Accepted Manuscript

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PII: S0301-2115(17)30604-8
DOI: https://doi.org/10.1016/j.ejogrb.2017.12.052
Reference: EURO 10183

To appear in: *EURO*

Received date: 21-8-2017
Revised date: 21-11-2017
Accepted date: 30-12-2017

Please cite this article as: Navaratnam Kate, Alfirevic Ana, Jorgensen Andrea, Alfirevic Zarko.Aspirin non-responsiveness in pregnant women at high-risk of pre-eclampsia.*European Journal of Obstetrics and Gynecology and Reproductive Biology* https://doi.org/10.1016/j.ejogrb.2017.12.052

Aspirin non-responsiveness in pregnant women at high-risk of pre-eclampsia

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Objectives: Low-dose aspirin is recommended for prevention of pre-eclampsia in high-risk pregnant women. Current doses provide a conservative risk reduction and some individuals demonstrate 'aspirin non-responsiveness', with insufficient antiplatelet effects. We aimed to determine if aspirin non-responsiveness could be identified in women at high risk of pre-eclampsia and assess for potential associations with placentally-mediated adverse outcomes.

Study design: Prospective cohort study. 180 women at high-risk of pre-eclampsia, by NICE criteria, prescribed 75mg dispersible aspirin daily were recruited from antenatal clinics of Liverpool Women's Hospital between 17/01/14 and 31/03/16. Platelet function (Multiplate™ impedance aggregometry, VerifyNow™ and 11-dehydrothromboxane B_2) and aspirin metabolites (nuclear magnetic resonance and liquid chromatography mass spectrometry) were assessed at $5+^{0}$-$20+^{6}$ and $33+^{0}$-$35+^{6}$ weeks. Pearson's chi-square test was used to assess for associations between longitudinal response to aspirin and (1) any pre-eclampsia (2) composite adverse placentally-mediated outcome (one, or combination of pre-eclampsia, placental abruption, IUGR and perinatal mortality). A Bonferroni correction was applied to correct for multiple analyses.

Results: 180 women were recruited, there were 4 withdrawals and no women were lost to follow-up. After 15 women delivered prior to the completion of follow-up, sufficient sample volumes for longitudinal platelet function and aspirin adherence testing were obtained from 156 women. There were no consistent aspirin non-responders in the cohort. 59% (n=92) women exhibited normal response to aspirin, 34% (n=53) variable response (switching response status between study visits) and in 7% (n=11) response could not be determined as they exhibited lack of platelet response on a background of undetectable aspirin metabolites. There was no significant association between indeterminate or inconsistent (variable or indeterminate) response to aspirin and either pre-eclampsia (p=0.59, p=0.84) or composite outcome (p=0.95, p=0.65).

Conclusions: When platelet function was assessed with COX-specific tests that measure the antiplatelet effects of low-dose aspirin and aspirin adherence is accurately accounted for aspirin non-responsiveness was not identified in pregnant women at high-risk of pre-eclampsia. Response to aspirin was not associated with placentally-mediated adverse outcomes. The high-degree of variable and indeterminate aspirin response indicates suboptimal adherence and/or dosing are more pressing factors to address to optimise aspirin effectiveness.

Keywords: Pregnancy; pre-eclampsia; aspirin; aspirin resistance; aspirin non-responsiveness

