Maternal Fetal Medicine Units Network

PROTOCOL

CLINICAL TRIAL OF LOW-DOSE ASPIRIN TO PREVENT PREECLAMPSIA IN HIGH RISK WOMEN

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Prepared by the

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Preface

Protocol Amendments

1. April 1st, 1992 - Section 5.1.3.

The following change to the definition of the primary study outcome was proposed by the High Risk Aspirin Study Subcommittee who are reviewing all potential cases of preeclampsia. For patients in the chronic hypertensive group with baseline proteinuria and for patients in the diabetic group with baseline hypertension and proteinuria, diagnosis of superimposed preeclampsia may be made if the patient had a diastolic blood pressure of $\geq$ 110 mg Hg on two occasions at least four hours apart in addition to at least one of a series of symptoms ($\geq$ 5 gm protein in a 24 hour collection, oliguria, severe headaches, pulmonary edema, epigastric pain). This is an additional category to the present criteria of thrombocytopenia or abnormal SGOT.

2. April 1st, 1992 - Sections 3.4, 4.1, 6.1.

The High Risk Aspirin Subcommittee also proposed that the exclusion criterion of proliferative retinopathy be removed. The original reason for this exclusion was based on a concern that there may be an increased risk of hemorrhagic complications. However, in view of the recent publications below, which indicate that low-dose aspirin is therapeutic in patients with retinopathy, this is no longer a concern. Recruitment to the diabetic subgroup and the overall cost of the study will be improved by including these patients.

Effects of Aspirin Treatment on Diabetic Retinopathy. Early Treatment Diabetic Retinopathy Study. Early Diabetic Retinopathy Group. Ophth. 1991; 98:757-765.

Effects of Aspirin alone and Aspirin plus Dipyidamole in Early Diabetic Retinopathy. Multicenter Randomized Controlled Clinical Trial. DAMAD Study Group. Diabetes 1989; 38:491-498.

These changes were passed by the NICHD Advisory Board on March 25th, 1992 and by the MFMU Network Steering Committee on March 31st, 1992.

3. May 28th, 1992

The Steering Committee approved the addition of an ancillary study to be conducted on all patients who participate in the High Risk Aspirin Study. Patients will be asked to consent to three blood drawings, on or before randomization, at 24-28 weeks gestational age and at 34-38 weeks gestational age. The timing of the blood collection will be coordinated with routine blood drawings wherever possible. Patients may still participate in the High Risk Aspirin Study without consenting to the blood drawing. The blood samples will be frozen as 1 ml serum samples and sent in batches to Dr. Walsh's laboratory at the Medical College of Virginia. The serum thromboxane results will be forwarded directly to the Biostatistical Coordinating Center, to maintain the masking.

The objectives of the ancillary study are

a. To document compliance. Serum thromboxane levels should decrease from baseline in aspirin patients and should not decrease in placebo patients.

b. To determine whether the degree of serum thromboxane reduction is predictive of the development of preeclampsia.

Full details regarding the methods of handling, storage and shipment of the samples are provided as separate instructions in the High Risk Aspirin Study Manual of Operations.

This change was approved by the NICHD Advisory Board on June 18th, 1992.

4. June 13th, 1994

The Data Monitoring and Safety Committee recommended that recruitment continue in all four risk groups until the diabetes group reaches 450 patients (rather than the originally planned goal in that group of 980). This goal will yield an approximate total sample size of 2,600, as originally assigned.

5. October 17th, 1994 - Sections 5, 7, 8

The following clarifications were made to the diagnostic criteria by the High Risk Aspirin Subcommittee:

- Intraoperative blood pressures (from the start of surgery to admission to the recovery room) will be ignored.

- All diagnoses that required two diastolic blood pressures $\geq$ 110 now also allow one diastolic $\geq$ 110 treated with anti-hypertensive medication.

- The criteria of oliguria was removed from all diagnoses.

- In the hypertensive, non-proteinuric group, SGOT was removed from the criteria for preeclampsia.

- In the hypertensive, proteinuric group, pulmonary edema was removed from the criteria for preeclampsia. Also in this group, the criteria for worsening hypertension was modified such that qualifying blood pressures must occur within the same time period and, if antepartum, within one week of delivery. The criteria for worsening proteinuria now reads as follows: either "at least a five-fold increase in proteinuria from baseline" or "at least a two-fold increase in proteinuria from baseline if the highest protein collection after baseline is $\geq$ 5 gm/ 24 hours".

Section 8 (Administration) has been updated to reflect current MFMU Network practices.

Table 2, "List of Data Collection Forms", in Section 7 (Data Entry and Management) has been updated to include the PE04B Supplement, the PE15 Adverse Effects Form, and the PE16 Serum/Plasma Catalog. To provide documentation of baseline eligibility, written justification (a PE04B Supplement) is required for patients in Group B with screening blood pressure < 140/90 and who are not on antihypertensive medication (this has been in effect since the start of the study).

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Introduction

1.1 Study Abstract

Recent clinical studies have suggested that daily low-dose aspirin (60 mg-150 mg) may be of value in preventing preeclampsia in women deemed to be at increased risk for the disease. The patients studied have had either a positive angiotensin II sensitivity test or a history of preeclampsia or a fetal death or hypertension in a previous pregnancy, or some combination of the above [33,2,3,23]. Although aspirin therapy appears to be beneficial, it is unclear if it is effective in all conditions which predispose women to preeclampsia or if it is effective in only a few specific situations. In the studies cited above, patients were not stratified by predisposing risk factor. This is largely a restriction imposed by the small sample size of each of these studies. A large sample size would enable stratification by risk category and allow for an evaluation of aspirin effectiveness in preventing preeclampsia in specific risk conditions. Currently the NICHD is evaluating the effectiveness of low dose aspirin in preventing preeclampsia in women without medical or obstetric complications. The purpose of the present study is to evaluate the effectiveness of 60 mg aspirin in preventing preeclampsia in four groups of women at risk for developing the disease. These groups include: 1) women with chronic hypertension, 2) women with insulin-dependent diabetes, 3) women with preeclampsia in a previous pregnancy, and 4) women with a multifetal gestation. The primary outcome variable is the development of preeclampsia. Additional outcome variables include routine maternal and perinatal outcomes.

1.2 Hypothesis

The primary hypothesis of this study is that daily administration of 60 mg of aspirin beginning between 13-26 weeks gestation will decrease the incidence of preeclampsia. This hypothesis is based on the assumption that early antiplatelet therapy will inhibit platelet thromboxane generation with minimal effects on vascular prostacyclin production, thus directing the fetoplacental prostacyclin/thromboxane A_2 balance to the dominance of prostacyclin. An increased prostacyclin/thromboxane A_2 ratio may inhibit platelet activation and prevent uteroplacental thrombosis and preeclampsia in a group of pregnant women judged to be at high-risk for these conditions [33,2,3,23,35].

2

Background

2.1 Risk of Preeclampsia

In women with chronic hypertension, the reported incidence of superimposed preeclampsia ranges from 10-50%, depending on the degree of hypertension at the onset of pregnancy [1]. In diabetic women, the likelihood of preeclampsia/eclampsia is increased about three-fold [30]; an increase in preeclampsia/eclampsia is noted even in the absence of demonstrated vascular disease [21]. In patients with a past history of preeclampsia, the incidence of recurrent preeclampsia is approximately thirty percent [7]. There is a two to five fold increase in the incidence of preeclampsia in pregnancies with more than one fetus [14].

2.2 Clinical Changes in Pregnancy and Preeclampsia

A rise in blood pressure of at least 30 mmHg systolic or 15 mmHg diastolic from a previous recording earlier in pregnancy has been used by several authors for the diagnosis of pregnancy induced hypertension. This definition causes considerable difficulties since a gradual increase in blood pressure from second to third trimester is usually seen in most pregnancies whether the subject was hypertensive prior to pregnancy or not. MacGillivray, et al., [16] found that an increase in diastolic pressure of $\geq$ 20 mmHg is present in 57% of normotensive patients. More recently, Moutquin, et al., [18] found that a rise of 30 mmHg systolic or 15 mmHg diastolic has a poor sensitivity for the diagnosis of preeclampsia in the primigravid patient. Similar findings were observed by Sibai, et al., [25] during the analysis of serial blood pressure findings in over 1000 nulliparous pregnancies. In addition, the above definition is influenced by gestational age at time of first observation, and frequency of blood pressure measurements. Hence, in this study, a rise in blood pressure with or without proteinuria will be recorded and analyzed but not used in the definition of preeclampsia.

