Title: Aspirin non-response in pregnant women at increased risk of pre-eclampsia.

Short Title: ASPIRE

Authors:

MRS RAYA VINOGRADOV, MClinRes, Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcastle upon Tyne, UK

MRS CLARE BOAG, MSc, Department of Nursing Midwifery and Health, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK

MR PAUL MURPHY, IBMS, Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcastle upon Tyne, UK

MR DAVID MCGEENEY, MMathStats, School of Mathematics, Statistics & Physics, Newcastle University, UK

DR VIJAY KUNADIAN, MD, Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcastle upon Tyne, UK and Translational and Clinical Research Institute, Newcastle University, UK

PROF STEPHEN C ROBSON, MD, Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcastle upon Tyne, UK and Population Health Sciences Institute, Newcastle University, UK

Authors Agreement

All authors have seen and approved the final version of the manuscript. This article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Disclosure of Interest:

All authors declare no interests that may have influenced the submitted work. EliTech has provided VerifyNow consumables for 100 patients. EliTech has not been involved in the design, conduct, management of the study, analysis or interpretation of the data, or the preparation, review or approval of this manuscript.

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Corresponding Author: Raya Vinogradov, The Reproductive Health Research Team Level 6, Leazes Wing, The Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK Raya.vinogradov@newcastle.ac.uk

Highlights

- Non-response to low dose aspirin, as defined by serum Trhomboxane$_2$, is common in high-risk women
- Adherence is a key factor in response

Tweetable abstract

Low dose aspirin is not always effective in preventing preeclampsia. We aimed to determine the prevalence of suboptimal response to 75 mg of aspirin and ascertain the effect of increasing the dose in non-responders.

166 women at risk of pre-eclampsia were asked to donate blood and urine at 18-24- and 32-36-weeks gestation. Aspirin dose was increased to 150 mg in participants with suboptimal response as assessed by bedside VerifyNow test.

The study showed that prevalence of non-response to 75 mg of aspirin was high and can be explained by non-adherence. VerifyNow test demonstrated moderate performance. Dose change could be useful to improve response to aspirin.

Contribution to authorship

SR, VK designed the study. CB, RV conducted the trial, P.M. were responsible for the TXB2 assays, RV, DM analysed and interpreted data. All authors contributed to the manuscript.

Acronyms

ARU – Aspirin Reactive Units, ASPI – a reagent used in the Multiplate test consist of a lyophilized preparation of arachidonic acid, LDA – low dose aspirin, LTA – Light Transmittance Aggregometry, NHS – National Health Services, NICE – National Institute for Health and Care Excellence, PE- preeclampsia, PFA- Platelet Function Analyser, PI – Pulsatory Index, ROC- Receiver Operating Curve, TXB$_2$- Thromboxane B$_2$.

Abstract

Objectives

Low dose aspirin (LDA) is recommended for women at increased risk of preeclampsia (PE), however it is not always effective. The study sought to determine the prevalence of non-response to LDA and to ascertain the effect of increasing aspirin dose in non-responders.

Study Design

Single centre, cohort study of 166 women at increased risk of PE was conducted in a large maternity unit in the UK between 2013 and 2016. All women were prescribed 75 mg of aspirin and invited to attend study visits at 18-24 weeks' and 32-36 weeks' gestation. Non-response was defined as a serum thromboxane B2 (TXB_2) ≤10 ng/mL. Aspirin dose was increased to 150 mg if a bedside VerifyNow test suggested non-response (test value ≥ 550 arachidonic acid reactive units [ARU]) at 18-24 weeks. Adherence was assessed by self-report.

Results

Based on serum TXB_2, response rates were 85.3% at 18-24 weeks and 79.3% at 32-36 weeks' gestation. Compared to serum TXB_2, the VerifyNow test demonstrated moderate test performance (AUC 0.79 95% CI 0.71-0.88, $p<0.0001$) to detect non-response. High prevalence of non-adherence (6/10) was evident in persistent non-response group. Dose change from 75 to 150 mg of aspirin in adherent participants improved response (VerifyNow: 598 [95% CI 550 – 665] ARU at 18-24 weeks on 75 mg aspirin, 509 [95% CI 350-667] at 32-36 weeks on 150 mg of aspirin, [$p<0.0001$]).

Conclusions

Non-response to LDA is common in pregnancy but appears to be largely attributable to non-adherence. Dose change could be useful to improve response to LDA in this cohort.

Key Words: aspirin, pre-eclampsia, aspirin response, platelet response, aspirin resistance, adherence.