INTRODUCTION

Low-dose aspirin (LDA) is a safe preventative treatment recommended for women at high-risk of pre-eclampsia [1]. LDA confers a relatively modest 10% reduction in the overall risk of pre-eclampsia, delivery prior to 34 weeks gestation and serious adverse pregnancy outcome [2]. Recent evidence suggests that, in carefully selected populations, the reduction in early onset pre-eclampsia may be greater than 50% [3]. However, over the last two decades it has become apparent that individuals may not have uniform antiplatelet action or clinical effects from aspirin. Such inadequate response is referred to interchangeably as aspirin resistance and aspirin non-responsiveness [4, 5]. Many causes have been proposed including pharmacokinetic, pharmacodynamic and genetic factors, though the most common is suboptimal aspirin adherence [4] Aspirin non-responsiveness ranges from 5-65% dependent on the platelet function assessment used and definition applied [6-10]. Biochemical aspirin non-responsiveness is defined as insufficient suppression of cyclo-oxygenase (COX)-mediated platelet function in individuals exposed to aspirin, with platelets retaining the ability to produce thromboxane A_2 (TXA_2), a potent pro-platelet aggregator. This may stem from incomplete COX blockade or the production of TXA_2 via alternative pathways. Unfortunately, there is widespread lack of consensus on an appropriate definition and diagnostic test for aspirin non-responsiveness [5]. Clinical definitions of aspirin non-responsiveness focus on recurrence of adverse events in aspirin-treated individuals.

Pregnancy is a state of enhanced platelet turnover, with immature platelets prone to activate and aggregate more readily, providing a plausible basis for diminished aspirin responsiveness in pregnancy [11]. Several observational studies suggested a direct link between aspirin non-responsiveness and adverse pregnancy outcomes [12-14]. In these high-risk obstetric populations, aspirin non-responsiveness was identified in 29-39% of women and associated with a higher risk of pre-eclampsia, preterm birth or of small for gestational age infants [12, 13]. Additionally, Rey et al undertook PFA-100^{TM} guided aspirin dose escalation and demonstrated that where escalation was necessary, women were at increased risk of pre-eclampsia (11/43, 25.6% vs. 6/68, 8.8%, p = 0.03) [12].

Our aim was to assess whether aspirin non-responsiveness could be identified in a prospectively recruited cohort of aspirin-adherent pregnant women at high-risk of pre-eclampsia, and if response to aspirin could be linked to placentally-mediated adverse pregnancy outcomes.

PARTCIPANTS AND METHODS

The Estimating Aspirin ResisTance in High-risk women (EARTH) study was a prospective cohort study, approved by the Liverpool Research Ethics Committee (13/NW/0764) and conducted in high-risk antenatal clinics of Liverpool Women's Hospital between January 2014 and March 2016.

Inclusion and exclusion criteria

Women viable singleton pregnancies, with at least one high risk factor or at least two moderate risk factors for pre-eclampsia and prescribed 75mg dispersible aspirin daily (LDA) were eligible for inclusion. (Table 1) Women were excluded if they were prescribed a higher dose of aspirin, if they were taking aspirin more than once daily, or if they were prescribed other antiplatelet or anticoagulant medication.

Study procedures

Maternal whole blood and urine were obtained at two separate visits between $5+^{0}$-$20+^{6}$ and $33+^{0}$ and $35+^{6}$ week's gestation. At each visit, aspirin's principal plasma (salicylic acid, SA) and urinary (salicyluric acid, SUA) metabolites were measured. At $33+^{0}$-$35+^{6}$ week's additional ultrasound examinations of fetal growth (including head and abdominal circumferences, and femur length) and Doppler velocimetry of the umbilical artery, middle cerebral artery, ductus venosus, and umbilical vein were conducted. All participants were asked to complete aspirin diaries for seven days prior to their third trimester visit, reporting the number of omitted doses.

Laboratory techniques

Blood samples for platelet function were analysed within 3 hours of collection. Maternal plasma and urine samples for measurement of aspirin metabolites were also prepared, aliquoted (600µL and 1ml, respectively) and stored at -80^{o}C within 3 hours of collection. The platelet function assays used provide COX-selective measures of platelet function that best reflect the effects of aspirin.

- Multiplate™ impedance aggregometry ASPI test (arachidonic acid) (Roche Diagnostics Limited, Switzerland) [15] operates with hirudin anticoagulated whole blood and is semi-automated for point-of-care use. Multiplate^{TM} detects the change in electrical impedance when platelets aggregate on electrode sensors. Change in impedance is transformed to aggregation units and plotted against time, to display area under the curve (AUC).