Edema occurs in about 80% of all pregnancies and generalized edema and excess weight gain are common in normal pregnancy. Dexter and Weiss [8] reported edema of the hands, face or both in 64 of 100 consecutive women examined during the third trimester of normotensive pregnancies. Significant edema of the hands, face or both was recognized at some stage of pregnancy in 30% of women studied in the Collaborative Perinatal Project. Sibai, et al., [25] studied total weight gain and magnitude of weight gain at four weekly intervals in preeclamptic women and a well-matched group of women who remained normotensive throughout pregnancy. There were no differences between the two groups regarding either magnitude or total weight gain. In addition, 40% of eclamptic women studied by Sibai, et al., [25] had no edema. Therefore, these variables, although recorded, will not be used to define patient outcome.

2.3 Vascular Changes in Pregnancy and Preeclampsia

The human placenta receives its blood supply from numerous uteroplacental arteries which are developed by the action of migratory interstitial and endovascular trophoblasts on the spiral arteries in the placental bed [3,4]. The conversion of the spiral arteries of the non-pregnant uterus to the uteroplacental arteries has been termed "physiological changes" by Brosens, et al., [4]. In normal pregnancy, these trophoblast-induced vascular changes extend all the way from the intervillous space to the origin of the spiral arteries from the radial arteries in the inner one-third of the myometrium. It is suggested that these vascular changes are affected in two stages: "the conversion of the decidual segments of the spiral arteries by a wave of endovascular trophoblast migration in the first trimester and the myometrial segments by a subsequent wave in the second trimester" [20].

In contrast, pregnancies complicated by preeclampsia and/or by fetal growth retardation demonstrate inadequate maternal vascular response to placentation. In these pregnancies, the above vascular changes are usually restricted only to the decidual segments of the uteroplacental arteries. Hence, the myometrial segments of the spiral arteries are left with their musculoelastic architecture, thereby rendering them responsive to vasometer influences. Additionally, the number of well-developed uteroplacental arteries is smaller than that found in normotensive pregnancies [12]. Furthermore, Robertson, et al., [22] postulated that this defective vascular response to placentation is due to inhibition of the second wave of endovascular trophoblast migration that normally occurs from about 16 weeks gestation onwards.

Normotensive pregnancies are characterized by an increased resistance to vasoactive substances such as angiotensin-II and norepinephrine. On the other hand, preeclamptic pregnancies have increased sensitivity to these peptides. Gant and co-workers [11] have found that the loss of refractoriness to angiotensin-II is not related to volume deficit or concentrations of renin or angiotensin-II. Everett, et al., [9] reported that the vascular response to angiotensin-II infusions in normotensive pregnancies and in pregnancies complicated by pregnancy induced hypertension can be modified by prostaglandin inhibitors (indomethacin, aspirin).

Preeclampsia is associated with vasospasm, pathologic vascular lesions within multiple organ systems including the placental bed, and activation of the coagulation system. McKay [15] has suggested that pregnancy is a state of chronic intravascular coagulation, and preeclampsia is an exaggerated state of this phenomenon. There is good evidence from several studies that preeclampsia is accompanied by endothelial injury (increased fibronectin), increased platelet activation with platelet consumption in the microvasculature (increased B-thromboglobulin) and enhanced clotting (low antithrombin III). Vascular endothelial damage may cause decreased prostacyclin production and activation of both clotting and fibrinolysis, resulting in the generation of thrombin and plasmin. Thrombin consumes antithrombin III resulting in intravascular fibrin deposition. Platelet activation leads to release of thromboxane A_2 and serotonin resulting in further vasospasm, platelet aggregation and endothelial injury.

2.4 Early Aspirin Trials

Beaufils, et al., [2] reported on 102 patients who because of a past history of hypertension, stillbirth, or fetal growth retardation were at increased risk for preeclampsia. Fifty-two patients were randomly treated with aspirin 150 mg/d plus dipyridamole a prostaglandin synthetase inhibitor, 300 mg/d starting from 12 weeks gestation onwards. The other 50 patients served as a control group. Preeclampsia, fetal deaths or fetal growth retardation did not occur in the treatment group, while there were 6 cases of preeclampsia, 5 fetal deaths, and 4 infants with severe growth retardation in the control group. The average duration of gestation, birth weight and plasma volume were significantly higher in

the treatment group. The incidence of fetal growth retardation was significantly higher in the control group than in the treated group (45% vs 8%).

Wallenburg, et al., [33] studied 207 primigravidas who were screened by angiotensin-II infusions at 28 weeks gestation. Forty-six patients with positive tests were randomized to receive either placebo (n=23) or aspirin 60 mg daily (n=23). At 33-35 weeks gestation, a venous blood sample was drawn for determination of thrombin induced production of malondialdehyde (MDA) by platelets. This dose caused 90% inhibition of platelet MDA synthesis. There were no differences between the two groups regarding average gestational age or birth weight at delivery. However, the treated group had no preeclampsia or severe PIH while in the placebo group there were 3 cases of severe PIH, 7 cases of preeclampsia, and 1 case of eclampsia. In addition, there were no premature deliveries in the treated group and the incidence of small for gestational age infants was 19% in the treated group compared with 39% in controls.

In a subsequent report Wallenburg, et al., [34] gave 60 mg/d aspirin to 34 normotensive primigravidas with a positive angiotensin-II sensitivity test at 28 weeks gestation. Sixteen control women were given matching placebo. The angiotensin-II sensitivity test was repeated at 34 weeks gestation. In the aspirin treated group the test became negative in 13 women and the sensitivity decreased or remained unchanged in 3. In the placebo treated group, 3 patients developed preeclampsia prior to 34 weeks gestation and the test was repeated in the remaining 15. Five of the 15 had a negative test, and 6 had increased sensitivity (all 6 developed preeclampsia or PIH later in pregnancy).

Schiff, et al., [23] screened 791 pregnant women at risk for preeclampsia with an angiotensin II sensitivity test at 28-29 weeks gestation. Sixty-five women with increased angiotensin II sensitivity were randomized to receive daily, in the third trimester, either a placebo or 100 mg aspirin.

Women receiving aspirin were significantly less likely to develop proteinuric hypertension (1/34 vs 7/31) than women receiving placebo. Furthermore, aspirin treated patients maintained their pregnancies significantly longer (272 vs 261 days) than the placebo treated subjects. These investigators demonstrated a good correlation (r=0.709) between the thromboxane B_2 /6-keto-prostaglandin F_1 α ratio and the mean blood pressure in the group receiving aspirin. Serum concentrations of thromboxane B_2 were higher in patients taking aspirin who developed pregnancy induced hypertension than in patients taking aspirin who did not develop pregnancy induced hypertension.

Benigni, et al., [3] randomized 33 pregnant women to receive either 60 mg aspirin or placebo from the 12th week of pregnancy onward. These women were thought to be at increased risk for preeclampsia because of hypertension or fetal death or early onset of preeclampsia or severe fetal growth retardation. Patients treated with aspirin delivered babies that were significantly heavier and older than the babies of the control group. The incidence of pregnancy induced hypertension was lower in the aspirin treated group (0/7 vs 3/16) but not significantly so. Maternal plasma thromboxane B_2 was almost completely inhibited by aspirin.

These studies suggest that low dose aspirin therapy reduces maternal thromboxane B_2 concentration. Pregnancy is prolonged, birthweight is increased and the incidence of proteinuria hypertension is reduced by aspirin therapy.

2.5 *Mechanism of Action of Aspirin*

For many years aspirin has been known to affect platelet function. It acetylates platelet cyclo-oxygenase thus inhibiting synthesis of thromboxane by platelets exposed to its action. The use of aspirin has been advocated and tested in cases of unstable angina, prevention of myocardial reinfarction and stroke.

