolic complications, and the span of reproductive life is 30 years;

3. Of the thrombotic episodes, 20% are complicated by pulmonary embolism which is fatal in 15% of cases. From assumptions 2 and 3, the mortality risk associated with thromboembolic complications is estimated to be 0.00063 (i.e., 0.0001*7*30*0.2*0.15) (3, 13).

Based on the above figures, the following calculation can be made of the magnitude of the selection effect. After one generation, 1.0094 times more survivors carry the mutation (i.e., 1.01*(1-0.00063) = 1.0094). Recently, Zivelin and co-workers (25) reported a probable single origin for the FV:Q^{506} mutation, using haplotype analysis, estimating it to have occurred approximately 21,000-34,000 years ago (i.e., 1,000-1,400 generations). Groube (26) estimated the world population to have been at least 2 million individuals 20,000 years ago. Thus, assuming the FV:$R^{506}Q$ mutation occurred in a Caucasian population of 200,000 individuals (and disregarding other selection mechanisms), after 1,000 generations, the prevalence of the mutation would be 11,500 times the original prevalence (i.e., $(1.0094)^{1000}$) and the prevalence of the mutation would be 6% (11,500/200,000). In 1967 Wiesenfeld (27) made similar calculations of evolutionary advantage based on a proposal by Allison and colleagues (28) of selective advantage for heterozygotes carriers of the sicklecell anaemia gene mutation, due to decreased mortality from malaria and increased fertility.

The findings in the present study suggest a possible survival advantage that might have been conferred by carriership of the FV:Q^{506} allele responsible for APC resistance, namely reduction in the risk of intrapartum bleeding complications. However, prospective studies in large series are needed to fully elucidate the implications of APC resistance.

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Note: "I hold the flux of blood deadly, if it be great, I never heard of any woman that escaped, but that they all perished.... Many have perished through this sad accident and usually it proves fatal to all women. If possible, I heartily could wish, that some worthy practicer would be pleased to direct some powerful ways, or medicines, to bridle this raging destroying evil. Women would have cause to acknowledge his worth, and all succeeding ages would give him thanks... I confess my ignorance, and I believe, that there is no other, but God alone, that can do this work, to help the woman."

Percivall Willughby (1596-1685). Observations in midwifery as also The Countrey Midwifes Opusculum or Vade Mecum. Edited from original ms. Henry Blekinsop 1863. S. R. Publishers Limited East Ardsley, Wakefield, Yorkshire England 1972, 199-202.

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Activated Protein C Resistance (FV:Q^{506}) and Pregnancy

Pelle G. Lindqvist[1], Peter J. Svensson[2, 4], Karel Maršál[3], Lars Grennert[1], Marie Luterkort[5], Björn Dahlbäck[4]

From the Departments of [1,3]Obstetrics and Gynecology, [2]Medicine [4]Coagulation Disorders, Lund University, University Hospital, Malmö[1, 2, 4] or Lund[3], and [5]Slottsstaden Group Practice, Malmö, Sweden

IV

Summary

Activated protein C (APC) resistance, due to a point mutation in the factor V gene (FV:Q^{506}), is a major risk factor for venous thromboembolism. To determine the prevalence of APC resistance in a large series of pregnant women, and to elucidate its obstetric consequences, we performed a prospective study in Malmö, Sweden, comprising 2,480 women enrolled in early pregnancy. The presence of APC resistance (the FV:Q^{506} allele) was determined. The women were interviewed about their medical histories including venous thromboembolic events (VTE) in relatives. The outcome variables were the VTE rate, intrapartum blood loss, and the prevalence of selected pregnancy complications such as fetal loss, pre-eclampsia, and intrauterine growth retardation.

The overall prevalence of APC resistance was 11% (270/2480). The APC-resistant subgroup did not differ significantly from the non-APC-resistant subgroup in terms of pregnancy complications, but was characterized by an 8-fold higher risk of VTE (3/270 vs. 3/2210), a lower rate of profuse intrapartum haemorrhage (3.7% vs. 7.9%) ($p = 0.02$), and less intrapartum blood loss (340 ml vs. 361 ml) ($p = 0.04$).

Despite the high prevalence of APC resistance in this series of gravidae (11%), its presence was unrelated to adverse pregnancy outcome apart from an 8-fold increased risk of VTE.

Introduction

Successful pregnancy outcome is dependent upon adequate uteroplacental circulation, which resembles venous circulation in terms of its low pressure and low flow velocity. The placental circulation may be particularly susceptible to thrombotic complications in thrombophilic women. Two conditions which further aggravate the shift in the hemostatic system towards hypercoagulation are pre-eclampsia and intrauterine growth retardation (IUGR). In both, spiral artery lesions with atherosclerosis and thrombosis are frequently seen in the placenta (1). This raises the question of whether inherited risk factors for thrombophilia might be predisposing to pregnancy complications. Acquired antithrombin deficiency is often seen in women with severe pre-eclampsia (2). Other less frequent inherited risk factors for venous thromboembolic events (VTE) are deficiencies of anticoagulant factors such as protein C, protein S, and antithrombin, all of which have been associated with fetal loss (3). Recently, several authors have reported the occurrence of adverse pregnancy outcome in activated protein C (APC) resistant women (4-8).

In recent years there have been rapid advances in our knowledge of hereditary thrombophilia. In Caucasians, APC resistance is the most common of the known inherited risk factors for VTE (9-11). APC-resistance is caused by a point mutation in the gene for coagulation factor V (FV), resulting in the replacement of arginine (R) at position 506 by glutamine (Q), (12). This results in decreased sensitivity to the anticoagulant action of APC, and consequently a hypercoagulable state. APC resistance due to the FV:Q^{506} allele is not only common among thrombosis patients, but is also highly prevalent (1%-15%) in Western societies generally (12-19). In northern Europe, it has been found in 40 to 60% of patients with pregnancy-related VTE (20, 21).

