CLINICAL TRIAL OF LOW-DOSE ASPIRIN (60MG)

AS A PREVENTATIVE OF PREECLAMPSIA

PROTOCOL

February 21, 1989

Maternal Fetal Medicine Units Network

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CHANGES TO THE PROTOCOL AFTER THE START OF THE STUDY

1. November 1st, 1990 - Sections 3.3.7, 3.3.8.

On October 16th 1990, the Steering Commitee agreed to revise the definition of pregnancy induced hypertension (PIH). The changes were proposed by the Aspirin Study Subcommittee who are reviewing all potential cases of preeclampsia and PIH. They found that the protocol definition of PIH could lead to cases which would technically qualify as PIH but where blood pressure elevation coincided with and was probably caused by labor. To rectify this they added two stipulations: that uric acid of at least 6 mg/dl must be measured for hypertension occurring intrapartum to qualify as PIH and that two diastolic readings of $\geq$ 110 at least 1 hour apart are required to upgrade intrapartum PIH from mild to severe. The actual definition of the primary study outcome, preeclampsia, remains unchanged as does the definition of PIH occurring antepartum or postpartum.

These changes were passed by the Data Monitoring Committee, who met on October 30th 1990.

2. March 15th, 1991 - Section 3.3.14

The study timetable has been revised to reflect reality.

3. March 15th, 1991 - Section 6

The data forms have been revised. Worksheet PE14 replaces most of PE07 and a separate form, PE13, has been designed to capture maternal or fetal death separately from other cases of early study termination (withdrawals and dropouts).

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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 and its associated complications. The purpose of this randomized, double-blind, placebo-controlled trial is to evaluate the effects of low-dose aspirin (60 mg per day) in preventing preeclampsia in nulliparous patients who begin treatment at 13-26 weeks gestation. The primary outcome is development of preeclampsia. Additional outcome variables include routine maternal and perinatal outcomes.

1.2 Hypotheses

The primary objective of this study is to determine if chronic daily administration of 60 mg of aspirin during pregnancy will decrease the incidence of preeclampsia in nulliparas. The study will compare the incidence of preeclampsia in two groups of patients: those receiving low-dose aspirin and those receiving placebo. In addition it will attempt to identify any adverse effects of chronic aspirin administration on the mother or the newborn.

1.3 Rationale for the Study

Early antiplatelet therapy with a low dose of aspirin (60 mg/d) given to the mother beginning in the second trimester will inhibit platelet thromboxane generation with minimal effects on vascular prostacyclin production, thus directing the fetoplacental prostacyclin/thromboxane A2 balance to the dominance of prostacyclin. An increased prostacyclin/thromboxane A2 ratio will inhibit platelet activation and prevent uteroplacental thrombosis and thus preeclampsia in a group of pregnant women judged to be at high risk for that condition.

This study is restricted to nulliparas because that population has a high incidence of preeclampsia. In the absence of any clinically useful biomarkers for predicting high-risk patients, it was not possible to restrict the population further. Angiotensin II sensitivity testing may have some predictive value, but that test is felt to be too invasive, time consuming, and expensive to screen large numbers of patients.

1.4 Background

Hypertension is the most common medical complication seen during pregnancy, with preeclampsia being the etiology of hypertension in about two-thirds of these patients. Preeclampsia is mainly a disease of the primigravida with an increased incidence in those who are less than 20 years old and those who are over 35 years of age. The incidence ranges between 5% and 10% of all deliveries [29]. It is usually more prevalent among single indigent patients. The incidence is about 15% in primigravidas and about 35% in young primagravidas with twin gestation. The disease is classified into mild or severe depending on the level of hypertension, amount of proteinuria, and the presence of abnormal laboratory findings or cerebral manifestations. The hallmarks of preeclampsia are increased sensitivity to vaso-active substances, activation of the coagulation system and reduced uteroplacental blood flow. Pregnancies complicated by preeclampsia are associated with increased perinatal mortality and morbidity. Most of the perinatal deaths are related to the increased incidence of intrauterine growth retardation, fetal hypoxia and premature deliveries. Additionally, the mother is at increased risk for the development of abruptio placentae, convulsions, renal failure and cerebrovascular accidents.

1.4.1 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 in the diagnosis of PIH. This definition causes considerable difficulties since a gradual increase in blood pressure from second to third trimester is usually seen in most normotensive pregnancies. MacGillivray et al [22] found that an increase in diastolic pressure of $\geq$ 20 mmHg is present in 57% of these patients. More recently, Moutquin et al [23] found that a rise of 30 mmHg systolic of 15 mmHg diastolic has a poor sensitivity for the diagnosis of preeclampsia in the primigravida patient. Similar findings were observed by Sibai et al during the analysis of serial blood pressure findings in over 1000 nulliparous pregnancies. In addition, the above definition is influenced by at least 3 factors: gestational age at time of first observation; frequency of blood pressure measurements; and the 2 observations which are selected. Hence, it is decided that 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 [10] 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 [30] 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 had no edema. Therefore these variables, although recorded, will not be used to define patient outcome.

1.4.2 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 [2,3]. The conversion of the spiral arteries of the non-pregnant uterus to the uteroplacental arteries has been termed "physiological changes" by Brosens et al [3]. 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" [25].

In contrast, pregnancies complicated by preeclampsia and/or by SGA infants 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 vasomoter influences. Additionally, the number of well-developed uteroplacental arteries is smaller than that found in normotensive pregnancies [19]. Furthermore, Robertson et al [26] 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.

It has recently been possible to study uterine vessels' (arcuate arteries') blood flow to the placenta using arterial flow velocity wave forms measured by Doppler ultrasound. It is well documented that the uterine artery S/D ratio and resistance index are increased in pregnancies complicated by preeclampsia and in most pregnancies with SGA infants without hypertension [34,12]. Campbell et al [4] suggested that measurements of blood flow velocity waveforms in arcuate arteries at 16-18 weeks' gestation may be used as screening tests to predict the future development of pregnancy-induced hypertension and/or future delivery of an SGA infant. In his study, 30 of 126 consecutive pregnancies screened with uterine artery Doppler at 16-18 weeks' gestation developed PIH and/or IUGR. The sensitivity of an abnormal test ($RI \geq 0.58$) was 68% and the specificity 69%; the predictive value of a positive test was 42% and that of a negative test was 87%. An abnormal arcuate arteries Doppler at 16-18 weeks' gestation predicted 64% of hypertensive pregnancies and 66% of SGA infants.

