# Platelet inhibition with variable dosages and frequency of aspirin in pregnant women: A randomized trial

# Protocol

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# 1. Introduction

## 1.1 Study Abstract:

Acetylsalicylic acid, or aspirin, is a cyclooxygenase inhibitor with antiplatelet and anti-inflammatory properties. Aspirin has been shown in multiple studies to be an effective medication to prevent or delay the onset of preeclampsia in pregnancy. While most guidelines recommend low-dose aspirin for this indication, there is no consensus on the optimal dose. Practitioners have used and described doses in the literature ranging from 50mg to 162mg daily.

This document describes a protocol of a randomized trial of women evaluating whether using 162mg of aspirin daily more adequately inhibits thromboxane-mediated platelet function, compared to a dose of 81mg daily. Within the population taking 162mg of aspirin daily, we will compare platelet function when taking a once-daily dose of 162mg of aspirin, compared to taking 81mg twice daily.

## 1.2 Study Hypothesis:

In women, taking 162mg of aspirin daily has improved inhibition of platelet function, compared to a dose of 81mg daily.

## 1.3 Purpose of the Study Protocol

This protocol describes the background, design and organization of the randomized clinical trial. Institutional Review Board (IRB) approval will be obtained prior to beginning recruitment.

## 2. Background

### 2.1 Introduction

Preeclampsia is a multi-system disorder affecting up to 10% of all pregnancies, and is part of the spectrum of hypertensive disorders of pregnancy.[1-2] There is well established evidence that low-dose aspirin is an effective medication for use to prevent or delay the onset of preeclampsia in patients at increased risk.[3-4] Low-dose aspirin is very safe to use in pregnancy, with no significant maternal or fetal risks.[4] This safety profile has been demonstrated in multiple large studies.[4]

However, there remains much controversy and lack of evidence surrounding the optimal dosing of aspirin for this indication.[1,5] Interestingly, studies have suggested a dose-response relationship between aspirin and preeclampsia reduction effect.[6]

### 2.2 Biological Mechanisms

The etiology and pathogenesis of preeclampsia are multifactorial, and are characterized by an abnormal vascular response to placentation, resulting in increased vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial cell dysfunction.[1-2] This is thought to be at least partially the result of an increased thromboxane A2 levels, and reduced prostacyclin levels.[7] These effects can be demonstrated as early as 13 weeks in at-risk pregnancies.[7] Low-dose aspirin has been shown to counteract this imbalance; aspirin inhibits cyclooxygenase-1 and its thromboxane production.[8] This explains aspirin's effectiveness as a preventative medication for preeclampsia.[8] Treatment with 81mg of aspirin, starting between 12 and 16 weeks of gestation, is associated with an at least 8% reduction in the onset of preeclampsia during that pregnancy.[3]

However, there is evidence to suggest that failure of low-dose aspirin in preventing preeclampsia may be related to incomplete inhibition of platelet aggregation at the recommended dosage, or "aspirin resistance".[9-10] In fact, by increasing using a dose of 162mg instead of 81mg, the rate of aspirin resistance drops from approximately 30% to around 5%.[11]

There is data to suggests that dosing aspirin at bedtime results in somewhat better morning platelet inhibition.[12] Also, twice-daily aspirin dosing, compared to once-daily dosing, has been persistently shown in the literature to be associated with improved platelet inhibition.[13-15]

**2.3 Aspirin and Obesity**

Obesity is known to be a risk factor for developing preeclampsia in pregnancy.[1,3,16] While the pathophysiology of this risk is not completely understood, the etiology is likely multifactorial.

The pathophysiologic changes resulting from obesity may alter the pharmacokinetics of medication in this population, including aspirin.[17-19] This may result in an increased likelihood of aspirin resistance in this population, resulting in incomplete inhibition of cyclooxygenase-2, and therefore persistent normal or elevated serum thromboxane levels.[18-20]

In addition, obesity in and of itself has been associated with platelet hyperreactivity, which is evident both with and without aspirin use. [20-21] This may in part be due to enhanced lipid peroxidation leading to low-grade inflammation, which in turn results in an increase in thromboxane-dependent platelet activation.[21-22]

Interestingly, mature human adipose tissue has also been found to express and release soluble fms-like tyrosine kinase 1 (sFlt-1), and both BMI and body weight have been shown to affect the rate of expression of this molecule in adipose tissue.[23] sFlt-1 is an anti-angiogenic molecule which has been correlated with and predictive of the onset of preeclampsia.[24] Aspirin has been shown to inhibit the production of sFlt-1 in cytotrophoblasts when induced by hypoxia,[25] and if these findings prove to be generalizable, this may suggest that different aspirin dosages are needed to inhibit a larger amount of circulating sFlt-1.