- VerifyNow™ is a closed point of care system that operates with citrated whole blood. VerifyNow™ Aspirin test (arachidonic acid) (Accumetrics, San Diego, CA) [16] measures the ability of activated platelets to bind to fibrinogen-coated micro particles. Light transmittance increases with the binding of activated platelets, measured as a change in optical signal (aspirin reaction units, ARU).
- TXB Cardio™ (Randox, UK) [17] measures urinary 11-dehydrothromboxane B_2, a stable metabolite of thromboxane A_2, production of which is inhibited by aspirin. TXB Cardio™ is a validated latex enhanced immunoturbidimetric method, with results expressed by sample concentration of 11-dehydrothromboxane B_2 relative to sample creatinine.

On the day of analysis, urine samples for Nuclear Magnetic Resonance (NMR) spectroscopy were thawed and centrifuged (21500 g, 4°C for 5 minutes). Spectra were acquired on a 700MHz Bruker spectrometer and SUA identified using Chenomx™ pattern recognition software (Chenomx, Canada). Plasma aliquots for Liquid Chromatography: Mass Spectroscopy (LC: MS) were thawed from -80°C and vortexed for five seconds prior to use. SA was identified with high performance LC: MS interfaced with a triple quadruple mass spectrometer. LCQUAN™ software (Thermo Fisher Scientific, UK) was used for data acquisition and processing.

Outcome measures

Multiplate™ and TXB Cardio™ results were assessed against 95% pregnancy-specific reference ranges calculated in our laboratory using samples from for healthy pregnant volunteers *(unpublished*). As a pregnancy-specific reference range for VerifyNow™, is not currently available, results were assessed against the manufacturer's cut-off for normal aspirin response (< 550 ARU). Appropriate aspirin adherence was defined as either evidence of appropriate platelet response or detection of aspirin metabolites by LC: MS or NMR. Our definitions of aspirin response are presented in Table 2.

Pre-eclampsia and gestational hypertension were defined according to NICE criteria as new onset (after 20 weeks) proteinuric or non-proteinuric hypertension, respectively, with systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90mmHg (Korotkoff V) on at least 2 occasions 4h apart, with proteinuria (spot urine ≥2+, protein: creatinine ratio ≥ 30 mg/mmol, or≥ 300 mg/24h using a validated 24 hour collection method). [1] Fetal growth restriction (FGR) was defined as customised birthweight < 5th centile [18] with one or more abnormal Doppler results (umbilical artery Pulsatility Index >95th centile, reduced, absent, or reversed end diastolic flow, middle cerebral artery Pulsatility Index <5th centile) or post-mortem classification

following intrauterine fetal death (IUFD). Composite placentally-mediated adverse outcome included pre-eclampsia, placental abruption (identified clinically or with histology), FGR or perinatal mortality.

Statistical analysis

From previous experience recruiting women at high-risk of pre-eclampsia in our centre we ascertained 200 women could feasibly be recruited during a 2 year study period. We assumed 12% of participants would develop pre-eclampsia and 20% would be aspirin non-responsive. With these assumptions, a sample size of 100 would give good estimated precision for aspirin non-responsiveness (i.e. 95% Confidence Intervals from 13%-29%). We hypothesised that approximately 40% of aspirin non-responders would develop pre-eclampsia, compared with less than 10% of responders. A sample size of 100 women would have adequate power (power of 80% with alpha error of 5%) to test this hypothesis. However, we estimated that a total of 180 participants would be required to ensure 100 aspirin adherent women would be included in the cohort.