Normotensive pregnancies are characterized by an increased resistance to vasoactive substances such an angiotensin-II and norepinephrine. On the other hand, preeclamptic pregnancies have increased sensitivity to these peptides. Gant and co-workers [16] found that the loss of refractoriness to A-II is an early event in the development of PIH since it precedes the development of hypertension by 8 to 12 weeks. It is further documented that the A-II pressor refractoriness is not related to either volume deficit or renin-angiotensin-II plasma concentration; most probably it was related to arteriolar vascular smooth muscle resistance to A-II. Everett et al [11] reported that vascular response to A-II infusions in normotensive and PIH pregnancies can be modified by prior use of PG inhibitors (indomethacin, aspirin).

Preeclampsia is associated with vasospasm, pathologic vascular lesions within multiple organ systems including placental bed, and activation of the coagulation system. McKay [21] 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 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.

1.4.3 Prostacyclin/Thromboxane Metabolism

It is well established that there is altered metabolism of the prostacyclin (PGI_2)-thromboxane A_2 (TxA_2) systems in preeclampsia. Hence, several authors used pharmacologic manipulation to alter these changes in an attempt to treat and/or prevent preeclampsia. Studies with the use of regular doses of aspirin before and after A-II infusion in pregnant women have shown a significant decrease in the effect or pressor dose of A-II, demonstrating increased A-II sensitivity in all patients studied [11]. Recently, Sanchez-Ramos et al [27] studied the effects of 80 mg of aspirin on vascular sensitivity to exogenous A-II in 13 normotensive pregnancies. Low-dose aspirin resulted in increased effective pressor dose of A-II from 17.4 $\pm$ 2.2 ng/kg/min. to 35.1 $\pm$ 4.2 ng/kg/min., demonstrating decreased A-II sensitivity in study patients.

1.4.4 Early Aspirin Trials

There is some evidence from case reports [17,18] and a survey of primigravid women [8] that aspirin may reduce and or treat preeclampsia. In one case, a preeclamptic patient with thrombocytopenia at 22 weeks' gestation was treated by heparin and aspirin 1800 mg/d. The pregnancy was complicated by oligohydramnios and delivery of an SGA infant at 34 weeks' gestation. In the other case, a patient had eclampsia with DIC at 21 weeks' gestation. She was treated with aspirin 500 mg/d. The pregnancy ended with fetal death one week later.

Beaufils et al [1] reported on 102 patients who were at risk for preeclampsia, IUGR or fetal demise. Fifty-two patients were treated with aspirin 150 mg/d plus dipyridamole (persantine) 300 mg/d starting from 12 weeks' gestation onwards. The other 50 patients served as a control group. It is unclear whether one drug or the combination is responsible for the results; however, the author reported absent preeclampsia and fetal deaths in the treatment group while there were 6 cases of preeclampsia and 5 fetal deaths 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 45% in the control group and 8% in the treated group.

Wallenburg et al [35] studied 207 primigravidas that were screened by A-II infusions at 28 weeks' gestation. Forty-six patients had positive tests, and thus randomized to either placebo (n=23) or aspirin 65 mg/d (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 causes 90% inhibition of platelets 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 the placebo group had 3 severe PIH, 7 preeclampsia, and 1 eclampsia. In addition, there were no premature deliveries in the treated group and the incidence of SGA infants was 19% compared to 39% in controls.

In a subsequent report Wallenburg et al [36] gave 65 mg/d aspirin to 34 normotensive primigravidas with a positive A-II infusion sensitivity test at 28 weeks' gestation. Sixteen women were given matching placebo. The A-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).

1.4.5 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 [37]. 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 [24] 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 platelets by aspirin. Cerletti et al [6] 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 [14] studied 5 pregnant women who were given various doses of aspirin (600 mg to 50 mg) 3 hours before fetal blood sampling. Platelet aggregation studies were performed in both mother and fetus. The use of a 50 mg dose resulted in 70% inhibition of maternal platelet aggregation and 100% inhibition of fetal platelet aggregation. Hence, aspirin crosses the placenta readily.

Recently, Ylikorkala et al [41] studied the effects of acetylsalicylic acid in healthy women given 100 mg (n=13) or 500 mg (n=14) 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 acetylsalicylic acid. Fetal thromboxane A_2 systhesis was inhibited in infants born to mothers receiving both doses. The authors concluded that small doses of maternal acetylsalicylic acid (100 mg) inhibit only the fetoplacental thromboxane A_2, but leave prostacyclin production unaffected.

1.4.6 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 [32] found no evidence of an increased risk of congenital defects. However, a recent report by Zieler et al [42] 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. Maternal and fetal risks included longer duration of pregnancy, and increased risks of antepartum and postpartum hemorrhage [7]. Neither Beaufils nor Wallenberg found this to be the case, however.

There are several adverse fetal-neonatal effects associated with the use of prostaglandin synthetase inhibitors during pregnancy. Patency of the ductus arteriosus in utero is maintained in part by prostaglandins E_2 and prostacyclin. Most of these effects are related to the use of indomethacin treatment late in pregnancy [40]. However, the Collaborative Perinatal Project [31] did not demonstrate such a risk in children of over 26,000 mothers taking aspirin during pregnancy. In addition, it is charged that the use of aspirin in pregnancy may lead to the development of oligohydramnios (30) and inhibition of fetal platelet aggregation [6] and neonatal bleeding disorders [33]. In the study by Stuart et al [33] neonatal bleeding problems were seen in mothers taking 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 [17,8,36].

1.4.7 Preliminary Data

In a pilot study Sibai et al evaluated the effects of 3 different low doses of aspirin versus placebo in 40 pregnancies during the third trimester. In this study 40 normotensive primigravid women were randomized to receive (n=10 each) placebo, 81 mg, 65 mg, or 20 mg of aspirin/d at 37 weeks' gestation. Serum levels of 6-keto-PGF_1 α and thromboxane B_2 (TXB_2) were obtained at enrollment, 1 week, 2 weeks, and from cord blood and neonatal blood (<12 hours old). In addition, platelet aggregation and platelet thromboxane B_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, at 1 week, at 2 weeks and in the neonate. All neonates were also studied by 2-dimensional Doppler echocardiography to verify patency of the ductus arteriosus and to noninvasively measure pulmonary artery pressure.