The high prevalence of APC-resistant women among Caucasians, particularly in northern Europe (10-15%), suggests that the potentially dangerous FV mutation may have conferred an evolutionary selection advantage. Recently, we found APC-resistant women to be characterized by a reduced incidence of severe bleeding episodes during delivery (22). The procoagulant effect of APC resistance may therefore constitute a survival advantage reflected in a lower mortality rate among APC-resistant gravidae, thus accounting for its high prevalence.

This prospective study was performed to investigate the prevalence of APC resistance among pregnant women and to elucidate its obstetric consequences.

Patients and Methods

The study design was approved by the Ethics Committee of Lund University, and informed written consent was obtained from all participants. Between February 1994 and June 1995, all pregnant women in Malmö were invited to participate in the study. At their first routine visit during pregnancy to one of the community or private antenatal care clinics in Malmö, the 2,496 women enrolled in the study were interviewed by midwives, and answered a detailed questionnaire (including medical history focussed on previous thrombosis, foetal loss and familial history of thrombosis). In cases of immigrants with language problems, an interpreter assisted. At this visit blood was drawn for APC-resistance testing.

Of the 2,496 gravidae, 16 were excluded from the analysis; 3 (one of them APC-resistant) were delivered at home, 11 were delivered abroad, one could not be identified from the interview form (reference number inadvertently omitted), and one died during pregnancy of meningococcal septicaemia. Of the remaining 2,480 women, 1,899 were delivered at University Hospital, Malmö, 485 at other Swedish hospitals, and 96 women had abortions [63 had early spontaneous abortions (before 13 weeks of gestation), 19 aborted late (at 13 to 27 weeks), and 14 underwent induced abortion, in eight cases on fetal indications (3 cases of chromosomal aberrations, 2 of central nervous system defects, and 3 of structural anomalies)]. Perinatal death was defined as intrauterine death during third trimester, or death during the first week of life. A history of spontaneous abortion was checked for both at the interview and by scrutiny of the patient´s medical records.

Correspondence to: Dr. Pelle Lindqvist, Department of Obstetrics and Gynecology, University of Lund, University Hospital, Malmö, 205 02 Malmö, Sweden – Tel: +46 40 332166; FAX Number: +46 40 158910; E-mail Pelle.Lindqvist@obst.mas.lu.se

APC resistance was defined as the presence of the FV:Q^{506} allele in either heterozygous or homozygous form. A modified functional APC resistance test using factor V deficient plasma, Coatest® APC-resistance-V (Chromogenix), was performed as previously described (23). This test has been shown to manifest close to 100% sensitivity and specificity for the FV mutation (24). Individuals with an APC-ratio below 1.86 were tested with PCR-based analysis for the presence of the FV:Q^{506} allele, as previously described (23). Women with an APC-ratio of ≥ 1.86 were assumed to be noncarriers, a conclusion supported by results of parallel analysis with the APC-resistance-V assay and PCR analyses. It was performed in 432 women, of whom 270 carried the FV:Q^{506} allele. All women with a normal FV genotype had an APC ratio > 1.67, and all those with an FV:Q^{506} genotype had an APC ratio ≤ 1.64. For technical reasons, in 167 patients only the PCR analysis for the FV:Q^{506} allele was made. The results of the APC resistance analysis were blinded until 3 months after all women had delivered. In four women with previous VTE the presence of heterozygous APC resistance was known to the patient and the doctor.

A venous thromboembolic event (VTE) was defined as a deep venous thrombosis or pulmonary embolism occurring in pregnancy or during the first three months postpartum. Familial VTE was defined as one or more VTEs in first-degree relatives (father, mother, or siblings), occurring before the age of 60 years.

The delivering midwife estimated intrapartum blood loss by measuring the volume of free blood, and in addition, approximating the amount of blood in swabs and cloths, and subtracting the amount of amniotic fluid mixed with it. The midwife filled in the estimated blood loss in the patient´s medical record immediately after delivery. Since the blood loss was estimated and recorded directly postpartum by the delivering midwife, and the results of the APC analysis were not known until all measurements had been recorded, the study was blind. Postpartum anaemia was defined as an Hb-value <100 g/l on the second day postpartum. Profuse haemorrhage during delivery was defined as a blood loss exceeding 600 ml (according to the International Classification of Diseases, Ninth revision) (25). Only patients delivered vaginally and those not treated with heparin were included in the analyses of intrapartum bleeding. Twelve women received low molecular weight heparin prophylaxis due to earlier confirmed (n = 7) or suspected (n = 1) or to present confirmed VTEs (n = 2), or severe familial history of VTE (n = 2). Four of the nine women with present or earlier VTEs were heterozygous APC-resistant.

The duration of hospitalization was defined as the number of full days in hospital care after delivery, at Malmö. In the analysis, only those who were delivered vaginally at term and who were not treated with heparin at Malmö were included. For women who left the hospital on the day of delivery (n = 227), the duration of postpartum hospitalization was recorded as 0.5 day.

Smoking habits were recorded at the first visit to the antenatal health clinic, mean 12th week of gestation (standard deviation 3.3 weeks), the gravidae being classified as nonsmokers or smokers. At this visit maternal weight was also measured and recorded. Overweight was defined as a body mass index [BMI (kg/m^2)] exceeding 27.6 [i.e., > 1 standard deviation (SD) above the mean for the series].