Data were analysed using SPSS v.24 for Windows (IBM, Chicago, IL). For platelet function tests, overall percent agreement (OPA) was calculated by dividing the sum of agreed positives plus agreed negatives by the total tests performed. OPA was calculated for the following comparisons at both study visits; VerifyNow and Multiplate, VerifyNow and urinary 11-Dehydrothromboxane B2 and Multiplate and urinary 11-Dehydrothromboxane B2. Descriptive statistics for the cohort, including mean and standard deviation or median and mode values, as appropriate were calculated for participant characteristics and clinical outcomes. Pearson's chi-square test was used to assess for associations between longitudinal aspirin response status and (1) any pre-eclampsia (2) composite adverse placentally-mediated outcome (previously defined). A Bonferroni correction was applied to correct for the effect of multiple analyses and the threshold for statistical significance was set at 0.025 (0.05/2).

RESULTS

Description of the cohort

A total of 180 women were recruited to the EARTH study, 177 of whom commenced testing for aspirin responsiveness and 156 of whom were available for longitudinal analysis (Figure 1, Table 3). Four women withdrew from follow-up prior to the third trimester visit as they did not wish to proceed with further study procedures. All women that withdrew consented to collection of their outcome data. Details for those with fetal losses/deliveries prior to completion of follow-up are presented in Table 4. The gestational age range of commencing aspirin varied from preconceptual to 18 weeks (median of 10 weeks gestation, Table 3). 85%

of participants returned completed aspirin diaries. There was no difference in the number of omitted doses reported (mode = 0, range 0-6) for those with detectable aspirin metabolites and those where no metabolites were detected.

Agreement between platelet function tests

There was evidence of good agreement between the two whole blood point-of-care tests, VerifyNowTM and Multiplate of 81 and 83% at the first and third trimester study visits, respectively. However, agreement between these tests and urinary 11-dehdrothromboxane B2 was more limited. OPA at the first visit was 74% and 76% with VerifyNowTM and MultiplateTM, respectively, and 72 and 78% at the third trimester visit.

Response to aspirin

For 156 participants sampled longitudinally, a large proportion of platelet function assay results fell outside the range expected for aspirin-treated individuals. For VerifyNow^{TM} Aspirin test 28% results were above the manufacturer's threshold. For Multiplate^{TM}, 29% of results fell within the pregnancy-specific reference range at the first visit and 30% at the third trimester visit. For TXB Cardio^{TM} the percentage of results within the aspirin naive reference range fell from 36% at the first to 26% at the third trimester visit.

Overall, a higher proportion of participants were found to be aspirin responsive at the third trimester visit (68% at the first, 76.4% at the third trimester visit). When examining longitudinal response to aspirin, the most striking finding was that no participants were consistently aspirin non-responsive (Figure 1). Of eleven participants with consistent indeterminate response to aspirin, only one had a placentally-mediated adverse outcome. Forty seven women had taken aspirin for less than a week prior to the first visit, of the remainder 68% (n=74) were aspirin responsive, 22% (n=24) were variable responders and 10% (n=11) had indeterminate response. Durations of aspirin treatment prior to the first visit were equivalent between groups; 1-16 weeks (median = 5 weeks) for responders, 1-13 weeks (median = 6 weeks) for variable responders and 1-18 (median = 5.5 weeks) for the indeterminate group.

Association with placentally-mediated adverse outcomes

There were no significant associations between aspirin response and clinical outcomes of pre-eclampsia or the specified composite of placentally-mediated adverse outcomes. (Table 5) This was true both when comparing aspirin responders to those with indeterminate response (P=0.948) or to those with inconsistent response (indeterminate responders plus variable responders, P=0.645).

DISCUSSION

When sampled at different time-points in pregnancy, women who appear non-responsive to aspirin can be identified (Figure 1). In contrast to others [5], we did not identify any woman who was consistently aspirin non-responsive. Most women who were initially non-responsive became responsive with continued dosing (Figure 1). Additionally, there were no significant associations between aspirin response and placentally-mediated adverse clinical outcomes.