1.4.7.1 Results and Conclusions

There were no significant differences among the 4 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 α levels (150-250 pg/ml) were not affected by these doses of aspirin. Maternal TXB_2 was decreased significantly by 81 and 65 mg of aspirin (see Table). However, cord and neonatal levels of 6-keto-PGF_1 α and TXB_2 were not different from placebo. Platelet aggregation with collagen was significantly inhibited by 65 mg ($p<0.001$) and 81 mg ($p<0.01$) of aspirin in the mother at 1 and 2 weeks of therapy. A smaller but significant inhibition of ADP-induced aggregation was noted with 65 mg of aspirin only ($p<0.002$). No inhibitory effect was noted with any other aspirin dose or placebo. On the other hand, ADP-induced platelet TXB_2 generation was 95% inhibited by 65 mg aspirin ($p<0.02$) and 99% by 81 mg of aspirin ($p<0.001$). The 20 mg dose and placebo demonstrated no inhibitory effect. Collagen-induced platelet TXB_2 generation was also significantly inhibited by 65 mg dose (98%, $p<0.002$) and 81 mg dose (98%, $p<0.004$). The 20 mg dose showed no inhibitory effect compared to placebo. All neonates had a PDA as expected and pulmonary artery pressures were not different among the 4 groups. Right ventricular systolic time interval ratios (PEP/ET) were 0.46 $\pm$ .02 (mean $\pm$ SEM), 0.47 $\pm$ 0.4, 0.43 $\pm$ and 0.44 $\pm$ .02 for the placebo, 81 mg, 65 mg, and 20 mg groups.

A 60-81 mg/day dose of aspirin given for one week inhibits maternal platelet TXB_2 generation while not affecting prostacyclin production. The neonate's ability to generate TXB_2 and prostacyclin and transitional circulation are not affected with such a dose. A dose of 20 mg/day is not adequate to inhibit either platelets aggregation or platelet-induced generation of TXB_2.

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
81 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.

2

STUDY DESIGN

2.1 Overall Design

The study will be a randomized, double-blind, placebo-controlled clinical trial, conducted by collaboration among the MFMU Network participants. The study procedures will be conducted in a uniform manner throughout the participating clinical sites. The medication to be employed is 60 mg aspirin or placebo to be prepared by the Glenbrook Laboratories.

2.2 Eligibility Criteria

All eligible nulliparous women will be randomized to provide reliable evidence about the effects of aspirin using a dose that will produce a full effect on maternal cycloxygenase-dependent platelet aggregation in almost all subjects.

2.2.1 Inclusion Criteria

Patients will be selected for participation in the study if they are nulliparas or had previous pregnancies terminating not later than 20 weeks who:

1. sign the consent form
2. have not taken medication containing aspirin in the past 2 weeks (A list of all over-the-counter medications containing aspirin will be available to the coordinators.)
3. are at 13-25 weeks' gestation by best obstetrical estimate by clinical and/or biophysical means
4. have blood pressure less than 135 mmHg systolic and less than 85 mmHg diastolic (Korotkoff phase IV, muffling) with no more than a trace of proteinuria and without antihypertension medication

2.2.2 Medical Exclusions

2.2.2.1 Maternal

1. active endocrine disease requiring medication and/or dietary modification
2. diabetes mellitus
3. chronic hypertension/renal disease, systolic $\geq$ 135 and/or diastolic $\geq$ 85 or receiving antihypertensive treatment
4. chronic pulmonary or heart disease
5. significant antepartum bleeding occuring after 20 weeks gestation
6. history of peptic ulcer
7. history of sensitivity to aspirin
8. epilepsy or other seizure disorders currently requiring medication
9. taking other platelet-active drugs or nonsteroidal anti-inflammatory agents
10. collagen vascular disease such as lupus
11. drug or alcohol abuse known by history

2.2.2.2 Fetal

1. known anomalies incompatible with life
2. abortion or fetal demise prior to entry
3. hydrops fetalis

3

STUDY PROCEDURES

3.1 Screening Procedures

All nulliparous patients in their second trimester, subsequent to a complete medical history and physical examination during this or a previous clinic visit, will be screened for inclusion and exclusion criteria and interviewed regarding medications used during the current pregnancy. Those who seem to be eligible will be told about the study and, if willing to participate, will be asked to sign an informed consent (see Appendix F for model informed consent). At this time, the baseline blood pressure will be recorded (per Appendix C) and the urine protein measured (per Appendix D).

If a woman is otherwise eligible but has taken aspirin-containing medication in the last two weeks or has at least 1+ proteinuria but also a UTI, then she may be rescreened at her next visit (provided it is not beyond 26 weeks of the pregnancy). The rescreening will duplicate the initial screening.

Participants will be given a week of placebo in a single-blind manner, and reevaluated in a week. For clinic patients, this will require a return visit. At the discretion of the Principal Investigator, patients of private physicians can be interviewed by telephone. At that time they will be checked for compliance and asked again about their willingness to participate. Non-compliers (those who have taken tablets on less than 50% of the days since the last visit or those who have taken aspirin-containing products), those who change their mind and those who fail to return will thus be eliminated from the study before randomization.

3.2 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 randomization scheme arranged by the Data Coordinating 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.

3.3 Study Procedures Post-Randomization

A carton of study drug with the corresponding drug number is then assigned to the study patient. The contents of the carton is either 60 mg aspirin or placebo tablets, as determined by the randomization scheme supplied to the drug company. The patient is given the first bottle from the carton. For those patients whose compliance check is conducted by telephone interview, arrangements will be made to have the initial bottle of study medication delivered to them. 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 and asked to avoid these products, and a supply of acetaminophen to take for pain and headaches. They will also 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.

3.3.1 Compliance

On return 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. A new bottle with the same identifying number will be supplied to the patient as needed. 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.

3.3.2 Sequence of Visits

Patients are to be seen at the scheduled prenatal visits of their clinics: usually every 4 weeks until 26-28 weeks, then every 2-3 weeks until 36 weeks' gestation and then once weekly until delivery. Blood pressure measurement, weight and urine protein dipstick will be recorded at each prenatal visit. Patients will also be questioned in a non-directed fashion about side effects from the study medication. 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 called by the research coordinator and seen at the earliest time possible.

3.3.3 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 [5]. 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 C. Each center is responsible for establishing its own quality assurance procedures to maintain the accuracy of its blood pressure measurements.