Finally, thromboxane B2 is a validated serum marker for measuring platelet response to aspirin therapy.[26] The levels of thromboxane B2 in obese women have been shown to be significantly

elevated when compared to a non-obese cohort, suggesting possible underlying increased platelet aggregation in the obese patient.[26]

### 2.4 Aspirin-Triggered 15-Epi-Lipoxins

One of the more recent proposed mechanisms of action for use of ASA is its ability to initiate biosynthesis of novel anti-inflammatory mediators by means of interactions between endothelial cells and leukocytes.[27] These mediators are classified as aspirin-triggered 15-epi-lipoxins (ATLs).[27] Such compounds may account at least in part for some aspirin's clinical benefits, which are distinct from the well-appreciated action of aspirin as a platelet inhibitor.[28] Thus, the protective actions of ATL are likely to underlie some of the therapeutic impact of aspirin when aberrant inflammation is a component of disease pathogenesis, such as in PE.[27-29]

### 2.5 Exosome and aspirin dosing

To date, professional societies recommend use of low-dose aspirin for preeclampsia prevention in pregnant individuals at high-risk of developing the disease, but the optimal dose is unknown. Furthermore, there are limited biomarkers available as candidates for monitoring therapeutic response to aspirin treatment in pregnant individuals at high-risk for PE, which ultimately may be used to generate desirable data to optimize aspirin dosing and improve pregnancy outcomes. Recent advancement in isolating specific extracellular vesicles (exosomes; 40–160 nm) from maternal blood and characterizing their cargo content have helped to better understand the response to therapeutic interventions. Although diagnostic potential of exosomes has been reported, no studies have examined the response to therapeutic interventions such as different doses of aspirin on exosomes.

### 2.6 Rationale for Randomized Trial and Hypothesis

Due to the known pharmacokinetic effects of aspirin, and the known increased incidence of aspirin resistance in this population, we hypothesize that the use of 162 mg daily will have lower incidence of aspirin resistance, when compared to a once-daily 81mg of aspirin.

## 3. Study Design

### 3.1 Primary Research Question

This randomized trial will address the primary research question:

In women, does taking 162mg of aspirin daily, starting between 12 and 16 weeks of gestation, result in a lower incidence of aspirin resistance, when compared to a daily dose of 81mg?

### 3.2 Secondary Research Questions

Secondary research questions that this study will address are whether in pregnant women, a daily aspirin dose of 162mg, compared to a daily dose of 81mg, starting between 12 and 16 weeks of gestation, will decrease the risk of:

- Preeclampsia;
- Gestational hypertension;
- Gestational diabetes;
- Composite maternal cardiovascular outcome;
- Composite maternal morbidity, including postpartum hemorrhage;
- Operative vaginal delivery, cesarean delivery;
- Neonatal morbidity and mortality.

### 3.3 Design Summary

This study is a non-blinded prospective randomized trial of pregnant women with a singleton pregnancy between 12 weeks 0 days and 16 weeks 0 days gestation. Participants will be randomized to receive either:

- Aspirin 81mg daily at bedtime until delivery;
- Aspirin 162mg daily at bedtime (2x 81mg tablets) until delivery;

### 3.4 Eligibility Criteria

### 3.4.1 Inclusion Criteria

- Females older than 18 years of age;
- The patient is physically and mentally able to understand the informed consent and is willing to participate in this study;
- The patient is between 12 weeks 0 days and 16 weeks 0 days of gestation at the time of enrollment. Gestational age will be determined by last menstrual period, confirmed with a first trimester ultrasound, per the recommended guidelines by the American College of Obstetricians and Gynecologists.
- One high risk factor or at least 2 moderate risk factors for preeclampsia

### 3.4.2 Exclusion Criteria

- Known allergy or reaction to aspirin, or concurrent medical condition where the use of aspirin is contraindicated;
- Use of aspirin prior to enrollment in the study;
- Known or suspected fetal anomaly or aneuploidy;
- Prisoners;
- Prenatal care or planned delivery outside the Ohio State University.

### 3.5 Informed Consent Criteria

Women will be approached at the time of one of their prenatal visits prior to 16 weeks to determine study eligibility. Voluntary oral permission will be obtained to participate in the screening process. Respondents who do not wish to participate in the screening process with be thanked and no further questions asked.