Pre-eclampsia was defined as pregnancy-induced hypertension and proteinuria > 0.3 g/l (Albustix® Boehringer Mannheim ≥ 1+). Pregnancy-induced hypertension was defined as a resting diastolic blood pressure > 90 mm Hg measured on two occasions at an interval of at least 5 hours, and developing after 20 weeks of gestation in a previously normotensive gravidity. IUGR was defined as a newborn small for gestational age at birth, i.e., with a birth weight lower than 2SD below the mean for a reference population (26). Preterm delivery was defined as delivery at less than 37 completed weeks of gestation. Gestational age was estimated by ultrasonographic measurements of biparietal diameter and femur length in 98% of cases, and from the date of the last menstrual period in the remaining 2%.

Student's *t*-test was used for the analysis of continuous variables, and the Chi-squared test or Fisher's exact test for categorical variables. The odds ratio for the risk of pregnancy complications or VTE was calculated by cross-tabulation and with a 95% confidence interval. All calculations were performed with SPSS software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, USA) and P-values < 0.05 were considered statistically significant.

Results

The overall prevalence of APC resistance was found to be 11% (270/2480), seven women (0.3%) carrying the FV:Q^{506} allele in its homozygous form and 263 being heterozygotes. The clinical characteristics of the APC-resistant and non-APC-resistant subgroups are presented in Table 1. No significant subgroup differences were found in the variables investigated. No significant relationship was found to exist between APC resistance and obstetric complications such as pre-eclampsia, IUGR, abruptio placentae, or fetal loss (Table 2). Early spontaneous abortions in former pregnancies were reported in 18.9% (51/270) of the APC-resistant women and 18.5% (409/2210) of the non-APC-resistant (p = 0.9). Corresponding data for late spontaneous abortions were 1.1% (3/270) and 2.3% (50/2210) (p = 0.2).

The incidence of VTE in the present pregnancy was 0.24% (6/2480) (3 antepartum and 3 postpartum VTEs) in the series as a whole, being 8-fold higher in the APC-resistant subgroup than in the non-APC-resistant subgroup [1.11% (3/270) vs 0.14% (3/2210), respectively] (Table 3). Among the nine women with a history of VTE, four VTEs were associated with pregnancies, four with oral contraceptive usage, two with operations, and one with no known risk factor. One woman in the non-APC-resistant group had experienced two earlier VTEs, and one of the APC-resistant women with an earlier VTE suffered a thrombosis in the present pregnancy despite low molecular weight heparin prophylaxis.

There were two cases of early spontaneous abortion in the present pregnancy among the seven APC-resistant homozygotes. None of these seven women had a VTE in her present pregnancy or during a previous pregnancy (n = 18). One of the seven homozygotes, who also had protein S deficiency, had a first-degree relative with a history of VTE. Moreover, she had a history of eight spontaneous abortions and one earlier intrauterine fetal death. In addition, she was now delivered by caesarean section, as she had been in three out of her four former term pregnancies. No other of the seven women had been delivered by caesarean section or had a history of spontaneous abortion.

Additional Risk Factors

Of the 127 women with familial VTE, four had VTEs in conjunction with pregnancy, one of them in her present pregnancy and four in earlier pregnancies, representing an 8-fold higher incidence than in the subgroup without familial VTE (Table 3). The familial VTE subgroup did not differ significantly from the nonfamilial VTE subgroup in the prevalence of APC resistance [14.2% (18/127) vs. 10.7% (252/2353); p = 0.2]. The results of the study also suggest that overweight is a risk factor for VTE and six of the 319 overweight women had had VTEs, two of them in the present pregnancy (Table 3).

Blood Loss Measurements

As compared with the non-APC-resistant subgroup, the APC-resistant subgroup was characterized by a significantly lower risk of profuse intrapartum bleeding (p = 0.02) and significantly less intrapartum blood loss (p = 0.04). (Table 4). Moreover, among women who delivered at Malmö (n = 1,899), the duration of postpartum hospitalization was significantly shorter in the APC-resistant subgroup than in the non-APC-resistant subgroup (2.5 and 2.8 days, respectively; p = 0.048).

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Table 1 Clinical characteristics of APC-resistant and non-APC-resistant subgroups of parturents

	APC resistance		Non-APC resistance		P-value
n	257	10.8%	2,127	89.2%	
Maternal characteristics					
Age (years)	29.1	±4.8	29.0	±4.8	0.8
Nulliparae	117	45.5%	986	46.4%	0.2
Smokers*	46	17.9%	405	19.0%	0.9
Weight (kg)	65.1	±10.7	65.1	±12.1	1.0
Mode of delivery					
Vaginal, spontaneous	207	80.5%	1,793	84.3%	0.1
Vaginal, operative	15	5.8%	136	6.4%	0.8
Cesarean section	23	8.9%	141	6.6%	0.2
Cesarean section for imminent fetal asphyxia	12	4.7%	57	2.7%	0.07
Neonates					
Female gender	116	45.1%	1,057	49.7%	0.1
Gestational age at birth (weeks)	39.2	±1.8	39.4	±1.9	0.4
Preterm delivery	15	5.9%	118	5.5%	0.8
Birthweight (g)	3,516	±595	3,515	±579	1.0
Birthweight deviation (%)**	1.6	±13.0	1.1	±13.0	0.5
5-min Apgar score <7	3	1.2%	29	1.3%	1.0
pH umbilical artery ***	7.23 (n=180)	±0.09	7.23 (n=1,454)	±0.08	0.9
pH umbilical vein ***	7.31 (n=195)	±0,08	7.31 (n=1,555)	±0,08	0.8

Means ± standard deviations, or numbers and percentages are given.