There are several important facets to our findings. First, platelet function tests alone cannot be used to assess for responsiveness to aspirin. At each time-point a quarter to a third of test results fell within the aspirin naïve range. Even when platelet function and aspirin metabolite data are combined, due to aspirin's rapid clearance, it may not always be feasible to assign a definitive response. In the EARTH cohort this resulted in an 'indeterminate' response group. Work by our group to develop protocols to detect stable plasma and urinary aspirin metabolites has indicated that metabolites could be reliably detected in healthy volunteers with a dose to sampling interval is 8 hours or less *(unpublished)*. The definition of indeterminate response means this group may contain individuals with suboptimal adherence both in the short-term (as evidenced by lack of aspirin metabolites) and medium-term (as evidenced by lack of expected platelet response). Due to the limitations of methods to detect aspirin metabolites, it is also feasible that the indeterminate group could contain genuinely non-responsive individuals (insufficient platelet response and dose to sampling interval outside the limits for the tests). Indeterminate response could be addressed by strengthening of adherence data, either by guiding the timing of aspirin dosing, to ensure the dose-sampling intervals are appropriate for detection, or by re-assessing platelet function following directly observed dosing.

Second, the high-degree of variation in individual response to aspirin across time-points is likely to reflect issues with platelet exposure to aspirin. Accurate assignment of response to aspirin has further important components: i) confirmation of platelet exposure to aspirin (adherence) and ii) assessment of platelet response to aspirin measured by platelet function. Variable response is most likely to stem from imperfect aspirin use during follow-up, but also calls into question the issue of aspirin dosing in pregnancy.

As EARTH was an observational study there is a possibility of confounding, both by recognised and unrecognised factors. Several characteristics, including BMI, diabetes and smoking have been reported by non-obstetric observational studies to be associated with aspirin non-responsiveness. However, there is a paucity of obstetric data and a lack of robust assessments of aspirin adherence in studies to date [5]. The EARTH study should be considered a preliminary investigation of aspirin non-responsiveness and we have conducted

an exploratory analysis to assess for associations with key clinical outcomes. Verification of our negative findings in a large randomised cohort would be desirable, within which there may be scope to investigate models to predict platelet and clinical response to aspirin. Whilst it remains possible that individuals who are genuinely aspirin non-responsive may be identified in larger cohorts, the lack of any cases in our cohort suggests that numbers expected may be of insufficient magnitude for aspirin non-responsiveness to be clinically significant at population level.

The significance of optimising aspirin dosing has recently been highlighted by the ASPRE trial [3]. In addition to focussing on dose, the trial protocol accounted for evidence of superior risk reduction in pre-eclampsia with aspirin commenced prior to 16 week's gestation and with doses scheduled 8 hours after wakening [19]. Participants underwent assessments of aspirin adherence with pill counts and interviews. 80% were designated as having 'good adherence' (≥85% of doses taken) [3]. EARTH included women prescribed and taking aspirin in accordance with their clinical care, reflective of current practice in our tertiary centre. However, it is feasible that gestational age at the onset of aspirin, duration of reliable treatment and dose schedule could influence measurable response to aspirin and should be controlled for in future studies.

Despite recent promising findings regarding aspirin dosing, there is currently a paucity of knowledge of pharmacokinetics and pharmacodynamics of aspirin, specific to pregnancy. In light of the ASPRE trial [3], there should be further opportunities to assess aspirin pharmacokinetics, pharmacodynamics and forgiveness and to strengthen short and long-term safety data for different doses. Whilst pharmacokinetic data are likely to be of use in determining the optimal safe and effective aspirin dose, evaluation of forgiveness can be utilised to define minimal adherence to ensure the intended pharmacodynamic and clinical effects and to optimise the use of aspirin in high-risk pregnancies.

Acknowledgements

We are grateful for the support of Wellbeing of Women who provided a Research Training Fellowship for Dr Kate Navaratnam (RTF 403).

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Figures/tables caption list

Table 1. Eligibility criteria based on NICE guidelines [1]. Women were eligible for inclusion with a single high-risk factor or ≥2 moderate risk factors.