Written informed consent will be obtained before enrollment into the study. A copy of the consent form will be provided to the patient. Women who are not fluent in English will be enrolled by a person fluent in their language, if possible. Both verbal and written informed consent and authorization will be obtained in that language; if this is not possible the patient will be excluded.

### 3.6 Randomization Method

Patients will be randomized to either 81mg of daily aspirin or 162mg of daily aspirin at bedtime, starting between 12 weeks 0 days and 16 weeks 0 days of gestation.

## 4. Study Procedures

### 4.1 Screening for Eligibility and Consent

Women will be approached at the time of one of their prenatal visits prior to 16 weeks to determine study eligibility. If the patient elects to participate in the study and signs the relevant consents, the patient's chart will be reviewed to ensure that the patient meets inclusion and exclusion criteria.

If a patient agrees to participate, and meets the inclusion and exclusion criteria, she will be asked to sign the consent form for participating in the study.

### 4.2 Randomization

Patients will be randomized to taking either 81mg daily or 162mg daily of aspirin. If the patient is below 12 weeks of gestation, she will be asked to start taking the medication at 12 weeks 0 days at bedtime. If the patient is between 12 weeks 0 days and 16 weeks 0 days gestation, she will be asked to start taking the medication at bedtime on the day of enrollment. The aspirin pills will be provided to the patient on the day of randomization.

### 4.3 Baseline Procedures

The patient's eligibility information will be collected, including gestational age and estimated date of delivery, in addition to the following:

- Demographics: age, race, insurance status;
- Medical history: pre-pregnancy weight, current weight, height, current blood pressure, past medical history;
- Social history: marital status, years of education, alcohol use, tobacco use and other maternal drug use;
- Obstetrical history: previous miscarriages and terminations, history of preeclampsia or other obstetric complications in prior pregnancies.

All patients have a baseline prenatal panel test via a lab draw and a urine specimen as part of their routine prenatal care. We will ask the patient to allow us to obtain additional blood tubes during the same draw, therefore not exposing the patient to added needle sticks. This will test for aspirin-triggered 15-epi-lipoxins, exosomes, placental growth factor levels, soluble fms-like tyrosine kinase 1 levels, thromboxane levels, serum creatinine, and liver function tests.

Patients also provide a urine sample at each prenatal visit. The patient will be asked to allow us to collect part of the urine sample to evaluate for baseline proteinuria by dipstick, and to measure urine thromboxane levels and the urine protein to creatinine ratio.

The patient will be asked to allow us to collect 10 inches of umbilical cord just after their delivery to measure prostaglandins and 15-epi-lipoxins.

### 4.4 Patient Management and Follow Up

Patients will continue to receive standard prenatal care with their treating physician. Patients typically have their blood pressure and weight measured at all prenatal visits, and this information will be collected.

Patients typically have a second trimester lab draw at approximately 27 weeks of gestation. We will ask the patient to allow us to obtain additional blood tubes during the same draw, therefore not exposing the patient to added needle sticks. This will test for aspirin-triggered 15-epi-lipoxins, exosomes, thromboxane levels, placental growth factor levels and soluble fms-like tyrosine kinase 1 levels. A urine sample will also be collected on that visit, to measure urine thromboxane levels.

Study personnel will meet with participants monthly, at the time of prenatal care visits, to ascertain compliance with study agent, query for side effects or complications, and to chart abstract medical/obstetrical complications and recorded blood pressure.

All other obstetric care is at the discretion of the primary provider, including but not limited to: treatment of maternal medical comorbidities or obstetric complications, timing and frequency of antenatal ultrasound, maternal and fetal surveillance, and timing and mode of delivery.

**4.5 Procedures on Labor and Delivery and Postpartum**

At delivery, study personnel will chart abstract maternal and infant delivery information and maternal and infant data will be collected until day of discharge or up to 30 days postpartum, whichever occurs first.

Patients typically have a blood draw in the third trimester. We will ask the patient to allow us to obtain additional blood tubes during the same draw, therefore not exposing the patient to added needle sticks if possible. These will test for aspirin-triggered 15-epi-lipoxins, exosomes, thromboxane levels, placental growth factor levels, and soluble fms-like tyrosine kinase 1 levels. A urine sample will also be collected to test for urine thromboxane levels.

**4.6 Adverse Event Reporting**

Detailed information concerning adverse events will be collected and evaluated throughout the conduct of the protocol. Adverse events will be reported to the Institutional Review Board per policy.