It has been suggested that the PGI_2/TxA_2 balance is important in regulating the uteroplacental circulation, and that any disturbance may lead to certain pathologic vascular occlusive lesions as a result of the opposing actions of the two eicosanoids on both blood vessels and platelet aggregation. In preeclampsia, the balance is tilted in favor of TxA_2 production [35]. The correct dose of aspirin for the prevention of preeclampsia remains unknown. The optimum dose would be the one which substantially inhibits platelet TxA_2 synthesis without affecting vascular endothelial PGI_2 production leading to an enhanced PGI_2/TxA_2 ratio.

There is evidence to suggest that doses of aspirin in excess of 80 mg/d will inhibit both PGI_2 and TxA_2 synthesis, and that only low doses of aspirin (20-40 mg) daily, would achieve an optimum balance. Pederson and Fitzgerald [19] reported 56% reduction in TxB_2 formation in serum following oral administration of 20 mg aspirin. The authors suggested that their findings are consistent with presystemic inhibition of platelet thromboxane production by aspirin. Cerletti et al. [5] studied 6 healthy men following oral administration of 20 mg of aspirin per day for one week. Serum thromboxane B_2 generation was reduced by 85-93%, and arachidonic acid induced platelet aggregation was completely suppressed.

Forestier, et al., [10] studied 5 pregnant women who were given various doses of aspirin (50-600 mg) 3 hours before fetal blood sampling. Platelet aggregation studies were performed in both mother and fetus. The 50 mg dose resulted in 70% inhibition of maternal platelet aggregation and 100% inhibition of fetal platelet aggregation. Hence, aspirin crosses the placenta readily.

Ylikorkala, et al., [37] studied the effects of acetysalicyclic acid in healthy women given 100 mg (n=13) or 500 mg (n=14) doses during labor at term. The fetal prostacyclin synthesis was significantly reduced in infants of mothers receiving the 500 mg dose, but it was unchanged in infants of mothers receiving 100 mg of acetylsalicyclic acid. Fetal thromboxane A_2 synthesis was inhibited in infants born to mothers receiving both doses. The authors concluded that small doses of maternal acetylsalicyclic acid (100 mg) inhibits only the fetoplacental thromboxane A_2 but leaves prostacyclin production unaffected.

Sibai, et al., [29] evaluated the effect of three doses of aspirin and a placebo in 40 pregnant women in the third trimester. In this study, 40 normotensive, primigravid women were randomized to receive (n=10 each) placebo, 80 mg, 60 mg, or 20 mg of aspirin/d for two weeks starting at 37 weeks gestation.

Serum levels of 6-keto-$PGF_1\ \alpha$ and thromboxane B_2 (TxB_2) were obtained at enrollment, at 1 week and 2 weeks after enrollment, and from cord blood and neonatal blood (<12 hours old). In addition, platelet aggregation and platelet thromboxane B_2 (TxB_2) generation in response to ADP (10^{-7} - 10^{-5} M final concentration) and collagen (0.375-3.0 ug/ml final concentrations) were assessed in the mother before treatment, at 1 and 2 weeks after the start of treatment and in the neonate. All neonates were also studied by 2-dimensional Doppler echocardiography to verify patency of the ductus arteriosus and to measure pulmonary artery pressure noninvasively.

There were no significant differences among the four groups regarding maternal height or weight, mean gestational age at enrollment or delivery, or mean birth weight and Apgar scores. The average duration of exposure to aspirin was 2.7 $\pm$ 1.4 weeks. Maternal 6-keto-$PGF_1\ \alpha$ levels

(150-250 pg/ml) were not affected by these doses of aspirin. Thromboxane B_2 generated during clotting of maternal blood or in response to adenosine diphosphate was decreased significantly by 80 and 60 mg of aspirin (see Table 1). Neonatal serum concentration of 6-keto $PGF_1 \alpha$ and thromboxane B_2 as well as platelet aggregation in response to collagen or adenosine diphosphate were not affected by any of the aspirin doses. All neonates had echocardiographic evidence of a patent ductus arteriosis and noninvasive estimates of pulmonary arterial pressure were similar among all groups of infants.

Benigni, et al., [3] reported that ingestion of 60 mg aspirin daily during the second and third trimesters reduced maternal serum thromboxane levels by 90% while not affecting prostacyclin activity. In the neonates of these mothers, serum thromboxane was reduced by 63% but there were no hemorrhagic complications reported. Similarly, in the study by Schiff, et al., [23], no neonatal hemorrhagic complications were noted among the infants of 34 women receiving 100 mg of aspirin during the third trimester.

2.6 Possible Adverse Effects of Aspirin in Pregnancy

Aspirin crosses the placenta and equilibrates between the mother and fetus within 60 minutes of ingestion. In a large epidemiological study involving about 14,000 pregnancies exposed to aspirin, Slone, et al., [31] found no evidence of an increased risk of congenital defects. However, a recent report by Zieler, et al., [38] found an increased risk of congenital heart disease from the use of regular doses of aspirin early in pregnancy. The risk increases from about 0.2% to 0.4%. This risk will be avoided if the drug is used after 12 weeks' gestation.

There are additional maternal and fetal-neonatal risks associated with chronic use of regular doses of aspirin during pregnancy. Potential maternal and fetal risks include longer duration of pregnancy, and increased risks of antepartum and postpartum hemorrhage [6]. Neither Beaufils, et al., Wallenberg, et al., Schiff, et al., or Benigni et al., found this to be the case.

Patency of the ductus arteriosus in-utero is maintained in part by prostaglandins E_2 and prostacyclin. Thus, there are several potential adverse fetal-neonatal effects associated with the use of prostaglandin synthetase inhibitors during pregnancy. Most of these effects are related to the use of these agents late in pregnancy [36]. However, the Collaborative Perinatal Project [24] did not demonstrate any significant risk in children of over 26,000 mothers who consumed aspirin during pregnancy. Aspirin use in pregnancy may lead to the development of oligohydramnios, inhibition of fetal platelet aggregation, or neonatal bleeding disorders [5,32]. In the study by Stuart, et al., [32] neonatal bleeding problems were seen after mothers took single doses of 300 mg-1500 mg of aspirin in the last five days before delivery. On the other hand, such complications were not reported in any of the studies in which aspirin was used to prevent preeclampsia [33,2,3,23].

Table 1: Maternal Prostanoids Levels (pg/ml)

	6-K-PGF1a (mean + SEM)		TXB_2 (mean + SEM)	
Placebo (initial)	274 ± 72		16045 ± 5067	
1 week	346 ± 116	(NS)	31048 ± 8934	(NS)
2 weeks	142 ± 67	(NS)	25263 ± 11644	(NS)
20 mg (initial)	156 ± 67		26552 ± 4892	
1 week	153 ± 43	(NS)	4201 ± 1259	(p=0.02*)
2 weeks	146 ± 45	(NS)	14584 ± 2892	(NS)
65 mg (initial)	125 ± 35		28395 ± 5159	
1 week	69 ± 10	(NS)	2670 ± 998	(p=0.0015*)
2 weeks	68 ± 18	(NS)	520 ± 165	(p=0.0035*)
80 mg (initial)	270 ± 46		20460 ± 4512	
1 week	127 ± 43	(NS)	721 ± 176	(p=0.008*)
2 weeks	83 ± 10	(NS)	1292 ± 562	(p=0.046*)

*compared to initial values in each group.

3

Study Design

3.1 Overall Design

The study will be a randomized, double-blind, placebo-controlled clinical trial. The medication to be employed is 60 mg aspirin or placebo to be prepared by the Sterling Laboratories.

3.2 Inclusion Criteria

Four groups of women at high risk for the development of preeclampsia will be studied.