* Smokers at first visit to the antenatal health care unit (mean 12th week of gestation).

** Birthweight as compared to a gestational age adjusted reference population (Marsal 1996).

*** Not investigated in all cases.

Discussion

The prevalence of APC resistance in this prospective study was 11%, a figure similar to those obtained in earlier studies at Malmö (18, 22) but slightly lower than that of 15% previously reported from Kristianstad which is also located in the south of Sweden (19). The lower figure for Malmö is perhaps to be explained by a lower prevalence of APC resistance among the 31% of the pregnant population who were of non-Swedish origin.

Pregnancy Complications

There was no significant difference between the APC-resistant and the non-APC-resistant subgroups in the incidence of pre-eclampsia, a finding consistent with those of two previous studies (22, 27), but in contrast to that of Dizon Townson and colleagues (5). We disagree with the view of Dizon Townson and colleagues that APC-resistance testing might be useful in genetic screening for predisposition to pre-eclampsia and other pregnancy complications (5). Its occurrence does not seem to be related to adverse pregnancy outcome, and if there is a connection with pre-eclampsia, the predictive value is too negligible to warrant screening. There has been some discussion as to whether APC resistance might be predisposing to placental insufficiency (6, 22). However, we found no differences between APC-resistant and non-APC-resistant subgroups in birth-weight deviation, or in the proportion of women with IUGR fetuses (Table 1). Thus, the existence of relationship between APC resistance and IUGR seems unlikely.

Since women were included at gestational age of mean 12 weeks of gestation, the incidence of early spontaneous abortion was presumably underestimated. Therefore, early and late spontaneous abortions of previous pregnancies were recorded. There were no subgroup differences in the risk of early or late spontaneous abortion, a finding in agreement with earlier reports (7, 22). Preston and colleagues found no subgroup difference in miscarriage, in a large cohort with maternal hereditary thrombophilia and a control population (pregnancies predominantly characterized by paternal hereditary thrombophilia) (7). In contrast, groups of women with recurrent abortions have been reported to be distinguished by a high prevalence of APC resistance (6, 8). Preston et al. reported increased risk of stillbirths among an APC-resistant subgroup (7). Due to the low numbers in our investigation, valid conclusions regarding perinatal death could not be drawn.

APC Resistance and VTE

The overall incidence of pregnancy-related VTE (0.24%) in the present series was slightly higher than that of 0.07% previously found in another Swedish study by Bergqvist and co-workers, though their series comprised only antepartum VTEs (28). Of those who suffered VTEs in the present series, three out of six were APC-resistant representing an 8-fold increased risk of VTE, which was in agreement of others (20, 21). Out of the 6 women with present VTE, one had the G20210A mutation in the prothrombin gene (29) and one had lupus anticoagulants (both were non-APC-resistant).

The risk of VTE has been estimated to be 50-100-fold greater among homozygous carriers of the FV:Q^{506} allele's (30). This suggests 7-14% of homozygous women to be at risk of VTE every time they are pregnant. However, this conclusion is not supported by the present finding that none of the 7 homozygote women had a VTE in their present or past pregnancies (n = 18).

A number of authors (31-33) have suggested that APC-resistant women should receive prophylactic treatment in association with preg-

Table 2 Pregnancy complications. Odds ratios and 95% confidence intervals (CI) are given

	APC resistance n=270*		Non-APC resistance n=2,210*		Odds ratio	95% CI
Pre-eclampsia	5	1.9%	34	1.5%	1.2	0.5-3.1
IUGR	9	3.3%	79	3.6%	0.9	0.5-1.9
Abruptio placentae	2	0.7%	11	0.5%	1.5	0.3-6.8
Fetal loss in present pregnancy						
Spontaneous abortion	9	3.3%	54	2.4%	1.4	0.7-2.8
Late spontaneous abortion	3	1.1%	16	0.7%	1.5	0.4-5.3
Legal abortion	1	0.4%	13	0.6%	0.6	0.08-4.8
Perinatal death	1	0.4%	9	0.4%	0.9	0.1-7.2
Number of women with former spontaneous abortions						
Early spontaneous abortion	51	18.9%	409	18.5%	1,0	0.7-1.4
Late spontaneous abortion	3	1.1%	50	2.3%	0.5	0.2-1.6

* Both parturients (n=2,384) and women with abortions (n=96) included.

IUGR = intrauterine growth retardation.

Early spontaneous abortion (before 13 weeks of gestation).

Late spontaneous abortion (after 12 weeks of gestation).

Table 3 Risk of venous thromboembolic events (VTEs) for women with; APC resistance, familial VTE*, or overweight. Odds ratios and 95% confidence intervals (CI) are given

	VTE in present pregnancy (n)		Odds ratio	95% CI	VTE in present pregnancy or in history (n)**		Odds ratio	95% CI
	Yes	No			Yes	No		
APC resistance								
Yes	3	267	8.3	(1.7-41.2)	5	265	4.6	(1.5-13.9)
No	3	2,207			9	2,201		
Familial VTE*								
Yes	1	126	3.7	(0.4-32.1)	4	123	7.6	(2.4-24.6)
No	5	2,348			10	2,343		
Overweight***								
Yes	2	315	3.4	(0.6-18.8)	6	311	5.2	(1.8-15.1)
No	4	2,159			8	2,155		

* Familial VTE = VTE among first-degree relatives (i.e., father, mother or siblings).