High risk factors	Moderate risk factors
Previous hypertensive disease in pregnancy	First pregnancy
Chronic hypertension	Maternal age >40 years
Pre-existing type 1 or 2 diabetes	BMI >35
Chronic renal disease	Pregnancy interval >10 years
Autoimmune disease	Family history of pre-eclampsia
e.g. Systemic Lupus Erythematosus, Antiphospholipid Syndrome	(first-degree relatives)

Table 2. Definitions of aspirin response.

Responsive	• Two or all three platelet function test results outside of normal range indicating • Above results seen at both visits. • Aspirin metabolites may or may not be present.
Non-responsive	• None or only one platelet function test (false positive) outside of normal range indicating lack of aspirin effect • Above results seen at both visits. • Detectable aspirin metabolites must be present.
Indeterminate response	• None or only one platelet function test (false positive) outside of normal range indicating lack of aspirin effect • Above results seen at both visits. • No detectable aspirin metabolites seen at both visits
Variable response	• The classification of aspirin response changed between two visits in the same participant.

Table 3: Demographic characteristics, antenatal and intrapartum care for EARTH participants completing study follow-up (n=156).
*HDU=high dependency unit, ITU=intensive therapy unit.

	Aspirin responder (n=92)	**Indeterminate response (n=11)**	**Variable response (n=53)**
Maternal demographic data			
Mean maternal age (SD)	31.4 (5.3)	29.6 (5.3)	31.3 (5.3)
Mean body mass index (SD)	28.3 (6.6)	29.1 (5.9)	30.9 (7.4)
Nulliparous	27.2% (n=25)	9.1% (n=1)	13.2% (n=7)
Smokers	5.4% (n=5)	9.1% (n=1)	17.0% (n=9)
Ethnicity Caucasian Afro Caribbean Asian Mixed Other Chinese	 82.6% (n=76) 4.3% (n=4) 4.3% (n=4) 4.3% (n=4) 1.1% (n=1) 3.3% (n=3)	 72.7% (n=8) 18.2% (n=2) 0.0% (n=0) 9.1% (n=1) 0.0% (n=0) 0.0% (n=0)	 83.0% (n=44) 9.4% (n=5) 5.7% (n=3) 1.9% (n=1) 0.0% (n=0) 0.0% (n=0)
Medical co-morbidities Chronic kidney disease Diabetes Hypertension	 12.0% (n=11) 9.8% (n=9) 28.3 (n=26)	 0.0% (n=0) 18.2% (n=2) 27.3% (n=3)	 5.7% (n=3) 7.6% (n=4) 30.2% (n=16)
Antenatal care			
Median gestational age of commencing LDA (range)	10 (0-16)	10.5 (2-18)	10 (3-17)
Antihypertensive treatment	32.6% (n=30)	9.1% (n=1)	32.1% (n=17)
Admissions due to pre-eclampsia, gestational hypertension, IUGR	14.1% (n=13)	0.0% (n=0)	17.0% (n=9)
Median length of admission (range)	2.0 (1.0-6.0)	0.0 (0.0-0.0)	2.0 (1.0-13.0)
Intrapartum care			
Admissions to HDU/ITU	4.3% (n=4)	0.0% (n=0)	11.3% (n=6)
Median length of HDU/ITU admission (range)	1.0 (1.0-1.0)	0.0 (0.0-0.0)	1.0 (1.0-2.0)
Acute antihypertensive treatment	12.0% (n=11)	0.0% (n=0)	18.9% (n=10)
Magnesium sulphate treatment	3.3% (n=3)	0.0% (n=0)	9.4% (n=5)
Spontaneous labour	13.0% (n=12)	18.2% (n=2)	15.1% (n=8)
Induction of labour	51.1% (n=47)	81.8% (n=9)	50.9% (n=27)
Delivery outcomes			
Spontaneous preterm birth <37 weeks	3.3% (n=3)	0% (n=0)	9.4% (n=5)
Caesarean section	51.1% (n=47)	18.2% (n=2)	39.6% (n=21)

Table 3: Demographic characteristics, antenatal and intrapartum care for EARTH participants completing study follow-up (n=156).
*IUGR=intrauterine fetal growth restriction, HDU=high dependency unit, ITU=intensive therapy unit.