3.3.4 Urine Protein Testing

(see Appendix D for details)

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 including contamination, specific gravity >1030; exercise or increased exertion; posture (orthostatic position) or pH$\geq$8.0, which cause a false positive; and large urine volume and specific gravity <1010, which can cause a false negative.

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

3.3.5 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.

3.3.6 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.

3.3.7 The Diagnosis of PIH

The diagnosis of PIH in any of the study patients will be classified according to the following criteria:

1. Mild PIH: 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 without proteinuria (<300 mg/24 hours).

2. Severe PIH: The presence of either a systolic BP≥160 mmHg and/or diastolic BP≥110 mmHg on at least two occasions 4 or more hours apart without proteinuria (<300 mg/24 hours).

3.3.8 The Diagnosis of Preeclampsia

The diagnosis of preeclampsia in any of the study patients will be classified according to the following criteria:

1. Mild preeclampsia: Mild PIH and proteinuria (≥2+ on 2 random samples 4 or more hours apart or >300 mg but less than 5g protein/24 hours).

2. Severe Preeclampsia:

   - Severe PIH and proteinuria as defined above
   - Mild PIH and proteinuria ≥5g/24 hours
   - BP≥140/90 mmHg and thrombocytopenia $<100x10^3/_{mm^3}$
   - PIH or Preeclampsia with oliguria <400 ml/24 hours
   - Pulmonary edema

3. Eclampsia: PIH or preeclampsia with convulsions

4. HELLP Syndrome: Preeclampsia with thrombocytopenia $<100x10^3/mm^3$ and AST or SGOT ≥70 and LDH ≥600 and total bilirubin ≥1.2

All patients diagnosed with preeclampsia should have uric acid measurements, hematocrit and platelet count. Additional evaluation will be obtained as needed. Records of all patients who develop preeclampsia will be verified by the PI of their Center as meeting the protocol requirements.

Patients with elevated liver enzymes and thrombocytopenia who do not meet blood pressure requirements for preeclampsia will be reviewed by the protocol subcommittee before inclusion in the HELLP category.

3.3.9 Suggested Guidelines for Evaluation and Management of Patients with Elevated Blood Pressure

The following guidelines can be applied to any patient beyond 20 weeks gestation whom the attending physician believes is developing preeclampsia either by virtue of rising blood pressure, excessive weight gain, edema, or any combination of the above.

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. In the presence of proteinuria with or without edema, the diagnosis of preeclampsia is made. Once made, the patient should be admitted (see below). If pregnancy-induced

hypertension is unassociated with proteinuria, the patient can be followed on an ambulatory basis. Management of such patients is defined below.

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, patients can be followed on an ambulatory basis as described below.

3.3.9.1 Ambulatory Management of Women Thought to be Developing Preeclampsia

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 excretion and creatinine clearance should be brought in by the time of the return visit. In the interval between visits, the patients 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, phenobarbitol 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.

3.3.9.2 Hospital Management of Patients with the Diagnosis of Mild Preeclampsia

Unlike women with severe preeclampsia (defined previously) who are generally hospitalized and treated by delivery, in 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 creatinine, a BUN, serum creatinine, uric acid 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.

3.3.10 Management and Pregnancy Outcome

Once preeclampsia is diagnosed patients will stop study medication, even if their symptoms improve following hospitalization. Centers will record when the disease occurred and its severity. In the absence of preeclampsia the study drug will be continued until delivery.

Management of patients with diagnosed preeclampsia will be left to the individual physicians. However, in order to address differences in patient management that affect time of delivery, a list of intervention criteria will be recorded and analyzed (see Appendix E).

3.3.11 Postpartum Measurements

All patients should have a measured blood pressure, urine protein dipstick results and hematrocrit recorded at time of discharge from the hospital.

3.3.12 Neonatal Exam

Each baby will be examined prior to discharge for evidence of aspirin effects on platelet function, ie petechiae over presenting fetal part, purpura over presenting fetal part, cephalhematoma, hematuria and excessive bleeding from circumcision.

3.3.13 Data to be Collected

Data will be collected on standardized data forms, on which nearly all responses have been precoded. The study forms are given in Appendix F.

3.3.14 Study Timetable

1989	February	Recruitment begins
1989	April/May	Conference call or Steering Committee meeting, evaluation of progress of study recruitment, procedures
1989	August	Data Monitoring Committee Meeting
1990	February	Interim analysis for efficacy
1990	August	Interim analysis for efficacy; Data Monitoring Committee Meeting
1991	February	Completion of pregnancy for last recruited patients, review of preeclampsia-diagnosed patients, start of final data analysis

4

OUTCOME MEASURES AND RATIONALE

4.1 Primary Outcome

The primary outcome is the appearance of mild preeclampsia, severe preeclampsia, eclampsia and HELLP syndrome as defined in section 3.3.8.

4.2 Secondary Outcomes

4.2.1 Maternal

1. incidence of antepartum bleeding
2. incidence of postpartum hemorrhage
3. incidence and duration of antenatal admission
4. significant drop in hematocrit postpartum (value to be determined)
5. need for transfusion
6. severe complications including CVA, renal failure, abruptio placentae
7. incidence and severity of PIH
8. death
9. incidence of labor induction
10. incidence of C-section delivery

4.2.2 Neonatal

1. mean gestational age at delivery
2. incidence of preterm (<37 weeks)
3. incidence of postterm (>41 weeks)
4. incidence of IUGR

5. mean birth weight at delivery

6. percent weighing <2500 grams

7. Apgar score

8. number of days in NICU

9. number of days in hospital

10. hematologic manifestations including prolonged bleeding, IVH, lowered hematocrit, petechiae

11. clinically diagnosed, symptomatic patent ductus

12. perinatal mortality (stillbirths or death during hospitalization)

13. retinopathy of prematurity

5

STATISTICAL CONSIDERATIONS

5.1 Sample Size and Power

The study will recruit at least 3000 patients, 1500 per group, in order to have adequate power (around 80%) to detect a 50-percent reduction in preeclampsia in the aspirin-treated group from the anticipated placebo group rate of 5-6 percent. The sample size calculation is based on a two-sided test of hypothesis with type 1 error level of 2 percent. Table 1 gives the power of the study to detect reductions of 40 to 50 percent in the treated group incidence rates of preeclampsia for type 1 error of both 5% and 1%.