** One APC-resistant woman got a VTE despite low molecular weight heparin prophylaxis and one of the non-APC-resistant women had two earlier VTEs.

*** Overweight was defined as a body mass index (kg/m2) > 27.6 (i.e., >1 standard deviation above the mean for the series.

Table 4 Intrapartum blood loss and postpartum anaemia in APC-resistant and non-APC-resistant subgroups

	APC resistance n=217*		Non-APC resistance n=1920*		P-value
Blood loss > 600ml (n)	8	3.7%	152	7.9%	0.02
Postpartum anaemia**(n)	13	6.0%	131	6.8%	0.6
Blood loss during delivery					
Geometric mean (ml)***	340		361		0.04
±1 standard deviation (ml)	235-494		232-562		

* Only vaginally delivered non-heparin treated women were included.

** Postpartum anaemia was defined as Hb <100 g/l on the second day after delivery.

*** Blood loss value was converted to its natural logarithm to normalise a skewed distribution.

nancy. The results of our prospective study argue against this recommendation, as the 1.1% risk of VTE among APC-resistant women hardly warrants prophylactic treatment. The risks of such treatment would probably outweigh any beneficial effect. Indeed, to the best of our knowledge there is no evidence that prophylactic treatment with heparin during pregnancy is efficacious. However, prophylaxis might be justified if additional risk factors for VTE are present in addition to APC resistance, such as a family history of VTE, caesarean section, overweight etc. Hitherto, only women with earlier VTEs have routinely been given prophylactic treatment with heparin in Sweden. Treatment of this high-risk group may reduce the risk of recurrence during pregnancy, but does not prevent de novo VTEs (21). In the future, estimation of the individual risk of pregnancy-related VTEs might be possible, based on the presence or absence of risk factors such as APC resistance, familial thrombosis, surgery or overweight.

APC Resistance and Bleeding Complications

The APC-resistant women had a reduced risk of severe bleeding episodes, as compared to those without APC resistance, which agrees with our earlier findings (22). In former times profuse haemorrhage during delivery was often lethal and the reduced incidence of this pregnancy complication among APC-resistant women might have conferred a survival advantage contributing to an increased prevalence of APC resistance. On the other hand the slightly increased risk of VTE among APC-resistant women have presumable not negatively influenced survival during evolution. To exclude confounding causes of profuse blood loss, the 12 women treated with low molecular weight heparins and the women delivered by caesarean section were excluded in analysis of blood loss. The use of blood transfusion is very restrictive in Malmö, therefore it could not be used as a marker of profuse blood loss.

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Screening for APC Resistance

General screening for APC resistance before pregnancy and oral contraceptive usage has been advocated by some authors (20, 30, 34, 35), whereas others have argued against it (36, 37). A beneficial effect of screening might be that all carriers would know that they are at a slightly higher risk of VTEs, knowledge which might be useful in choosing contraceptives, in association with major surgery, and in other VTE-predisposing situations. In addition, screening would identify homozygotes who are at a 50-100-fold increased risk of VTEs. On the other hand critics maintain that screening for APC resistance is not cost-effective, and women may be unduly alarmed on being informed that they are APC-resistant. Our prospective study provides basic data which may be of value in discussing pros and cons of APC resistance-screening.

Conclusions. In this prospective study of 2,480 pregnant women, the prevalence of APC resistance was found to be 11%. APC resistance did not seem to be related to adverse pregnancy outcome, apart from an 8-fold increased risk of a VTE.

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Thrombotic Risk During Pregnancy: A Population Study

PELLE LINDQVIST, MD, BJÖRN DAHLBÄCK, MD, AND KAREL MARŜÁL, MD

Objective: **To determine the incidence of pregnancy-related venous thromboembolic events and the relationship to selected risk factors such as maternal age, parity, smoking, preeclampsia, or cesarean delivery.**

Methods: **All Swedish women reported as having pregnancy-related venous thromboembolic events during 1990–1993 (608 of 479,422 deliveries) were compared with all thrombosis-free Swedish pregnant women during 1993 (114,940).**

Results: **The incidence of pregnancy-related venous thromboembolic events was 13 per 10,000 deliveries. Cesarean delivery was associated with a fivefold increased risk of venous thromboembolic events. Advanced age was not a significant risk factor itself, but was associated with an age-related increase in frequency of cesareans. Women with preeclampsia were at a threefold higher risk postpartum, but at no increased risk before delivery. There was a tobacco consumption–dependent increase in the risk of thrombosis among smokers.**

Conclusion: **The incidence of pregnancy-related thrombosis was 13 per 10,000 and provided new insights to important risk factors such as age, cesarean delivery, smoking, and preeclampsia. (Obstet Gynecol 1999;94:595–9. © 1999 by The American College of Obstetricians and Gynecologists.)**

Pregnancy-related venous thrombosis is a major cause of maternal morbidity and mortality worldwide.[1–3] Clinical diagnoses of deep venous thrombosis are often inaccurate,[4] and it has been estimated that if diagnosis is based on clinical criteria alone, two of three cases will receive unnecessary anticoagulant treatment.[5] However, there have been very few reports of clinically significant thrombosis verified by objective methods. In a prospective Swedish study in which diagnosis was confirmed by phlebography and plethysmography, the incidence of antepartum venous thromboembolic events was 7 per 10,000.[6]

From the Departments of Obstetrics and Gynecology, and Coagulation Research, University of Lund, University Hospitals, Malmö and Lund, Sweden.