The following are the four risk groups.

a. Insulin-dependent diabetes antedating pregnancy. Insulin dependent diabetes developing after the start of pregnancy does not qualify for the study. Women with insulin dependent diabetes and multifetal pregnancy are excluded from the study.

b. Chronic hypertension. This is defined as the presence of sustained blood pressure of 140/90 or higher either before the twentieth week of pregnancy or prior to pregnancy. A woman who is on antihypertensive medication or who was on antihypertensive medication prior to pregnancy but taken off when she became pregnant will be considered to have chronic hypertension even though she may not fulfill the blood pressure criterion above. Women may be included in this group if they have non-insulin dependent diabetes but not if they have insulin-dependent diabetes. Women with chronic hypertension and multifetal pregnancy are excluded from the study.

c. Multifetal pregnancy. Women may have non-insulin dependent diabetes but not insulin-dependent diabetes. They cannot satisfy the criteria for chronic hypertension nor can they have proteinuria of 300 mg/24 hours or more at entry. They may have had previous preeclampsia.

d. Previous preeclampsia. They cannot have chronic hypertension as defined above, nor insulin-dependent diabetes nor a multifetal pregnancy nor proteinuria of 300 mg/24 hours or more at entry.

Women within each risk group will be randomized between 13-26 weeks gestation to one of two treatment groups. One treatment group will receive placebo, whereas the other will receive a daily dose of 60 mg aspirin. The medication will be continued until the onset of term ($\geq$ 37 weeks) labor or until preeclampsia occurs. Consenting patients should not have taken aspirin medication between screening and randomization. (A list of all over-the-counter medication containing aspirin will be available.)

3.3 Estimation of Gestational Age

Gestational age will be determined by integration of clinical and laboratory information including the last menstrual period, hormonal tests, ultrasound, etc. Upon enrollment into the study, a firm gestational age and estimated delivery date should be established. If there is uncertainty about the gestational age, ancillary testing should be obtained.

Gestational age will be considered as firmly established in women with a reliable last menstrual period in addition to one of the clinical or one of the laboratory criteria listed below.

Clinical

- exam by 16 weeks gestation
- auscultation of the fetal heart beat by 14 weeks with a doppler apparatus or by 20 weeks with a stethoscope

Laboratory

- an ultrasound evaluation by the 26th week
- a positive HCG test by the 8th week

In women not fulfilling the criteria above, ancillary tests should be obtained as needed. The PI or his designee must make a determination of gestational age by the 28th week of pregnancy. The best estimation of gestational age must be clearly documented in the patient's medical record.

3.4 Exclusion Criteria

Maternal

a. Delivery elsewhere

b. Gestation >26 weeks

c. Significant antepartum bleeding

d. Active peptic ulcer disease

e. Allergy to aspirin

f. Women requiring other platelet active drugs or nonsteroidal anti-inflammatory agents

g. Drug or alcohol abuse in current pregnancy

h. Women with a bleeding disorder

i. Renal failure

j. Active hepatitis

k. Uncontrollable hypertension

Fetal

a. Known anomalies incompatible with life

b. Hydrops fetalis

For the insulin dependent diabetes group:

a. Multifetal pregnancy

For the chronic hypertensive group:

a. Insulin dependent diabetes

b. Multifetal pregnancy

For the multifetal group:

a. Insulin dependent diabetes

b. Chronic hypertension

c. Proteinuria > 300 mg/24 hours at screening

For the previous preeclamptic group:

a. Insulin dependent diabetes

b. Chronic hypertension

c. Proteinuria > 300 mg/24 hours at screening

d. Multifetal pregnancy

4

Study Procedures

4.1 Screening Procedures

Pregnant women in the second trimester will be screened for inclusion and exclusion criteria and interviewed regarding medications used during the current pregnancy. At this time, the baseline blood pressure will be recorded and the urine protein measured by dipstick. A negative or trace urine protein on dipstick is sufficient evidence of absent proteinuria. If urinary protein excretion is $\geq$ 1+ on dipstick, a 24 hour urine collection for total protein and creatinine clearance must be ordered. All women with chronic hypertension or diabetes with nephropathy should have a baseline serum BUN, creatinine, SGOT, and uric acid. Those who are eligible, pending review of their medical charts or the results of the lab tests will be told about the study and, if willing to participate, will be asked to sign an informed consent. Participants will be given a 10 day supply of placebo tablets in a single-blind manner.

Ideally, confirmation of eligibility (other than multifetal pregnancy, which is determined by ultrasound) should be based on a review of the medical record and every effort to obtain the record prior to randomization should be made. If that effort is successful, that fact is noted on the randomization form. If the record is not available soon enough it still may be possible to make a reasonably firm determination. Thus, a woman on antihypertensive medication, or who was on antihypertensive medication but taken off when she became pregnant, is presumptively a chronic hypertensive. Otherwise a record review is required for assignment to the chronic hypertensive group.

Assignment to the previous preeclampsia group should be based on the previous medical record wherever possible. The medical record must contain documentation to satisfy the basic study criteria for preeclampsia, eclampsia or HELLP syndrome (see section 5.1.1). If the chart is absolutely impossible to obtain, patient history and toxemia/hypertension resulting in an iatrogenic pre-term delivery (i.e. a cesarean section or attempted induction $<$37 weeks) is acceptable.

By the time of the patient's return visit, her eligibility should be established by the necessary documentation. Those deemed still eligible will be checked for compliance and asked again about their willingness to participate. Those who have taken aspirin-containing products or have taken less than half of their placebo tablets and those who change their mind will be eliminated from the study before randomization.

4.2 Randomization

Patients will be randomly assigned with equal probability to the aspirin treatment group or the placebo group in a double-blind fashion according to a blocked randomization scheme. The randomization will be stratified by risk group and by Network center. For each center, a list of drug numbers is provided. By writing the patient's name on the list, beside the next drug number, the patient is assigned to that number. This defines the point at which randomization takes place.

4.3 Study Procedures Post-Randomization

A carton of study drug with the corresponding drug number is then assigned to the patient. She will then be given the first blister pack from the carton containing either 60 mg aspirin tablets or identical placebo tablets. The tablets are to be taken once daily up to delivery or until preeclampsia develops. All participants will be given a list of common over-the-counter products that contain aspirin (with instructions to avoid them) and a supply of acetaminophen to take for pain and headaches. They will be informed that ingestion of regular doses of aspirin would confound the effects of the study and perhaps result in adverse fetal and neonatal effects.

4.3.1 Sequence of Visits

Patients are to be seen at the scheduled prenatal clinic visits. Blood pressure measurement, weight, and urine protein (by dipstick) will be recorded at each prenatal visit. If urinary protein excretion is $\geq$ 1+ on dipstick on 2 occasions at least 4 hours apart or 2+ or more on a single occasion, a 24 hour urine collection for total protein must be ordered. This test should be repeated as clinically necessary to make the diagnosis of preeclampsia.

For patients in the chronic hypertensive group or chronic hypertensives in the diabetic group, if there are signs of worsening hypertension ($\geq$160/110 or initiation or increase in antihypertensive medication), increase in protein excretion (2 points on dipstick), excessive weight gain (5 lbs or more in a week), or edema (2+ or more) then liver function tests and a platelet count must be ordered.

Patients will also be questioned about study medication side effects in a non-directed fashion. In addition, patients will be contacted by the research coordinators as needed during the study to check on compliance and ensure that they will appear for their scheduled prenatal visits. Each patient who does not appear for her regular clinic visit will be contacted by the research coordinator and seen at the earliest time possible.

4.3.2 Compliance

At these visits patients will be questioned as to how they took the tablets and a tablet count will be made and recorded on data forms for later analysis. The patient should return her pack and a new blister pack with the same identifying drug number will be supplied to her (unless she is close to term and running out of new packs in which case she may use a pack for more than one visit). Each patient will be questioned regarding any medications used during the previous time period, and will have the importance of clinic attendance and treatment adherence reinforced so as to minimize noncompliance and dropouts. Patients will be kept on the study even if compliance is less than optimal. If they refuse further treatment, follow-up information will still be collected.

4.3.3 Blood Pressure Measurement

The diagnosis of preeclampsia and the severity of disease are generally based upon maternal blood pressure measurements as ascertained by a variety of medical personnel who regularly measure blood pressures in prenatal clinics, local physicians' offices and in hospital antepartum and labor and delivery units. Other than personnel, factors that may influence measurement of the blood pressure include type of equipment, size of cuff, environment of patient, position and arm used, and Korotkoff phase used. While both Korotkoff phase IV and phase V diastolic sounds should be recorded, the phase IV (muffling) will be used in this study for the diastolic blood pressure. Detailed instructions for measuring blood pressures for this protocol are contained in Appendix B.