Table 4. Participants delivered prior to completion of follow-up visits.

Case	Risk factors	Aspirin response	Gestation at birth	Clinical outcomes
1	Previous PE, family history of PE	Responder	$22+^{0}$	Placental abruption, maternal collapse, hysterotomy, fetal loss.
2	CH, chronic kidney disease, nulliparous	Responder	$25+^{0}$	IUFD, SGA
3	CH, nulliparous	Intermediate responder	$26+^{6}$	Spontaneous preterm birth, Poor neonatal weight gain
4	CH, chronic kidney disease, previous PE	Responder	$29+^{0}$	Spontaneous preterm birth, periventricular leukomalacia.
5	CH, previous PE, BMI>35	Responder	$31+^{1}$	Severe PE, EMCS, FGR, MVUP.
6	CH, previous PE	Responder	$31+^{4}$	Severe PE, EMCS, SGA
7	CH nulliparous, family history of PE	Non-responder	$32+^{1}$	Severe PE, EMCS, SGA
8	Previous PE, BMI>35	Non-responder	$32+^{2}$	Spontaneous preterm birth, EMCS
9	CH, previous PE	Responder	$32+^{4}$	Spontaneous preterm birth, EMCS
10	Diabetes, nulliparous, BMI>35	Responder	$33+^{4}$	Moderate PE, EMCS, HIE grade III, MVUP.
11	Diabetes, nulliparous, BMI>35	Responder	$33+^{6}$	Moderate PE, EMCS
12	Previous PE	Non-responder	$34+^{3}$	Fetal distress, fetal duodenal atresia, EMCS
13	Chronic kidney disease, nulliparous	Responder	$35+^{4}$	Severe PE, Labour induction, SGA

CH=chronic hypertension, PE=preeclampsia, FGR=fetal growth restriction, IUFD=intrauterine fetal death, SGA=small for gestational age, EMCS=emergency caesarean section, MVUP=placental histology showing maternal vascular under perfusion, HIE=hypoxic ischaemic encephalopathy.

Table 5. Maternal, fetal and neonatal outcomes.

	Aspirin responder (n=92)	Indeterminate response (n=11)	Variable response (n=53)
MATERNAL CLINICAL OUTCOMES			
Pre-eclampsia	15 (16%)	1	8 (15%)
≥37 weeks with AGA	8 (9%)	1	4 (7.5%)
≥37 weeks with SGA 5-9th customised centile	1	0	0
≥37 weeks with SGA <5th customised centile	1	0	1
<37 weeks with AGA	2	0	1
<37 weeks with SGA 5-9th customised centile	0	0	0
<37 weeks with SGA <5TH customised centile	1	0	1
<37 weeks with IUGR	1	0	1
Gestational hypertension	5 (5%)	0	4 (7.5%)
Placental abruption	1	0	0
FETAL AND NEONATAL OUTCOMES			
IUFD	1	0	1
Isolated FGR	1	1	2
Isolated SGA	11 (12%)	1	6 (11%)
<5th customised centile	4 (4%)	0	1
5-9th customised centile	7 (8%)	1	5 (9%)
Admissions to NICU	6 (6.5%)	2	10 (19%)
Median length of NICU admission (range)	1.5 (1.0-11.0)	30.0 (1.0-59.0)	3.0 (1.0-11.0)
COMPOSITE PLACENTALLY MEDIATED ADVERSE OUTCOME	16 (17%)	2 (18%)	11 (21%)

**AGA=appropriate for gestational age, SGA=small for gestational age, IUFD=intrauterine fetal death, FGR=fetal growth restriction, NICU=neonatal intensive care unit.*

Figure 1. EARTH study flow diagram.