### 4.7 Study Outcome Measures

#### 4.7.1 Primary Outcome

The primary outcome is complete platelet inhibition by aspirin. This will be evaluated with measured maternal serum thromboxane levels at randomization, in the second trimester, and on admission to labor and delivery.

#### 4.7.2 Maternal Secondary Outcomes

- Diagnosis of eclampsia, HELLP, atypical HELLP, preeclampsia, superimposed preeclampsia or antepartum gestational hypertension, using standard American College of Obstetricians and Gynecologists criteria[1], during the pregnancy or up to 30 days postpartum;
- Placental growth factor levels and soluble fms-like tyrosine kinase 1 levels, to evaluate whether the values are predictive of aspirin resistance, as the ratio of both values has been validated as a predictor of preeclampsia[20];
- Aspirin-triggered 15-epi-lipoxins;
- Gestational diabetes by oral GTT criteria performed after randomization;
- Preterm birth < 34 weeks and <37 weeks, both spontaneous and indicated;
- Operative vaginal delivery and cesarean delivery;
- Estimated and quantitative blood loss;
- Blood transfusion;
- Epidural hematoma;
- Maternal morbidity and adverse maternal outcomes, including venous thromboembolism, peripartum cardiomyopathy, massive transfusion and postpartum hemorrhage, ICU admission, and maternal death.

The maternal plasma exosome proteome profile

### 4.7.3 Neonatal Secondary Outcomes

- Antepartum, intrapartum, or neonatal death
- Intubation, continuous positive airway pressure (CPAP) or high-flow nasal cannula (HFNC) for ventilation or cardiopulmonary resuscitation within first 72 hours
- Birth weight
- Neonatal encephalopathy as defined by the NICHD Neonatal Research Network criteria
- Seizures
- Shoulder dystocia
- Birth trauma (bone fractures, brachial plexus palsy, other neurologic injury, retinal hemorrhage, or facial nerve palsy)
- Intracranial hemorrhage (intraventricular hemorrhage, subgaleal hematoma, subdural hematoma, or subarachnoid hematoma)
- Hyperbilirubinemia requiring phototherapy or exchange transfusion
- Hypoglycemia (glucose < 35 mg/dl) requiring IV therapy
- NICU admission

## 5. Statistical Considerations

### 5.1 Data Relevant to the Primary Outcome

As discussed in the background, pregnancy is associated with an increased prevalence of aspirin resistance. The literature suggests that women have an aspirin resistance rate of 26% with low-dose aspirin, compared to 20.5% in non-pregnant women.[26]

### 5.2 Sample Size and Power

For this calculation, we performed a power analysis comparing patients taking a total of 81mg of aspirin daily, to patients taking 162mg of aspirin daily.

We predicted that taking 162mg of aspirin daily will be associated with a 35% reduction in aspirin resistance. Assuming a two-sided alpha of 0.05, it is estimated that 128 women/group (256 total) would provide 80% power to detect the above described reductions in the frequency of aspirin resistance in the 2 groups. Assuming an approximately 30% rate of drop out and loss to follow up, we will collect data on a total of 400 patients to achieve the required statistical power. Patients will be randomized in a 1:1 ratio.

**6. Data collection and security**

Data collection will be done using REDCap database. Only individuals who are included as investigators or study personnel will be granted access to the database. The REDCap database will NOT include any protected health information; patients will be identified by a unique Study ID. There will be a separate secure database at each site linking Study ID with patient identification. User access will be granted by the PI only. When study personnel/investigators are no longer part of the institution or study, the PI will be notified and their access to the database will be immediately discontinued.

We will send de-identified maternal blood samples to University of Texas Medical Branch (UTMB) for exosome analysis and they will send the data back to us for analysis at the OSU Biomedical Informatics department.