4.3.4 Urine Protein Testing

The diagnosis of preeclampsia requires the presence of elevated blood pressure with proteinuria. The presence of proteinuria is usually detected by the use of dipsticks. In random samples it is highly variable and can be influenced by several factors. Contamination, specific gravity >1.030, exercise or increased exertion, posture (orthostatic position) or pH $\geq$ 8.0 can cause falsely positive protein readings. Large urine volume and specific gravity <1.010, alternatively can cause a false negative result. Details are given in Appendix C.

This protocol requires obtaining a midstream urine sample and recording urine protein at each prenatal visit. Significant proteinuria is defined as $\geq$ 2+ on dipstick in the absence of a urinary tract infection or 300 mg or more of protein excretion during a 24-hour period. Because protein levels fluctuate widely, significant proteinuria by dipstick should be present in at least 2 random samples at least 4 hours apart. For patients having $\geq$ 1+ proteinuria in 2 random samples at least 4 hours apart, a 24-hour urine collection must be obtained to substantiate the presence of significant proteinuria.

4.3.5 Weighing Patients and Testing for Edema

Patients should be weighed in street clothes without shoes or coats at each visit on scales in good working order. In addition, generalized edema of hands and face should be recorded.

4.3.6 Adverse Effects

In addition to the non-directed interview at each study visit, potential adverse effects will be monitored by recording hematocrit prior to delivery and the lowest hematocrit postpartum, and reporting antepartum bleeding, postpartum hemorrhage, and transfusion.

4.4 Neonatal Exam

Each baby will be examined prior to discharge for evidence of platelet dysfunction, i.e. petechiae, purpura, cephalhematoma and excessive bleeding from circumcision.

5

Outcome measures and rationale

The primary outcome is the appearance of preeclampsia, including eclampsia and HELLP syndrome, as defined below. Baseline is defined as 20 weeks gestation or randomization, whichever is later. Three time periods are considered: antepartum (baseline to onset of labor), intrapartum (onset of labor to delivery), and postpartum (delivery to discharge).

5.1 The Diagnosis of Preeclampsia

5.1.1 Multifetal Pregnancy and Previous Preeclampsia Groups

a. Mild Preeclampsia: The presence of either a systolic BP $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg (Korotkoff phase IV, muffling) on two occasions 4 or more hours apart (within the same time period) with proteinuria ($\geq$ 2+ on 2 random samples 4 or more hours apart or $\geq$ 300 mg but less than 5g protein/24 hours) after baseline.

b. Severe preeclampsia: In the presence of mild preeclampsia (vide supra), any of the findings listed below constitute severe disease:

   1. The presence of either a systolic BP $\geq$ 160 mmHg or diastolic BP $\geq$ 110 mmHg on at least two occasions 4 or more hours apart within the same time period. If the diagnosis is based on intrapartum pressures, two diastolic pressures $\geq$ 110 are needed to qualify (or one diastolic BP $\geq$ 110 treated with antihypertensive medication).

   2. Proteinuria $\geq$ 5 g/24 hours

   3. Pulmonary edema

   4. Thrombocytopenia (platelet count $< 100 \times 10^3/mm^3$)

c. HELLP Syndrome: The development of all of the following:

   Thrombocytopenia (platelet count $< 100 \times 10^3/mm^3$)

   Abnormal SGOT (SGOT or AST $\geq$ 70 Iu/l)

   Hemolysis (LDH $\geq$ 600 Iu/l or total bilirubin $\geq$ 1.2 mg/dl or hemolytic anemia on peripheral smear)

d. Eclampsia: Elevated blood pressure with convulsions

5.1.2 *Chronic Hypertensive group - Without Baseline Proteinuria*

In a woman with chronic hypertension, whether or not on antihypertensive medication, superimposed preeclampsia will be diagnosed when the following conditions exist. Negative or trace urine protein(s) on dipstick prior to baseline is sufficient evidence of absent proteinuria. For this group we do not make a distinction between mild and severe preeclampsia.

a. Preeclampsia: Any of the findings listed below constitute severe disease:

   1. The development of proteinuria ($\geq$300 mg/24 hours or two dipsticks $\geq$ 2+ at least 4 hours apart) after baseline.

   2. Thrombocytopenia (platelet count $< 100 \times 10^3/mm^3$).

b. Eclampsia: Convulsions

c. HELLP Syndrome (see 5.1.1.c)

5.1.3 *Chronic Hypertensive group - With Baseline Proteinuria*

Again, for this group we do not make a distinction in severity of preeclampsia. If a 24 hour urine collection shows $\geq$300 mg/24 hours prior to baseline superimposed preeclampsia will be diagnosed as follows:

a. Preeclampsia: Any of the following

   1. Diastolic BP $\geq$ 110 on at least two occasions 4 or more hours apart within the same time period (or one diastolic BP $\geq$ 110 treated with antihypertensive medication), but no more than one week before delivery AND at least one of the following symptoms:

      - Worsening proteinuria (at least a five-fold increase from baseline or at least a two-fold increase from baseline if the highest protein collection after baseline is $\geq$ 5 grams/24 hours)

      - Severe headaches

      - Epigastric pain

   2. Thrombocytopenia (platelet count $< 100 \times 10^3/mm^3$).

   3. Abnormal SGOT ($\geq$70 Iu/l)

b. Eclampsia: Convulsions

c. HELLP Syndrome (see 5.1.1.c)

5.1.4 *Diabetes Group - Without Baseline Proteinuria or Hypertension*

As before, negative or trace urine protein(s) on dipstick before baseline is sufficient evidence of absent proteinuria. The usual criteria for preeclampsia, eclampsia and HELLP will be used (as defined in 5.1.1 a-d).

5.1.5 *Diabetes Group - Without Hypertension With Proteinuria at Baseline*

In normotensive diabetics with a 24 hour urine collection before baseline demonstrating $\geq$ 300 mg protein/24 hours the diagnosis of preeclampsia will be based on the development of hypertension, specifically:

a. Mild Preeclampsia: The presence of either a systolic BP$\geq$140 mmHg and/or diastolic BP$\geq$90 mmHg (Korotkoff phase IV, muffling) on two occasions 4 or more hours apart within the same time period.

b. Severe Preeclampsia: The presence of either a systolic BP$\geq$160 or a diastolic BP$\geq$110 on at least two occasions at least 4 hours apart within the same time period. If the diagnosis is based on intrapartum pressures, two diastolic BP$\geq$110 are required (or one diastolic BP $\geq$ 110 treated with antihypertensive medication).

c. Eclampsia: Elevated blood pressure with convulsions.

d. HELLP Syndrome (see 5.1.1c)

5.1.6 *Diabetes Group - With Hypertension Without Proteinuria*

As before, negative or trace urine protein(s) on dipstick before baseline is sufficient evidence of absent proteinuria. The criteria for preeclampsia, eclampsia and HELLP are as in 5.1.2 a-c.

5.1.7 *Diabetes Group - With Hypertension With Proteinuria at baseline*

In diabetics with hypertension and proteinuria $\geq$300 mg/24 hours prior to baseline, preeclampsia, eclampsia and HELLP are diagnosed as defined in section 5.1.3 a-c.