The power calculations incorporate the assumption of 10% non-adherence to treatment in both groups. That is, they give the resulting study power if 10 percent of the aspirin group do not adhere to the study medication, and if 10 percent of the placebo group do take some form of aspirin during their pregnancy. The effect of such nonadherence is to dilute the effect of treatment as observed in the incidence rates in the two groups, so that the estimated powers shown in the table are lower than the power expected if both groups had 100% adherence to treatment.

If the study achieves its recruitment goal of 1500 patients per group, it will have adequate power to detect a 50% decrease in preeclampsia in the treated group, as summarized in the following table. Since the incidence of PIH is much greater than the incidence of preeclampsia alone, the proposed sample size will yield excellent power for the between-group comparison on the incidence of PIH.

After an initial recruitment, if the percent non-adherence is higher than anticipated, and if the recruitment rate is adequate, we will consider extending the duration of recruitment to achieve adequate sample size.

Table 2: Aspirin and Preeclampsia Study

Power of the Study to Detect Reductions in Incidence
Type 1 Error = .05, Two-sided Test,
Assuming 10% Non-adherence in Each Group

Incidence		Sample Size Per Group						
Placebo	Aspirin	800	1000	1500	2000	3000	4000	5000
		(40% reduction)						
0.05	.030	45	54	71	83	95	98	100
0.06	.036	52	62	79	89	97	99	100
0.10	.060	76	84	95	99	100	100	100
0.15	.090	91	96	100	100	100	100	100
		(50% reduction)						
0.05	.025	66	75	90	96	100	100	100
0.06	.030	74	83	95	98	100	100	100

Power of the Study to Detect Reductions in Incidence
Type 1 Error = .01, Two-sided Test,
Assuming 10% Non-adherence in Each Group

Incidence		Sample Size Per Group						
Placebo	Aspirin	800	1000	1500	2000	3000	4000	5000
		(40% reduction)						
0.05	.030	23	30	48	63	84	94	98
0.06	.036	29	38	58	73	91	97	99
0.10	.060	53	65	86	95	99	100	100
0.15	.090	77	87	98	100	100	100	100
		(50% reduction)						
0.05	.025	42	53	75	88	98	100	100
0.06	.030	51	63	84	94	99	100	100

5.2 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.

The following plan for two interim analyses of efficacy was devised using the method of Lan and De Mets [20], where the boundary function, which characterizes the rate at which the error level is spent, was 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. The following critical values for type 1 error of 1% will 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) when one-third, two-thirds and the total sample have been accumulated.

Percentage of Sample Recruited	Z
33	4.81
67	3.25
100	2.59

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 because the treatment risk is judged to be unacceptable. The trial may also be stopped early because recruitment is well below acceptable levels or the incidence of preeclampsia is well below that expected.

5.3 Planned Analyses

The analytical program will involve a collaboration among the principal investigators, the data coordinating center, and the NICHD. It will consider the following major topics:

5.3.1 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:

1. Has randomization yielded comparable groups?

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

5.3.1.1 Key variables to consider are these:

1. Race
2. Age
3. Alcohol and cigarette usage
4. Height and weight
5. Age of mother
6. Fetal age at first prenatal visit
7. Fetal age at time of randomization
8. Blood pressure at baseline
9. Rescreening because of UTI
10. Rescreening because of aspirin usage

5.3.2 Follow-up and compliance

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

5.3.3 Blood pressure trends

Trends of blood pressure in the two groups will be compared. Women will enter the study at varying times during the second trimester, and will deliver and leave the study at varying times, thus effects of blood pressure on fetal age will be assessed both by linear and quadratic regression taking into account the varying numbers over each interval of observation.

5.3.4 Incidence of the primary outcome

The incidence and time of appearance of the primary outcome in the two groups will be compared. The primary outcome includes both preeclampsia and eclampsia, as well as HELLP syndrome, and is determined by a case review. Each individual component, as well as the composite, will be reported separately for each group. The incidence of preeclampsia as defined by study blood pressures and proteinuria will also be compared between the two groups. 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.

5.3.5 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.

Interim reports which are prepared for the data monitoring committee will be evaluated with allowance for the effect on type 1 error of repeated looks at the data.

6

DATA ENTRY AND MANAGEMENT

A list of data collection forms for the study is given in Table 3 on page 26. The purpose of each form is described briefly below. Each data collection form will also form 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 will be recorded for each patient excluded.

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 bottle 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 bottles 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 blood pressure and protein urine dipstick measurements are recorded. After the patient has completed her one-week placebo trial, the compliance results are recorded. If the patient is compliant, 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 bottles of study drug) is reserved for this patient.

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, a new study visit form is filled out with clinical monitoring data (blood pressure, urine protein, presence of edema, weight) and with compliance information (pill count, symptoms or complaints and whether commercial aspirin has been taken since the previous visit). 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, as defined by the protocol, this is recorded. If she does not yet meet the criteria for preeclampsia, but goes on to develop the condition at a future visit, this will be noted on the form for the later visit.

Once a patient meets the criteria for preeclampsia, form PE07, the Preeclampsia Diagnosis form is required. On this form, the blood pressure and urine protein measurements necessary for making the diagnosis are documented. The final diagnosis at delivery is also recorded, so that the severity of the condition may be obtained. Some details of the management of the patient are included. A code list on the form, derived from Appendix E, allows the investigator to document symptoms and indications for delivery.

The next form is the labor and delivery record, which is filled in when the mother is discharged from hospital. Similarly, the neonatal summary describes the baby's hospital course. (Note that a neonatal exam has been proposed, which would be recorded on the latter form.) Finally, only 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.

A dispensing form for each patient, to which the double-blind label is attached, records the dates of dispensing of the study drug bottles.

Table 3: List of Data Collection Forms

ASPIRIN PROTOCOL

PE01. Screening log

PE02. Compliance Check Log

PE03. Randomization Log

PE04. Randomization form

PE05. Baseline interview

PE06. Study visit form

PE07. Preeclampsia diagnosis form

PE08. Labor & delivery record

PE09. Neonatal summary and exam

PE10. Treatment termination form

PE11. NICU summary

PE12. Dispensing form

6.1 Microcomputer Data Entry System

The microcomputer data entry system consists of a network of microcomputers, one at each clinical center, and one at the Data Coordinating Center. Hardware for the network includes IBM PC AT microcomputers and auto-answer, auto-dial modems. Data entry software corresponding to the study forms are developed and maintained by the staff of the Data Coordinating Center. Data will be entered by clinical center staff, and transmitted weekly via a telecommunications link to the Data Coordinating Center. Detailed instruction for entering and transmitting data are provided in the MFMU Network Distributed Data Entry System Handbook.