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Normal pregnancy is associated with a manifest shift of coagulation and fibrinolytic systems towards hypercoagulability. Although these changes are of physiologic importance in minimizing the risk of blood loss during delivery, they also increase the risk of thrombosis.[7] Other predisposing factors are advanced maternal age, higher parity, operative delivery, immobilization, obesity, heart disease, malignancy, white race, history of thrombosis, thrombophilia, or familial thrombosis.[1,8]

V

In Sweden, there is an established pretreatment routine for verifying clinically suspected thrombosis related to pregnancy. A diagnosis of deep venous thrombosis is almost always based on findings at phlebography or ultrasonography. Pulmonary embolism is diagnosed by perfusion and ventilation lung scan or by pulmonary angiography. Treatment is routinely an inpatient procedure. Births are registered in the national birth registry and hospitalizations in the national patient registry. By merging the two registries, it was possible to make reliable estimates of the national incidence of thrombosis and of the magnitude of several risk factors.

The purpose of the present retrospective population-based study was to determine the incidence of pregnancy-related thrombosis in Sweden and its relationship to selected risk factors.

Methods

Over 99% of deliveries in Sweden are registered in the national birth registry,[9] and all patients who have been hospitalized (even if only overnight) are registered in the national patient registry. The national birth and patient registries were used to identify all women with pregnancy-related thromboses during the 4-year period 1990–1993. Pregnancy-related thrombosis was defined as deep-vein thrombosis or pulmonary embolism. The diagnosis numbers classified as thrombosis (according to International Classification of Diseases, ninth revision) were deep-vein thrombosis (671D, 671E, and 671F) or pulmonary embolism (673C) related to pregnancy or

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Table 1. Bivariate Analysis of Selected Risk Factors and Pregnancy-Related Venous Thromboembolic Events

	Thrombosis cases (n = 608)	Controls (n = 114,940)	OR	95% CI
Maternal age (y) (classification 1)				
≤19	26 (4.3)	2817 (2.5)	1.9	1.3, 2.9
20–24	125 (20.6)	23,006 (20.0)	1.1	0.9, 1.4
25–29	216 (35.5)	44,763 (38.9)	1.0	Reference
30–34	151 (24.8)	30,135 (26.2)	1.0	0.8, 1.3
≥35	90 (14.8)	14,219 (12.4)	1.3	1.0, 1.7
Maternal age (y) (classification 2)				
≤19	26 (4.3)	2817 (2.5)	1.8	1.2, 2.7
20–34	492 (80.9)	97,904 (85.2)	1.0	Reference
≥35	90 (14.8)	14,219 (12.4)	1.3	1.0, 1.6
Parity				
Para 0	304 (50.0)	47,425 (41.3)	1.8	1.5, 2.2
Para 1	142 (23.4)	40,734 (35.4)	1.0	Reference
Para 2	93 (15.3)	18,113 (15.8)	1.5	1.1, 1.9
≥Para 3	69 (11.3)	8429 (7.3)	2.4	1.8, 3.1
Missing data	0 (0)	239 (0.2)		
No. of cigarettes daily				
0	423 (69.6)	87,408 (76.0)	1.0	Reference
1–9	80 (13.2)	14,295 (12.4)	1.2	0.9, 1.5
≥10	57 (9.4)	8177 (7.1)	1.4	1.1, 1.9
Missing data	48 (7.9)	5060 (4.4)		
Multiple pregnancy				
No	593 (97.5)	113,330 (98.6)	1.0	Reference
Yes	15 (2.5)	1610 (1.4)	1.8	1.1, 3.0
Preeclampsia				
No	562 (92.4)	111,788 (97.3)	1.0	Reference
Yes	46 (7.6)	3152 (2.7)	2.9	2.1, 3.9
Cesarean delivery				
No	420 (69.1)	102,181 (88.9)	1.0	Reference
Yes	188 (30.9)	12,759 (11.1)	3.6	3.0, 4.3

OR = odds ratio; CI = confidence interval.
Data presented as n (%).

the corresponding nonpregnant diagnosis numbers (451B, 452, 453 [C, D, W, or X]) or (415B), respectively, when in conjunction with pregnancy (ie, from 240 days before delivery to 6 weeks postpartum). Age, parity, weight, and height also were collected from registry data. Complete information on weight and height was available for less than 30% of the women, so those two variables were not included in analysis. Data were also collected on occurrence of cesarean delivery, multiple pregnancy, and preeclampsia.

The risk patterns before and after delivery might differ, so thromboses were divided into antepartum and postpartum subgroups. From 479,422 deliveries, a subgroup of 608 women accounted for 625 cases of thromboses. Of 44 women who gave birth more than once during the 4-year study period, 16 had recurrent thrombosis (one woman had thrombosis three times), 15 had one pregnancy-related thrombosis but a thrombosis-free pregnancy in 1993, and 13 women delivered twice during 1993. To avoid the same patient being included twice, only data on first pregnancies were included in logistic regression analysis. Of the 608 women with thromboses (308 antepartum, 300 postpartum) 90 had pulmonary embolisms and 518 had deep-vein thromboses (of which five had cerebral thromboses). Using logistic regression analysis, the women with thrombosis (n = 608) were compared with all thrombosis-free pregnant women in the country during 1993 (n = 114,940).

Data on smoking habits, routinely recorded at initial maternity unit appointments, were available in 96% of cases. Smoking was classified in terms of daily cigarette consumption, ie, at least 10 (heavy smokers), 1–9 (moderate smokers), or 0 (nonsmokers or not regular smokers) used as the reference class. Preeclampsia was defined as the combination of blood pressure higher than 139/89 mm Hg and albuminuria (at least 0.3 g/L). Parity was classified as para 0, para 1, para 2, or at least para 3, para 1 chosen as the reference class because bivariate analysis showed it to be associated with the lowest odds ratio (OR) for thrombosis. Maternal age was first classified according to five age groups (under 20, 20–24, 25–29, 30–34, and at least 35 years of age), but because bivariate analysis found no significant differences in ORs among the 20–24-, 25–29-, and 30–34-year-old age groups, in subsequent analysis they were combined as a 20–34-year-old age group and used as the reference class.