5.2 *Secondary Outcomes (Efficacy Related)*

It is hypothesized that the following secondary outcomes will be more favorable in the aspirin group than placebo:

5.2.1 *Maternal*

a. Incidence of PIH: hypertension ($\geq$140/90) without proteinuria in a woman who was normotensive prior to baseline (possible only for the risk groups of multifetal gestation and previous preeclampsia and the subgroup of diabetics without hypertension or proteinuria)

b. Incidence of aggravated hypertension: extreme hypertension (diastolic BP$\geq$110) or pulmonary edema without satisfying the other criteria for preeclampsia, in women who were hypertensive at baseline (chronic hypertensive group and diabetics with hypertension)

c. Incidence and duration of antenatal admission

d. Significant drop in hematocrit postpartum (value to be determined)

e. Severe complications including CVA, renal failure, abruptio placentae

f. Death

g. Incidence of Cesarean section delivery

h. Need for antihypertensive pharmacotherapy in chronic hypertensive-women

5.2.2 Neonatal

a. Mean gestational age at delivery

b. Mean birth weight at delivery

c. Apgar scores

d. Incidence of small for gestational age infants (SGA)

   1. < 10th percentile

   2. < 3rd percentile

e. Incidence of preterm delivery (< 37 weeks)

f. Incidence of postterm delivery (> 42 weeks)

g. Percent weighing < 2500 grams

h. Number of days in NICU

i. Number of days in hospital

j. Perinatal mortality (stillbirths or death at < 7 days age)

k. Retinopathy of prematurity

l. Incidence of fetal distress prior to or during labor. This includes persistent severe variable decelerations, repetitive late decelerations, or a scalp pH < 7.20

5.3 Secondary Outcomes (Safety Related)

The following outcomes may be less favorable in the aspirin group and must be carefully monitored:

5.3.1 Maternal

a. Incidence of antepartum bleeding

b. Incidence of postpartum hemorrhage

c. Need for transfusion

5.3.2 Neonatal

a. Hematologic manifestations including prolonged bleeding, IVH, lowered hematocrit, petechiae, cephalhematoma, excessive bleeding from circumcision

b. Clinically diagnosed, symptomatic patent ductus

6

Statistical Considerations

6.1 Sample Size and Power

For this trial, four separate sample sizes are calculated to give sufficient power within each of the risk categories to detect a reduction in preeclampsia by aspirin therapy from the anticipated placebo group rate. Each risk group is allowed to have a different placebo or background rate: the rationale for the choices made is given below.

To determine sample sizes, information on the incidence of preeclampsia is necessary. However, it is difficult to find references where the diagnosis of preeclampsia is as strict as that proposed in this clinical trial. For example, the definition of preeclampsia often does not require significant proteinuria. Therefore, in the discussion below, care has been taken to account for this. While the incidence chosen in each risk category tends to be conservative given the published data, it is expected that these figures will be more realistic.

In recent data obtained on 231 women with diabetes at one of the participating centers [17], the incidence of preeclampsia based on blood pressure criteria is approximately 10% amongst B and C class diabetics and 19% amongst D, R and F class diabetics. If proteinuria is taken into account, as in this protocol, the incidence drops to 6% among class B and C diabetics and 12% among D, R and F diabetics. In the proposed study, patients who have proteinuria at baseline are diagnosed on the basis of blood pressure alone. Based on the above information, a rate of 10% is considered appropriate for the placebo group.

In women with chronic hypertension, the incidence of superimposed preeclampsia ranges from 10-50%, depending on the severity of the hypertension [1]. Amongst those with mild disease it was found that 16% developed preeclampsia [26]. Therefore 15% has been chosen as a moderately conservative estimate of the incidence of preeclampsia in hypertensives.

The incidence of preeclampsia/pregnancy induced hypertension was studied in 642 women with twin gestation and found to be approximately 35% amongst nulliparas and 20% amongst primiparas [14]. Of those diagnosed, about 71% of the primiparas and 53% of the multiparas had proteinuria, resulting in 10% of multiparas and 24% of primiparas with disease diagnosis corresponding to this protocol. Assuming approximately 50% of the sample will be multiparas, an incidence rate of 17% for this risk category is postulated.

The incidence of preeclampsia in women who have previously had preeclampsia has been found as high as 65% [28,27] if the disease was severe with onset in the second trimester, although it is more commonly considered to be in the order of 30% [7]. Again, because the diagnostic criteria in this protocol are strict, a conservative figure of 20% has been chosen as being more realistic.

A review of the literature indicates that low dose aspirin therapy may reduce the incidences of preeclampsia by as much as 60% [2,33,34]. In calculating the sample sizes, a 50% reduction is assumed. In addition, a correction is made to account for 'dropouts' (those assigned to

aspirin who do not take any study medication and therefore are effectively on placebo). The dropout rate is set at 5% since it is expected that the women eligible for this study will be relatively compliant compared with the subjects of the MFMU Network clinical trial of aspirin to prevent preeclampsia in women at low risk for the disease. It is assumed that there will be no 'drop-ins' (those assigned to placebo who take some form of aspirin). Sample sizes, rounded to the nearest 10 for each risk category, were estimated assuming that the probability of a Type I error (alpha) is 0.05 (two-sided test) and that of Type II error (beta) is 0.2 (80% power).

Sample Sizes

	Preeclampsia Incidence Placebo	Preeclampsia Incidence Aspirin	Sample Size Per Group	Total
Insulin Dependent Diabetic	10%	5%	490	980
Chronic Hypertensive	15%	7.5%	315	630
Multifetal Pregnancy	17%	8.5%	270	540
Previous Preeclampsia	20%	10%	225	450

Thus the total sample size over all four groups is estimated to be 2600 patients. After an initial recruitment period, if the non-adherence is higher than anticipated, an extension to the sample size may be considered.

6.2 Monitoring

While the trial is ongoing it will be continuously monitored for data quality and at intervals there will be an examination of interim results. It is well recognized that repeated examination of accumulating data modifies the meaning of Type I error. We will therefore, formally test results for efficacy only twice during the trial and modify the significance levels accordingly. We shall use the method of Lan and De Mets [13], where the boundary function, which characterizes the rate at which the error level is spent, is chosen to be the horizontal boundary for Brownian motion. This tends to be conservative at the beginning so that the trial is unlikely to stop at the first decision point.

A plan is proposed for two interim analyses: when one-third and two-thirds of the total sample have completed the study. This yields the following critical values for Type I error of 5% to be used for the formal tests of efficacy (comparison of the proportion developing preeclampsia in the two groups using the normal approximation to the binomial distribution).

Percentage of Sample Having Completed Study	Z	Critical p-value
33	3.50	.0005
67	2.51	.0121
100	1.99	.0466

In practice when an interim analysis takes place, the proportion of total sample size accumulated may not be exactly as specified; and, therefore, the critical value may not correspond with this plan. The critical value will be calculated at the time of the interim analysis based on the actual proportion accumulated and the number of prior interim "looks".

Examination of evidence of toxicity will occur more frequently and, for this and other reasons, formal statistical tests will play only a minor part in judging possible hazards from the treatment. The trial may be stopped early either for reason of clear early evidence of treatment efficacy or for evidence of treatment risk which is judged to be unacceptable; but it also may be stopped early because recruitment is well below acceptable levels or the incidence of preeclampsia is well below that expected amount.

6.3 Planned Analyses

6.3.1 Baseline comparisons

The two groups (aspirin and placebo) will be compared with respect to variables collected by the baseline interview and examination. Two questions will be addressed:

a. Has randomization yielded comparable groups?

b. Are the differences between the two groups such that they may impinge significantly on the outcome comparisons?

Key variables to consider are these:

a. Race

b. Age

c. Alcohol and cigarette usage

d. Height and weight

e. Age of mother

f. Fetal age at first prenatal visit

g. Fetal age at time of randomization

h. Blood pressure at baseline

i. Urine protein at baseline (in the diabetic and chronic hypertensive groups)

6.3.2 Follow-up and compliance

The two groups will be compared with respect to the distribution of length of follow-up, percent of dropouts and drop-ins (evidence of other aspirin usage), and pill counts.

6.3.3 *Incidence of proteinuria*

The incidence of proteinuria in the two groups will be compared. Moreover, the gestational age of appearance of proteinuria in the two groups will be compared.

6.3.4 *Incidence of the primary outcome*

The incidence and time of appearance of the primary outcome in the two groups will be compared. The primary outcomes, preeclampsia, eclampsia and HELLP syndrome, will be determined by a case review. Each individual component, as well as the composite, will be reported separately for each group. To evaluate the effect of differences between the two groups in baseline characteristics, a covariate adjustment will be made as well, and comparisons with and without covariate adjustment will be considered. Separate analyses restricted to good compliers (in both aspirin and placebo groups) who have complete follow-up will be examined.