7

ADMINISTRATION

7.1 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 among nine institutions: the Institute, seven clinical centers and a Data Coordinating Center. The clinical centers and the Data Coordinating Center 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.

7.1.1 Participating Centers

7.1.1.1 Clinical Centers

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

7.1.1.2 Data Coordinating Center

The Data Coordinating Center is responsible for all aspects of biostatistical design, analysis and data management of the study. In concert with the Steering Committee, the DCC is responsible for forms development and testing. The DCC 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 DCC reports to the Steering Committee and Data Monitoring and Safety Committee.

7.1.2 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.

7.2 Committees

Figure 1 on page 30 illustrates the relationships between working components of the MFMU Network.

7.2.1 Steering Committee

This committee is comprised of twelve members: the principal investigator from each of the seven clinical centers and the Data 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 Patient Safety Monitoring Committee.

The list of the participating institutions, Steering Committee members and alternates is given in Appendix A.

7.2.2 Study Committees

7.2.2.1 Aspirin Protocol Subcommittee

The Aspirin Protocol Subcommittee is responsible for the preparation of the aspirin protocol, and for the conduct of the study. This subcommittee will report the progress of the aspirin study to the Steering Committee.

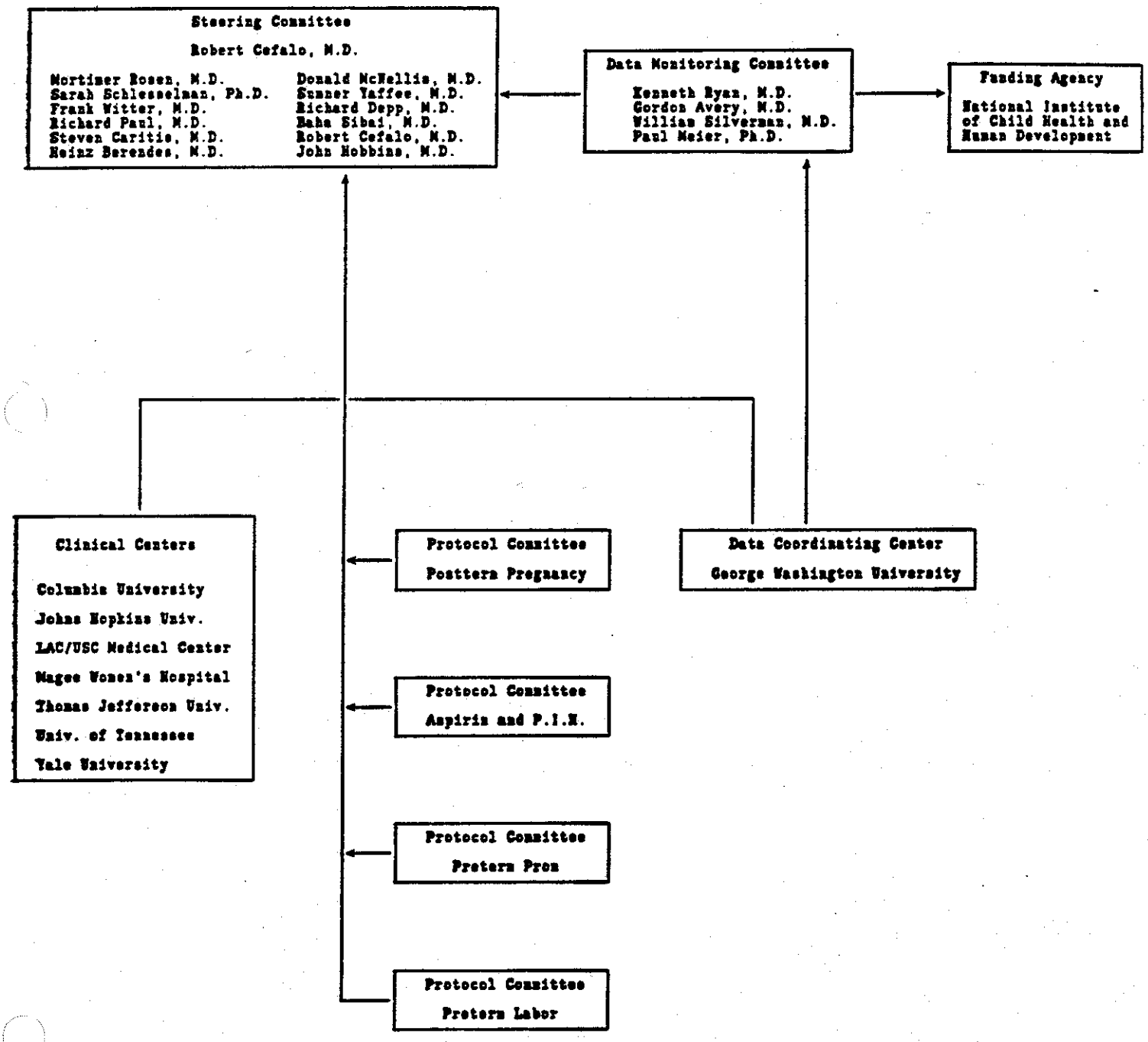
7.2.2.2 Clinical Review Subcommittee

The Clinical Review Subcommittee, composed of the Chair of the Aspirin Committee, a second clinical investigator, a representative from the DCC, and a representative from NICHD, will be responsible for reviewing case reports of adverse outcomes and possible adverse effects of aspirin. The committee will monitor the classification of study outcomes to assure that clinical centers submit the proper documentation for each reported outcome, and that the patient outcomes are classified according to the definitions as stated in the Protocol.

7.2.3 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.