Bivariate and multiple logistic regression analyses were used to determine relationship between the outcome variable (the occurrence of thrombosis) and the explanatory variables (smoking, parity, maternal age, multiple pregnancy, preeclampsia, and cesarean delivery). All explanatory variables were included in analysis of postpartum thrombosis, and all except cesarean delivery in analysis of antepartum thrombosis. No significant interactions were found between any of the explanatory variables. Relative risk was determined in terms of ORs and 95% confidence interval (CI). The degree of linear association was calculated with the Mantel-Haenszel χ^2 test. All statistical calculations were done by computer, using SPSS software (Statistical Package for the Social Sciences; SPSS Inc, Chicago, IL), $P < .05$ was considered statistically significant.

Results

The incidence of pregnancy-related thrombosis was 13 per 10,000 pregnancies. The results of bivariate analysis of explanatory variables are shown in Table 1. The results of logistic regression analysis of antepartum

Table 2. Logistic Regression Analysis of Selected Risk Factors and Antepartum Thromboembolic Events

Variable	Thrombosis cases* (n = 308)	Controls* (n = 114,940)	Bivariate OR	Bivariate 95% CI	Multivariate† OR	Multivariate† 95% CI
Maternal age (y)						
≤19	10 (3.2)	2817 (2.5)	1.3	0.7, 2.5	1.0	0.5, 1.9
20–34	262 (85.1)	97,904 (85.2)	1.0	Reference	1.0	Reference
≥35	36 (11.7)	14,219 (12.4)	0.9	0.7, 1.3	1.0	0.7, 1.4
Parity						
Para 0	178 (57.8)	47,425 (41.3)	2.6	1.9, 3.4	2.9	2.1, 3.9
Para 1	60 (19.5)	40,734 (35.4)	1.0	Reference	1.0	Reference
Para 2	36 (11.7)	18,113 (15.8)	1.3	0.9, 2.0	1.3	0.8, 2.0
≥Para 3	34 (11.0)	8429 (7.3)	2.7	1.8, 4.2	2.8	1.8, 4.4
Missing data	0 (0)	239 (0.2)				
No. of cigarettes daily						
0	221 (71.8)	87,408 (76.0)	1.0	Reference	1.0	Reference
1–9	39 (12.7)	14,295 (12.4)	1.1	0.8, 1.5	1.1	0.8, 1.5
≥10	28 (9.1)	8177 (7.1)	1.3	0.9, 1.9	1.3	0.9, 2.0
Missing data	20 (6.5)	5060 (4.4)				
Multiple pregnancy						
No	299 (97.1)	113,330 (98.6)	1.0	Reference	1.0	Reference
Yes	9 (2.9)	1610 (1.4)	2.1	1.1, 4.1	2.1	1.0, 4.6
Preeclampsia						
No	301 (97.7)	111,788 (97.3)	1.0	Reference	1.0	Reference
Yes	7 (2.3)	3152 (2.7)	0.8	0.4, 1.7	0.8	0.4, 1.6

OR = odds ratio; CI = confidence interval.
* Data are given as n (%).
† Adjusted for maternal age, partiy, multiple pregnancy, smoking, and preeclampsia.

thrombosis are shown in Table 2. Only parity differed significantly in regard to risk of thrombosis, and neither preeclampsia nor advanced age (at least 35 years of age) was associated with increased risk of antepartum thrombosis.

The results of multiple logistic regression analysis of postpartum thrombosis are shown in Table 3. Cesarean delivery was associated with a fivefold increased risk of postpartum thromboses. There was an increase in the rate of cesarean deliveries with increasing age (9% in the age group below 20 years, 10% in the 20–34-year-old group, and 18% among those 35 years or older). The risk of postpartum thrombosis was twice as great in the para 2 and para 3 subgroups compared with the para 1 (reference) subgroup, and threefold greater in the preeclampsia subgroup and the youngest age group (under 20 years old) than in their respective reference classes.

In the thrombosis series as a whole, (ie, antepartum and postpartum subgroups), smoking was associated with a significantly increased risk of thrombosis (OR 1.24; 95% CI 1.02, 1.51). There was a statistically significant association between increased risk of thrombosis and higher tobacco consumption (P = .007), but the difference between nonsmokers and smokers was significant only for heavy smokers (OR 1.41; 95% CI 1.04, 1.82 versus 1.11; 95% CI 0.87, 1.41 for moderate smokers).