6.3.5 *Incidence of secondary outcomes*

The incidence of each secondary outcome in the two groups will be compared. Analysis of secondary outcomes will follow the same pattern indicated for the primary outcome, except that there will be no composite secondary outcome. Those secondary outcomes which are intended to identify adverse effects will be carefully monitored.

7

Data Entry and Management

A list of data collection forms for the study is given in Table 2. The purpose of each form is described briefly below. Each data collection form will also comprise the basis for data entry screens for the microcomputer data collection system.

The Screening Log lists all patients screened for the study. Age, race, date of screening, and the reason for exclusion (if applicable) will be recorded for each patient.

The Compliance Check Log lists all patients who are eligible and have consented. The drug code number for this portion of the study is assigned by this list. A pack with this number (containing 10 placebo tablets) is given to the patient for the compliance check.

The Randomization Log lists all patients who have passed the compliance check and are still eligible. The study drug code number, which is the randomization number, is assigned by this list. A box with this number (containing 8 packs of study drug) is reserved for this patient.

The Randomization Form will be completed for every patient who meets the screening criteria and who consents to the study. Baseline laboratory and blood pressure measurements as specified in 4.1 are recorded. After the patient has completed her placebo trial, the compliance results are recorded. If the patient is compliant and still eligible, she is randomized. Her study drug number (i.e., randomization number) is recorded on this form from the Randomization Log, which serves to assign the study drug number. A box with this number (containing 8 packs of study drug) is reserved for this patient.

Written justification (a PE04B Supplement) is required for patients in Group B whose blood pressure is $\leq$ 140/90 and who are not on antihypertensive medication. The PE04D Supplement is a form for reporting the basis for diagnosis in the index pregnancy, and is to be completed for every patient in Group D whose medical record is available.

The Baseline Interview Form is also completed at this time, recording a few background characteristics such as height, pre-pregnancy weight, smoking and drinking habits.

At every visit and every time a pack is mailed if there was no visit, a new study visit form is filled out. Clinical monitoring data (blood pressure, urine protein, presence of edema, weight) and compliance information (pill count, symptoms or complaints and whether commercial aspirin has been taken since the previous visit) are recorded. In addition, there is a section to record whether a work-up has been initiated as a result of the patient's condition, such as an increase in blood pressure. If the patient is diagnosed as having preeclampsia or PIH, this is recorded.

If a patient is diagnosed with preeclampsia or PIH (even if the study criteria are not met), form PE07 should be filled out after delivery to record the patient management.

Table 2: List of Data Collection Forms

Aspirin Protocol

PE01A-D	Screening logs for the four groups
PE02	Compliance check log
PE03A-D	Randomization logs for the four groups
PE04A-D	Screening data and randomization by group
PE04B SUPP	Documentation of chronic hypertension
PE04D SUPP	Previous preeclampsia diagnosis report
PE05	Baseline data
PE06	Study visit form
PE07	Patient management summary
PE08	Labor & delivery record
PE09	Neonatal summary and exam
PE10	Treatment termination form
PE11	NICU summary
PE12	Drug dispensing record
PE13	Fetal or maternal death
PE14	Preeclampsia diagnosis worksheet
PE15	Adverse effects form
PE16	Serum/Plasma catalog

The next form is the labor and delivery record, which is completed when the mother is discharged from hospital. If the mother was diagnosed with suspect or confirmed preeclampsia or PIH, whether ante-, intra- or postpartum, the Preeclampsia diagnosis worksheet is required. On this form, the blood pressure and urine protein and other lab measurements necessary for making the diagnosis are documented. This form will not be entered on the computer system but will be mailed to the Biostatistical Coordinating Center along with a copy of the patient's chart for review.

The neonatal summary describes the baby's hospital course and the results of the neonatal exam. For those babies who have sufficient complications to be admitted to a NICU or Intermediate Care Nursery for more than 12 hours, the NICU form records more severe morbidity.

A treatment termination form has also been devised which will record when a patient stops taking the study medication and the reason. In addition, there is a separate form for fetal or maternal death.

A drug dispensing form for each patient, to which the double-blind label is attached, records the dates on which study blister packs were dispensed.

7.1 Microcomputer Data Entry System

The microcomputer data entry system consists of a network of microcomputers, one at each clinical center, and one at the Biostatistical Coordinating Center (BCC). Data entry software corresponding to the study forms are developed and maintained by the staff of the BCC. Data will be entered by clinical center staff, and transmitted weekly via a telecommunications link to the BCC. Detailed instructions for entering and transmitting data are provided in the MFMU Network Microcomputer Data Management System Users' Manual.

8

Administration

8.1 Organization and Funding

The Aspirin Study is a clinical trial being conducted by the Maternal Fetal Medicine Units Network. The Network is funded by the National Institute of Child Health and Human Development (NICHD) and is conducted as a Cooperative Agreement: the Institute, a number of clinical centers and the Biostatistical Coordinating Center (BCC). The clinical centers and the BCC are each represented by a Principal Investigator who is a member of the Network's Steering Committee. The Center for Research for Mothers and Children (NICHD), the Prevention Research Program (NICHD), and the Pregnancy and Perinatology Branch (NICHD) are each represented by one member on the Steering Committee. The Steering Committee Chairman, a person independent of the participating institutions, was appointed by NICHD. The Steering Committee has the responsibility for establishing the study protocol and monitoring its implementation.

8.2 Participating Centers

8.2.1 Clinical Centers

The clinical centers presently participating in the Cooperative Agreement are listed in Figure 1. The Principal Investigators representing these clinics have agreed to abide by the study protocol and, in addition, to have comparable staff, facilities, and equipment.

8.2.2 Biostatistical Coordinating Center

The Biostatistical Coordinating Center (BCC) is responsible for all aspects of biostatistical design, analysis and data management of the study. In concert with the Steering Committee, the BCC is responsible for forms development and testing. The BCC conducts the interim and final statistical analyses and collaborates with the Steering Committee members in the preparation of publications based on the study results. The Principal Investigator of the BCC reports to the Steering Committee and Data Monitoring and Safety Committee.

8.2.3 NICHD

In addition to its role as funding agency, the NICHD participates in the activities of the Cooperative Agreement by being represented by three members on the Steering Committee. NICHD staff also participate in the development of protocols, in assisting the Steering Committee in the coordination of the studies conducted by the Network and in reporting study results.

8.3 Steering Committee

This committee is comprised of: the principal investigator from each of the clinical centers and the Biostatistical Coordinating Center, three members from NICHD and Chairman of the Steering Committee. The Steering Committee has the responsibility for identifying topics for Network studies, for designing study protocols, and for monitoring study implementation, recruitment and protocol adherence. The Steering Committee will also make recommendations for changes to study protocols if it deems them necessary. This committee receives recommendations from the Data and Safety Monitoring Committee.

8.4 The High Risk Aspirin Protocol Subcommittee

The High Risk Aspirin Protocol Subcommittee is responsible for the preparation of this protocol and for the conduct of the study. The subcommittee reviews case reports of adverse outcomes and possible adverse effects of aspirin. This group also reviews the medical record of every case of suspected preeclampsia or PIH to determine primary outcome according to the study criteria. Progress reports are made to the Steering Committee.

8.5 Data Monitoring and Safety Committee

The Data Monitoring and Safety Committee, a group of individuals not affiliated with any of the participating institutions, is established by NICHD. This committee is charged with reviewing the protocol with respect to ethical and safety standards and making recommendations if necessary. This committee will also be privy to statistical data and case reports which it may require for its deliberations.

Appendix A

Patient Management

A.1 Outpatient Management

The following guidelines are suggested but are not required of the attending physician whose patient is developing preeclampsia:

a. In Previously Normotensive Patients

If the basis of suspicion is a blood pressure of 140/90 or greater, the blood pressure should be repeated at 4 or more hours. If it remains elevated, the diagnosis of pregnancy induced hypertension can be made. If, in addition, there is significant proteinuria ($\geq$300 mg/24 hr) with or without edema, the diagnosis of preeclampsia is made. Once the diagnosis of preeclampsia is made, the patient should be admitted to the hospital (see below). In women with pregnancy induced hypertension unassociated with proteinuria, the patient can be followed on an ambulatory basis.