Figure 1: MFMU Network Organization



Appendix A

MATERNAL FETAL MEDICINE UNITS NETWORK

Institution	Steering Committee Member	Coordinator
Columbia University	Mortimer Rosen, M.D. 212-305-2377	Angela Portale 212-305-2169
George Washington Univ.	Sarah Schlesselman, Ph.D. 301-881-9260	Julia Zachary 301-881-9260
Johns Hopkins Univ.	Frank Witter, M.D. 301-955-6293	Laura Rocco, R.N., M.S. 301-955-8240
LAC/USC Medical Center	Richard Paul, M.D. 213-226-3306	Catherine Walla, R.N. 213-226-3306
Magee Women's Hospital	Steven Caritis, M.D. 412-647-4220	Peggy Cotroneo, R.N. 412-647-4220
NICHD/PRP	Heinz Berendes, M.D. 301-496-5064	
NICHD/PP	Donald McNellis, M.D. 301-496-5575	Susan Herbert, M.A. 301-496-5575
NICHD/CRMC	Sumner Yaffe, M.D. 301-496-5097	
Thomas Jefferson Univ.	Richard Depp, M.D. 215-928-6920	Susan Tannenbaum 215-928-6931
Univ. of Tennessee	Baha Sibai, M.D. 901-528-5771	Eileen Bray, R.N. 901-577-8341
Univ. North Carolina	Robert Cefalo, M.D. * 919-966-1601	
Yale University	John Hobbins, M.D. 203-785-2173	Ellen Capiello, R.N. 203-785-6598

* Chairman, Steering Committee

Alternates Members of the Steering Committee

Institution	Steering Committee Member
NICHD/PRP	George Rhoads, M.D. 301-496-1711
Magee Women's Hospital	Eberhard Mueller-Heubach, M.D. 412-647-4222
Thomas Jefferson	Ronald J. Wapner, M.D. 215-928-7996
Yale University	Roberto Romero, M.D. 203-785-2173
NICHD/PP	Charlotte Catz, M.D. 301-496-5575
Johns Hopkins Univ.	Timothy Johnson, M.D. 301-955-6207
LAC/USC Medical Center	Janet Horenstein, M.D. 213-226-3306
Columbia University	Harold Fox, M.D. 212-305-2169
George Washington Univ.	Tavia Gordon 301-881-9260
Univ. of Tennessee	Garland Anderson, M.D.
NICHD/CRMC	James Kavanagh, Ph.D. 301-496-5097

Appendix B

PUBLICATION POLICY

B.1 Purpose

The Maternal Fetal Medicine Units Network will generate data from randomized clinical trials and other evaluations of maternal-fetal therapies and managements. The Steering Committee, consisting of the principal investigators, NICHD participants, Data Coordinating Center (DCC) representative, and the independent chairman of the Steering Committee, intend that these data be presented and published in as complete and mutually acceptable fashion as possible, so that the primary hypotheses of the studies can be answered most meaningfully. These policies and procedures are defined to serve this intention.

B.2 Process

B.2.1 Proposals

B.2.1.1 Primary publications

The Aspirin Protocol Subcommittee will have the responsibility of proposing specific written and oral communications, intended for publication and meeting presentation, respectively. A written proposal shall include the title, authors, hypotheses and rationale, anticipated findings, limitations of the data, timetable, and probable conclusions. Also, the most likely journals and meetings shall be identified. Submissions dealing with the primary hypotheses of the study will have priority over other related submissions, both in terms of timing and of study resources for data analysis.

B.2.1.2 Ancillary publications

After the primary hypotheses have been reported, other presentations and publications may be proposed by any Steering Committee member on his/her own behalf, or on behalf of other qualified investigators from any of the participating institutions or from NIH. The format for these proposals should be the same as that for the proposals for primary submissions, listed above.

B.2.2 Review, Approval, and Monitoring

The full Steering Committee will approve or disapprove, by majority vote, each proposal to communicate any protocol information and data, and will monitor progress according to the proposed timetable.

B.2.3 Data Analysis

Analysis of the pooled data will be performed at the Data Coordinating Center for the Network (Biostatistics Center of George Washington University), unless exceptions are agreed to by the Steering Committee. Pooled data will not be released prior to the submission of the primary results papers for publication.

B.2.4 Publication

Final manuscripts must be reviewed and approved by the Steering Committee before any major presentation or prior to submission for publication. Unapproved presentations or manuscript submissions are inconsistent with the spirit of collaborative research, and cannot be identified with the NICHD-supported Maternal Fetal Medicine Units Network. Disregard of this policy may lead to withdrawal of access to data and support.

B.3 Credits and Authorship

Submitted manuscripts should acknowledge that the data were generated from the Maternal Fetal Medicine Units Network, and cite participating institutions. Where possible, individual investigators who were active participants in the study should be cited. Those investigators contributing to the preparation of the manuscripts should be designated as authors, journal guidelines permitting. Significant contributions other than preparation of manuscripts will also be recognized by authorship. An equitable distribution of authorship among its members will be consciously sought and promoted by the Steering Committee. The Steering Committee also has the option of not designating authors for any specific, corporate publication.

B.4 Data Monitoring and Safety Committee

The Steering Committee may receive comments and suggestions regarding communications from the Data Monitoring and Safety Committee. This advice should be carefully considered with regard to publication and presentations. This committee should have privileged access to the data, but should not use any privileged information or data for purposes of public disclosure or publication.

B.5 Ancillary Studies

Plans for conducting ancillary studies using Network data or Network study patients from one or more participating institutions should be submitted to the Steering Committee. The Steering Committee review of such plans should assure that the ancillary study will not interfere with the conduct of the Network studies, and that publications arising from the ancillary study will not conflict with the reporting of the major results of the Network studies.

Appendix C

STANDARD METHOD FOR BP MEASUREMENT BY SPHYGMOMANOMETRY

(MODIFIED FROM DAVEY AND MACGILLIVRAY, CLINICAL & EXPERIMENTAL HYPERTENSION: HYPERTENSION IN PREGNANCY B5(1):97, 1986)

C.1 Sphygmomanometer

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

2. 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 ante-cubital fossa.

3. Application of cuff

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

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

   c. 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).

   d. 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.

C.2 Patient

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

2. Inquire about possibility of smoking and time from last cigarette.

3. 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.

C.3 Taking the Blood Pressure

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

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

3. 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.

4. 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.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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 D

STANDARD METHOD FOR URINE PROTEIN TESTING

(MODIFIED FROM DAVEY AND MACGILLIVRAY, CLINICAL & EXPERIMENTAL HYPERTENSION: HYPERTENSION IN PREGNANCY B5(1):97,1986)

D.1 Urine Testing

1. Patient should not drink large quantities of fluid before passing urine samples - diluted urine may give false negative results.

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

3. Test urine protein and record this value.

4. If specific gravity is obtained and found to be <1010 or >1030, then obtain another sample and retest again if the dipstick method is used.

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

D.2 Interpretation of Results

Negative or trace	<0.1 g/l	
1+	0.3 g/l	(significant proteinuria)
2+	1.0 g/l	
3+	3.0 g/l	
4+	$\geq$5.0 g/l	

D.3 Diagnosing Proteinuria

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

2. 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.