Discussion

A notable finding was that preeclampsia was associated with increased risk of thrombosis postpartum but not antepartum. The increased thrombin generation in preeclampsia has been assumed to be evidence for a prothrombotic state,[10] but it has also been suggested that fibrinolysis is more pronounced than fibrin formation in women with severe preeclampsia.[11] The routine recommendation of bed rest before and after delivery for women with preeclampsia might add to the risk of postpartum thrombosis. We noted that smoking was a significant risk factor for pregnancy-related thrombosis. The reason for the cigarette consumption-dependent increase in the risk of thrombosis is not known, but inhibited or defective fibrinolysis during pregnancy among smokers might be one explanation.[12]

Advanced maternal age has been reported as a risk factor for pregnancy-related thrombosis,[8,13] but our results do not support that (Tables 2 and 3). Instead, the youngest women (under 20 years of age) were at threefold higher risk of postpartum thrombosis (Table 3). The higher prevalence of thrombosis among women above 35 years of age in bivariate analysis (Table 3) was not significant after adjustment for other variables, mainly because of an increased rate of cesarean delivery with increasing maternal age. When plethysmography

Table 3. Logistic Regression Analysis of Selected Risk Factors and Postpartum Thromboembolic Events

Variable	Thrombosis cases* (n = 300)	Controls* (n = 114,940)	Bivariate OR	Bivariate 95% CI	Multivarite† OR	Multivarite† 95% CI
Maternal age (y)						
≤19	16 (5.3)	2817 (2.5)	2.4	1.5, 4.0	2.5	1.4, 4.4
20–34	230 (76.7)	97,904 (85.2)	1.0	Reference	1.0	Reference
≥35	54 (18.0)	14,219 (12.4)	1.6	1.2, 2.2	1.2	0.9, 1.6
Parity						
Para 0	126 (42)	47,425 (41.3)	1.3	1.0, 1.7	1.1	0.8, 1.5
Para 1	82 (27.3)	40,734 (35.4)	1.0	Reference	1.0	Refernce
Para 2	57 (19.0)	18,113 (15.8)	1.6	1.1, 2.2	1.7	1.2, 2.4
≥Para 3	35 (11.7)	8429 (7.3)	2.1	1.4, 3.1	1.8	1.2, 2.9
Missing data	0 (0)	239 (0.2)				
No. of cigarettes daily						
0	202 (67.3)	87,408 (76.0)	1.0	Reference	1.0	Reference
1–9	41 (13.7)	14,295 (12.4)	1.2	0.9, 1.7	1.2	0.8, 1.7
≥10	29 (9.7)	8177 (7.1)	1.5	1.0, 2.3	1.4	1.0, 2.1
Missing data	28 (9.3)	5060 (4.4)				
Multiple pregnancy						
No	294 (98.0)	113,330 (98.6)	1.0	Reference	1.0	Reference
Yes	6 (2.0)	1610 (1.4)	1.4	0.6, 3.2	0.6	0.2, 1.4
Preeclampsia						
No	261 (87.0)	111,788 (97.3)	1.0	Reference	1.0	Reference
Yes	39 (13.0)	3152 (2.7)	5.3	3.8, 7.4	3.0	2.0, 4.4
Cesarean delivery						
No	177 (59.0)	102,181 (88.9)	1.0	Reference	1.0	Reference
Yes	123 (41.0)	12,759 (11.1)	5.6	4.4, 7.0	4.9	3.8, 6.3

OR = odds ratio; CI = confidence interval.
* Data are given as n (%).
† Adjusted for age, parity, multiple pregnancy, smoking, cesarean delivery, and preeclampsia.

was used to screen for thrombosis, Bergqvist et al[14] found an association between cesarean delivery and a 1.8% incidence of thrombosis. In the present series, the prevalence of clinically significant thromboses among those delivered by cesarean was 0.9%, which was fivefold greater than among those who delivered vaginally. The proportion of postpartum thromboses associated with cesarean delivery was high (41%). Among women who died after delivery due to postpartum pulmonary embolism, the figure was even higher (76%).[1]

Women with postpartum thrombosis might be treated in internal medicine wards, so that group is difficult to identify because such cases tend to be inadequately classified (eg, all thromboses during pregnancy, or during the first 42 days postpartum, should be classified as 671 [D, E, or F], or 673C, but they often have nonpregnant thrombosis diagnosis numbers). By merging the two national registries we were able to identify women with thrombosis, irrespective of where they were treated. Previously, postpartum thromboses have been far more common than antepartum thromboses,[15] which is in contrast to the predominance of antepartum thrombosis in a study by Macklon and coworkers,[13] and with the even distribution found in our study. The lower proportion of postpartum thromboses in the latter two studies might be attributable to a change in obstetric practice that minimizes the duration of hospitalization and postpartum immobilization. Our findings are consistent with those of the Confidential Enquiry into Maternal Death during the period 1985–1993,[1] showing deaths due to pulmonary embolism to be equally distributed between antepartum and postpartum subgroups.

Only pregnancies that resulted in birth were included; extrauterine pregnancies and spontaneous or legal abortions were excluded. We relied on diagnoses recorded in the national birth and patient registries, so clinically insignificant thromboses, or cases treated in outpatient settings were not included. In Sweden, thrombosis is routinely diagnosed on the basis of objective criteria, and it is unlikely that many thromboses were treated in outpatient clinics. The design of the study, ie, including 4 years of cases and 1 year of controls, and the inclusion of only the first pregnancy for thrombosis cases with multiple pregnancies during the 4-year study period might have introduced a selection bias; however, we believe that bias is small compared with the gain of power in analysis by using 4 years of cases.

Our results are supported to some extent by the findings of Bergqvist and coworkers[5] whose prospective study in Sweden found the incidence of antepartum thrombosis to be 7/10,000. The relative homogeneity of the Swedish population and the national uniformity of obstetric practices made confounding of the results, eg, due to demographic factors, likely to be minimal. Details of cigarette consumption are routinely recorded at initial appointments at maternity units throughout the country, so any recall bias in that respect was precluded. The design of the study did not allow analysis of other genetic or acquired risk factors of thrombosis.

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Address reprint requests to:
Pelle Lindqvist, MD
Department of Obstetrics and Gynecology
University Hospital, Malmö
S-20502 Malmö
Sweden
E-mail: pelle.lindqvist@obst.mas.lu.se

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