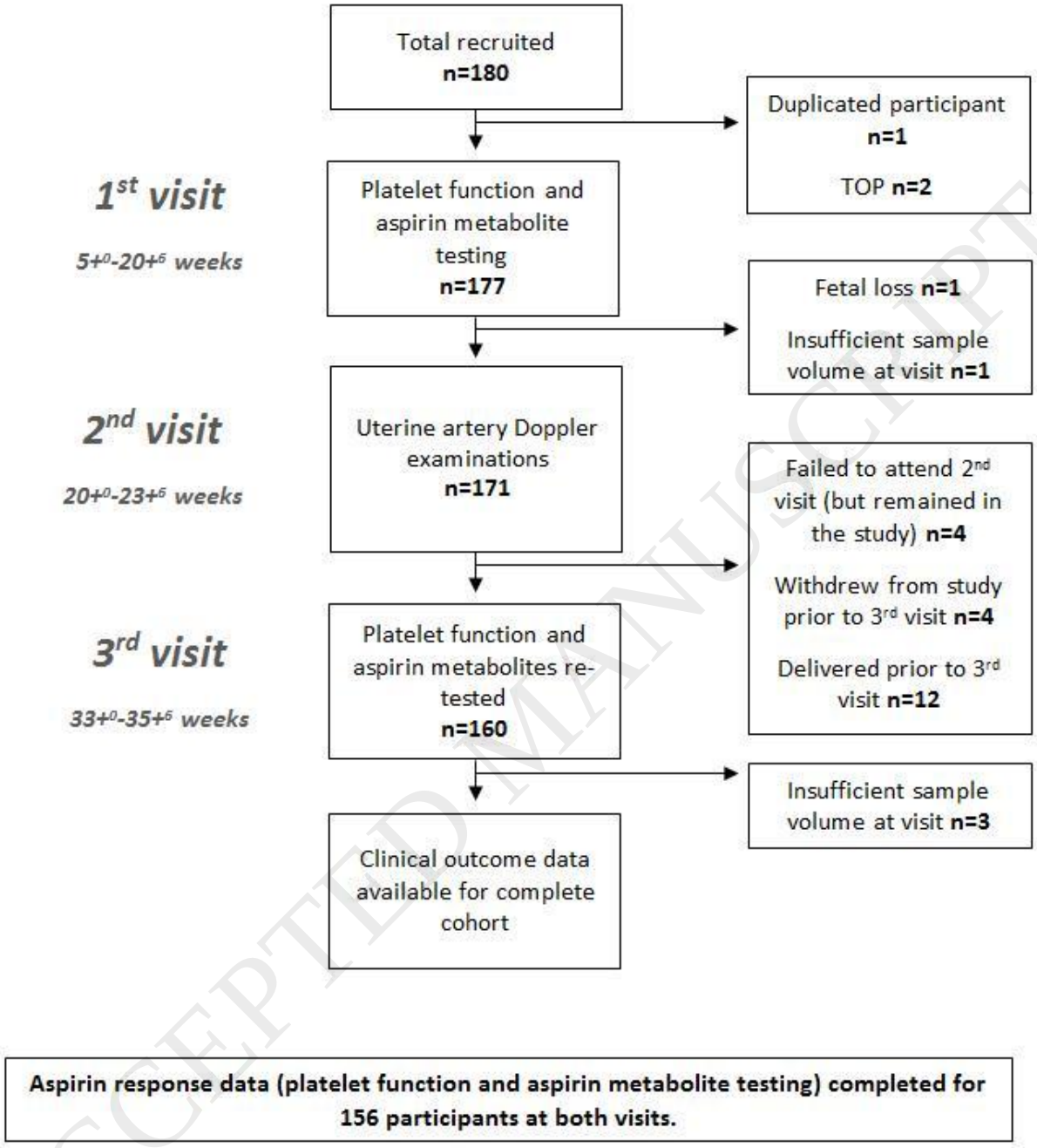

Figure 2. Participants' Longitudinal response to aspirin during the EARTH study.