3. For making 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.

Appendix E

POSSIBLE INDICATIONS FOR INTERVENTION

E.1 Maternal

1. Spontaneous labor/delivery
2. Eclampsia
3. Pulmonary edema/cyanosis
4. Oliguria less than 400 ml/24 hours (in absence of other cause)
5. Epigastric discomfort
6. Recurrent vomiting
7. Systolic blood pressure 130-139
8. Systolic blood pressure 140-159
9. Systolic blood pressure at least 160
10. Diastolic blood pressure 80-89
11. Diastolic blood pressure 90-109
12. Diastolic blood pressure at least 110
13. Blood pressure unstable on hospital bed rest
14. Blood pressure unstable on home bed rest
15. Weight gain on hospital bed rest
16. Weight gain on home bed rest
17. Weight gain, no bed rest
18. Compliance unlikely
19. BUN 10-15 mg
20. BUN 16-20 mg

21. BUN >20 mg

22. Platelets: 150-200K

23. Platelets: 100-149K

24. Platelets: 50-99K

25. Platelets: <50K

26. Proteinuria: 2+ (on two or more occasions 4 hours apart)

27. Proteinuria: 3-4+ (on two or more occasions 4 hours apart)

28. Proteinuria: .1 gram-.34 gram in 24 hour collection

29. Proteinuria: .35 gram-.49 gram in 24 hour collection

30. Proteinuria: .5 gram-.99 gram in 24 hour collection

31. Proteinuria: 1.0 gram-4.99 gram in 24 hour collection

32. Proteinuria: 5.0 gram-9.99 gram in 24 hour collection

33. Proteinuria: at least 10.0 gram in 24 hour collection

34. SGOT (or AST) 40-79

35. SGOT (or AST) 80-159

36. SGOT (or AST) at least 160

E.2 Fetal

1. Abruption
2. Positive OCT/CST
3. Gestational age at least 37 weeks
4. Gestational age 34-36 weeks
5. Surfactant transitional
6. Surfactant mature
7. IUGR suspected
8. NST: non-reactive
9. Oligohydramnios

Appendix F

INFORMED CONSENT

CLINICAL TRIAL OF LOW-DOSE ASPIRIN AS A PREVENTATIVE OF PREECLAMPSIA

You are being asked to participate in a research study designed to evaluate the effects of low-dose aspirin in preventing preeclampsia, a type of hypertension that affects only pregnant women. Women in their first pregnancy are at particularly high risk for this condition, which can cause serious complications for both the pregnant woman and her unborn baby.

Starting in the middle third of your pregnancy you will be asked to take once a day a certain pill dispensed to you in a 4-week supply. The pill might contain either 60mg of aspirin (pediatric tablet) or a placebo (containing no aspirin). The pills are to be taken every day until delivery. Otherwise your visit schedule and other prenatal care will follow the usual routine for this institution.

There are no known risks associated with taking aspirin at the low doses used in this study. Aspirin in higher doses can cause bleeding, stomach upsets, delayed onset of labor, and can affect one of the vessels in the baby's heart. You and your baby will be monitored for these complications.

The data collected in this study will be maintained in strict confidence. There will be 3000 women involved from 7 different clinical centers. The study is sponsored by the National Institute of Child Health and Human Development of the National Institutes of Health.

By participating in this study you will help us determine the benefit of low-dose aspirin in preventing hypertension in pregnancy. The value of the aspirin to you is to be proven; there is no value to you in taking the placebo.

You are free to withdraw your consent to participate at any time during the study. You will not be denied medical care if you refuse participation.

If you have any questions concerning the nature of this study or your participation in it and in case of complications you can contact _____ at _____ or the staff physician in labor and delivery at _____ at any time.

Appendix G

DATA COLLECTION FORMS

Appendix H

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MATERNAL FETAL MEDICINE UNITS NETWORK

Randomized Clinical Trial of Low-Dose Aspirin as a Preventative of Preeclampsia

OBJECTIVE

To establish whether daily administration of low-dose (60 mg) aspirin to nulliparous obstetrical patients will decrease the incidence of preeclampsia.

ORGANIZATION

Clinical centers:
* Johns Hopkins U.
* U. of Pittsburgh
* Columbia U.
* U. of Tennessee
* Thomas Jefferson U.
* LAC/USC
* Yale U.

Subcommittee: * Dr. Sibai (Chair)

DESIGN

Type: Placebo controlled, randomized clinical trial

Major Eligibility Criteria:
* Nulliparous
* 13 - 26 weeks gestation
* BP < 135/85 w/o antihypertensive meds
* No aspirin meds during prior 2 weeks
* None or trace proteinuria
* Compliance with 1 week placebo run-in

Groups:
* Experimental = 60 mg aspirin (daily)
* Placebo = 60 mg placebo (daily)

Random Allocation: One of the two treatment groups using an unbiased coin

Level of masking: Double masked

Pre-stratification: Clinical center

Sample size:
* Goal = 3,000 (1500/group)
* Assumptions:
  - Outcome event = preeclampsia
  - Placebo group event rate = 5-6%
  - Experimental group event rate $\leq$ 2.5-3% (1/2 reduction in placebo event rate)
  - Type I error = 2% (two-sided)
  - Power = 80%
  - Non-compliance = 10% per group

Interim analysis: Group sequential method

SCHEDULED EVALUATIONS

Pre-Randomization:
* History and physical (second trimester)
* One week placebo run-in period

Post-Randomization:
* Routine prenatal visits
* Labor and delivery
* Post-partum prior to discharge

MANAGEMENT PROTOCOLS

Coded medication: * 60 mg once daily up to delivery

Coded medication discontinued: * Development of preeclampsia

Preeclampsia: * Suggested guidelines for outpatient management

BP Measurement: * Standardized method (appendix C)

Urine Protein: * Standardized method (appendix D)

OUTCOME MEASURES

Primary:
* Preeclampsia:
  - Mild preeclampsia
  - Severe preeclampsia
  - Eclampsia
  - HELLP syndrome

Secondary:
* Mild and severe PIH
* Adverse effects of aspirin

TIMETABLE (current)

Recruitment (n=3135):	3/ 1/89 -	9/30/91 (2 years, 7 months)
Data Collection:	3/ 1/89 -	3/31/92 (3 years, 1 month)
Closeout/Final analysis:	4/ 1/92 -	11/30/92 (8 months)

3/92;VM=LLMFU