## References

1. American College of Obstetricians and Gynecologists. Hypertension in pregnancy: report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstet Gynecol 2013; 122:1122
2. Cunningham FG, Leveno KJ, Bloom SL *et al*. Williams Obstetrics. 24th edition. New York: McGraw-Hill Education, 2014
3. LeFevre ML, U.S. Preventive Services Task Force. Low-dose aspirin use for the prevention of morbidity and mortality from preeclampsia: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2014; 161:819
4. ACOG Committee Opinion No. 743: Low-Dose Aspirin Use During Pregnancy. Obstet Gynecol 2018; 132:e44
5. Askie LM, Duley L, Henderson-Smart, DJ, Stewart LA. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet 2007; 369: 1791–1798
6. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. Am J Obstet Gynecol 2017; 216:110–120.e6
7. Walsh SW. Preeclampsia: An imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985; 335-340
8. Walsh SW. Low-dose aspirin: treatment for the imbalance of increased thromboxane and decreased prostacyclin in preeclampsia. Am J Perinatol. 1989;6:124–132
9. Syrbe G, Redlich H, Ludwig J *et al*. Individual dosing of ASA prophylaxis by controlling platelet aggregation. Clin Appl Thromb Haemost 2001; 7:209–213
10. Hovens MM, Snoep JD, Eikenboom JC *et al*. Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review. Am Heart J 2007; 153:175–181
11. Caron N, Rivard GE, Michon N *et al*. Low-dose ASA response using the PFA-100 in women with high-risk pregnancy. J Obstet Gynaecol Can 2009; 31(11):1022–7
12. Bonten TN, Snoep JD, Assendelft WJ *et al*. Time-dependent effects of aspirin on blood pressure and morning platelet reactivity: a randomized cross-over trial. Hypertension 2015; 65(4):743-50

13. Patrono C, Rocca B. Type 2 diabetes, obesity and aspirin responsiveness. J Am Coll Cardiol 2017; 69(6):613-615
14. Bethel MA, Harrison P, Sourij H *et al*. Randomized controlled trial comparing impact on platelet reactivity of twice-daily with once-daily aspirin in people with Type 2 diabetes. Diabet Med 2016; 33(2):224-30
15. Larsen ML, Pedersen OH, Hvas AM *et al*. Once- versus twice-daily aspirin treatment in patients with essential thrombocytosis. Platelets 2018; Feb 14:1-7 (epub ahead of print)
16. Bodnar LM, Ness RB, Markovic N, Roberts JM. The risk of preeclampsia rises with increasing prepregnancy body mass index. Ann Epidemiol 2005; 15(7):475–482
17. Jain R, Chung SM, Jain L *et al*. Implications of obesity for drug therapy: limitations and challenges. Clin Pharmacol Ther 2011; 90(1):77-89
18. Rocca B, Dragani A, Pagliaccia F. Identifying determinants of variability to tailor aspirin therapy. Expert Rev Cardiovasc Ther 2013; 11(3):365-79
19. Peace A, McCall M, Tedesco T *et al*. The role of weight and enteric coating on aspirin response in cardiovascular patients. J Thromb Haemost 2010; 8:2323-5
20. Maree AO, Curtin RJ, Dooley M *et al*. Platelet response to low-dose enteric-coated aspirin in patients with stable cardiovascular disease. J Am Coll Cardiol 2005; 46(7):1258-63
21. Beijers HJ, Ferreira I, Spronk HM *et al*. Body composition as determinant of thrombin generation in plasma: the Hoorn study. Arterioscler Thromb Vasc Biol 2010; 30: 2639–2647
22. Santilli F, Vazzana N, Liani R *et al*. Platelet activation in obesity and metabolic syndrome. Obes Rev 2012; 13(1):27-42
23. Herse F, Fain JN, Janke J *et al*. Adipose Tissue-Derived Soluble Fms-Like Tyrosine Kinase 1 Is an Obesity-Relevant Endogenous Paracrine Adipokine. Hypertension 2011; 58(1):37-42
24. Zeisler H, Llurba E, Chantraine F *et al*. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. N Engl J Med 2016; 374(1):13-22
25. Li C, Raikwar NS, Santillan MK *et al*. Aspirin inhibits expression of sFLT1 from human cytotrophoblasts induced by hypoxia, via cyclo-oxygenase 1. Placenta 2015; 36(4):446-453

26. Bordeaux BC, Qayyum R, Yanek LR *et al*. Effect of obesity on platelet reactivity and response to low-dose aspirin. Prev Cardiol 2010; 13(2):56-62
27. Gil-Villa AM, Norling LV, Serhan CN *et al*. Aspirin triggered-lipoxin $A_4$ reduces the adhesion of polymorphonuclear neutrophils to endothelial cells initiated by preeclamptic plasma. Prostaglandins Leukot Essent Fatty Acids 2012; 87(4-5):127-134
28. Chiang N, Bermudez EA, Ridker PM *et al*. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. PNAS 2004; 101(42):15178-15183
29. Cadavid AP. Aspirin: The mechanism of action revisited in the context of pregnancy complications. Frontiers in Immunology 2017; 8:261