When blood pressure does not consistently exceed 140/90 yet there are other clinical manifestations suggesting the likely development of preeclampsia, such as excessive weight gain, edema or proteinuria, the patient should be reevaluated by a physician within 72 hours of the suspicious examination. At that follow-up evaluation, blood pressure, weight, and the presence of edema should be evaluated. A 24-hour collection of urine for protein and urinary creatinine excretion should be obtained by the time of the return visit. In the interval between visits, the patient should be at reduced activity including at least 3 to 4 hours of rest in the lateral decubitus position daily. The patient should not receive diuretics, phenobarbital, or antihypertensive agents. Salt should not be added to food, and foods containing heavy salt such as pretzels, potato chips, ham, sausage and pizza should be avoided. If the worrisome signs of impending preeclampsia have resolved, office visits can be spaced out to once every 1-2 weeks.

b. In Women with Chronic Hypertension who are not on Antihypertensive Medication Prior to Pregnancy

An increase in blood pressure alone is not sufficient to diagnose superimposed preeclampsia (see criteria in section 5.1.2 and 5.1.3). In the absence of criteria for the diagnosis of superimposed preeclampsia, sustained hypertension (more than 160 mmHg systolic or more than 100 mmHg diastolic on two or more occasions four hours apart), can be treated with antihypertensive medication. It is suggested that alpha-methyl dopa (Aldomet), up to a dose of 3 gm daily, be the agent of first choice. If this is insufficient to control hypertension, a B1 specific blocking agent can be added or used alone. Before initiating antihypertensive therapy, sufficient laboratory tests including liver function tests and a platelet count must be obtained to rule out superimposed preeclampsia (see section 5.1.2-3). The general principles outlined in section A.1.a should be applied where appropriate.

The initiation of antihypertensive therapy is not a primary outcome variable but will be recorded.

c. In Women on Antihypertensive Therapy Prior to Pregnancy

A careful history regarding the circumstances that led to antihypertensive therapy prior to pregnancy should obtained. In those with severe hypertension ($\geq$160/110 mmHg) requiring medication prior to pregnancy, it is acceptable to continue therapy during pregnancy. Diuretics, however, should be stopped, and either alpha-methyl dopa or a B_1 specific blocker should be the primary agent used. In women with mild-moderate essential hypertension (i.e. < 160/100 mmHg), antihypertensive therapy can be discontinued prior to pregnancy. If hypertension exceeding 160/100 mmHg develops during pregnancy, antihypertensive therapy may be reinitiated after superimposed preeclampsia has been excluded (see section 5.1.2).

The development of severe hypertension or the need to increase the dosage of, begin, change, or add additional antihypertensive medications are not primary outcome variables, but these occurrences will be recorded. The diagnosis of superimposed preeclampsia required one or more of the outcomes listed in section 5.1.2-3, and therefore liver function tests and a platelet count must be obtained.

The general principles outlined in section A.1.a relating to management of patients thought to be developing preeclampsia should be applied where appropriate.

d. In Diabetics

The principles outlined for previously normotensive and for hypertensive subjects (sections A.1.a and A.1.b) are applicable to women with diabetes.

e. In Women with Multifetal Gestation

The principles outlined for previously normotensive subjects in section A.1.a are applicable for women with multifetal gestations. Increased bedrest in the second and third trimesters may be advisable in women with multifetal gestations.

A.2 Hospital Management of Patients with the Diagnosis of Mild Preeclampsia

Unlike women with severe preeclampsia (defined previously) who are hospitalized and treated by delivery, for women with mild preeclampsia, bed rest can ameliorate the disease and subsequently the patient may be followed on an ambulatory basis. Women admitted to the hospital with mild preeclampsia should have, at a minimum, a 24-hour collection of urine for protein and total urinary creatinine, a hematocrit, SGOT, and platelet count. If the objective criteria of preeclampsia disappear with hospitalization, the patient can be followed on an ambulatory basis with weekly office visits.

Appendix B

Standard Method for BP Measurement by Sphygmomanometry

(modified from Davey and MacGillivray, Clinical & Experimental Hypertension: Hypertension in Pregnancy B5(1):97, 1986)

B.1 Sphygmomanometer

a. Make sure the equipment is properly zeroed, has no leaks or faulty control valves.

b. Position the equipment so that the 90 mmHg mark is at eye level when the BP is taken with the stethoscope fixed in the ears and the bell applied to the antecubital fossa.

c. Application of cuff

1. Remove any tight clothing so that the right arm is fully exposed and the cuff can be easily applied.

2. Apply the cuff evenly and firmly but not tightly around the arm with the connecting tubes pointing upwards and the antecubital fossa free.

3. Use a cuff appropriate for the arm circumference of the patient (12cm in width and 23cm in length for average adult; 14cm in width and 35cm in length for obese women).

4. Place the center of the bladder in the cuff directly over the brachial artery on the inner side of the right arm with the cuff at the same level as the sternum at the 4th intercostal space.

B.2 Patient

a. Allow a rest period of at least 10 minutes prior to blood pressure measurements.

b. Inquire about the possibility of smoking and time from last cigarette.

c. The woman should be seated comfortably with legs uncrossed and with the right arm in a roughly horizontal position at heart level supported on a desk. Do not take blood pressure with woman in supine position as this reading will be lower.

B.3 Taking the Blood Pressure

a. Avoid the presence of noise in the surrounding area.

b. Palpate the brachial artery in the antecubital fossa and place the stethoscope directly over the artery and hold in place without undue pressure.

c. Rapidly pump up the pressure in the cuff to 20-30 mmHg above the point at which pulsation in the brachial and radial arteries ceases and the Korotkoff sounds disappear.

d. Let the air out of the cuff without delay so that mercury falls steadily at 2-3 mmHg/sec. Rapid deflation of the cuff will lead to under-estimation of diastolic pressure.

e. Take the systolic BP as the point where first clear tapping sound is heard, read top of mercury meniscus, record to the nearest 2 mmHg.

f. Take diastolic blood pressure as the point where the Korotkoff sounds first become muffled (phase IV), read top of mercury meniscus to nearest 2 mmHg. Then record point of disappearance of sounds (phase V). If one point is recorded only, note which point is taken. Ideally both points should be recorded.

g. Let down pressure in the cuff completely as soon as BP is taken to minimize patient discomfort and to allow free flow of blood in and out of arm.

h. The first blood pressure reading will be used as the official reading. However, if the BP reading is uncertain always repeat the measurement. Let cuff down completely and wait for 2 minutes before re-inflating cuff and repeating measurement. Repeated and prolonged inflation of the bladder cuff will result in false elevations of both systolic and diastolic BP levels.

i. Make sure that the person taking the blood pressure has adequate hearing and visual acuity and is a person trained and responsible for measuring blood pressure in the clinic or office.

Appendix C

Standard Method for Urine Protein Testing

(Modified from Davey and MacGillivray, Clinical & Experimental Hypertension: Hypertension in Pregnancy B5(1):97, 1986)

C.1 Urine Testing

a. The patient should not drink large quantities of fluid before passing urine samples - diluted urine may give false negative results.

b. Obtain a clean fresh urine sample in a clean uncontaminated container without preservatives and note any abnormal deposit.

c. Test urine protein and record this value.

d. If specific gravity is obtained and found to be <1.010 or >1.030, then obtain another sample and retest again if the dipstick method is used.

e. If urine pH is $\geq$ 8.0 (alkaline urine), then obtain another sample and retest again if the sulphosalicylic acid cold test is used.

C.2 Interpretation of Results

Negative or trace	<0.1 g/l
1+	0.3 g/l
2+	1.0 g/l
3+	3.0 g/l
4+	≥ 5.0 g/l

C.3 Diagnosing Proteinuria

a. The definitive test for diagnosing proteinuria is the quantitative measurement of total protein excretion over 24-hour period.

b. If 24-hour urine data is not available or possible it is recommended that the diagnosis of proteinuria should be based on the finding of proteinuria of $\geq$2+ on dipstick in at least 2 random midstream samples at least 4 hours apart.

c. For making a diagnosis of severe preeclampsia based on proteinuria it is required that 24-hour urine excretion of protein be available. Random urine test results are not adequate for such diagnosis.

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