1 Introduction

Pre-eclampsia (PE) affects 2-8% of pregnancies and is one of the leading causes of maternal death (1). PE also increases women's and children's risk of developing cardiovascular disease (2, 3).

Low-dose aspirin (LDA), up to 150 mg, prevents PE but it is not always effective (4). Following recent publication of the ASPRE trial (5), the UK guidance has been updated and recommends women at increased risk of PE receive 75-150 mg of Aspirin daily from 12 weeks' gestation (6).

Aspirin inhibits release of cyclooxygenase and subsequently impedes thromboxane A_2 synthesis in platelets (7). Aspirin's effect on platelets can be measured by several functional and biochemical tests (8). Near complete (>95%) inhibition of thromboxane B_2 (TXB_2) is necessary for effective antiplatelet response to aspirin (9). Individuals who adhere to aspirin treatment but fail to achieve an effective platelet response are often classified as non-responders. Depending on the test and cut-off used, 5-65% of non-obstetric patients are reported to be non-responsive to 75-80 mg aspirin (10-12).

Less is known about the prevalence and implications of aspirin non-response in pregnant women; studies report rates of 7% to 39% (13-15). Given the frequency of aspirin prophylaxis, further studies are required to investigate aspirin response in women at increased risk of PE.

The study aimed to determine the prevalence of non-response to 75 mg aspirin, as assessed by serum TXB_2 and a bedside platelet function test (VerifyNow), to assess agreement between the tests, and to ascertain the effect of increasing aspirin dose in non-responders.

2 Methods

NHS Research Ethics Committee (13/NE/0106) approval was obtained. All women attending large maternity unit in the UK between August 2013 and March 2016, identified at increased risk of PE based on the presence of a major risk factor in their medical history (16) and prescribed 75 mg of aspirin, were invited to participate. During two study visits at 18-24 and 32-36 weeks gestation participants were asked to provide 12 mL of blood and a urine sample.

2.1 Tools

(a) VerifyNow (Accriva Diagnostics©, USA) aspirin assay was used to assess inhibition of thromboxane in citrated whole blood. VerifyNow is a bedside optical detection system which measures platelet induced aggregation as an increase in light transmittance. Test values of <550 ARU (arachidonic acid reactive units) were used to indicate inhibition of the COX-1 pathway by aspirin (17).

(b) Impedance aggregometry measured platelet activation in a sub-sample of participants using a supplementary Multiplate Analyser (Roche Diagnostics International LTD, Switzerland). Test values <40 U were used to indicate inhibition of the COX-1 pathway by aspirin (18).

(c) Thromboxane B_2 levels were measured in serum and urine using an Enzyme-Linked Immunosorbent Assay-ELISA (R&D systems Inc., USA) as the gold standard method to assess inhibition of the COX-1 pathway. Response was defined as a serum TXB2 ≤10 ng/ml (12, 19). Where VerifyNow result suggested non-response (i.e. ≥550 ARU) at 18-24 weeks, participants were advised to increase aspirin to 150 mg daily.

Aspirin adherence was assessed by self-reported questionnaire. Participants were asked about adherence to aspirin in the 7 days prior the study visit; those reporting taking ≥ 5 tablets of aspirin were deemed to be adherent (20, 21).

Demographic information, blood pressure and heart rate were recorded. Maternal height, weight, age, parity, uterine artery Doppler Pulsatility Index (PI), notching, and level of

adherence at each research visit were studied as predictors of non-response. Clinical outcomes were collected from medical records.

2.2 Statistical analysis

Data was analysed using SPSS v.24 for Windows (IBM, Chicago, IL). Agreement between tests was assessed using Cohen's Kappa and McNemar's test, a modification of the Chi-square test (22). Receiver Operating Curves (ROC) were used to investigate performance of VerifyNow and Multiplate assays. Predictors of non-response were explored using stepwise logistic regression (backward elimination). Response to aspirin dose change was analysed by Wilcoxon signed-rank test. A p-value of <0.05 was considered to be statistically significant.

3 Results

3.1.1 Population

Out of 252 women approached,, 178 given written consent to participate. Ten participants withdrew and two were excluded from the data analysis (Figure1). Due to sample loss and non-attendance serum TXB_2 results were only available for 150 participants at 18-24 weeks and 113 participants at 32-36 weeks (Figure 1). Participant's characteristics are described in Table S1.

3.1.2 Prevalence of non-response

The prevalence of non-response (serum TXB_2 >10 ng/ml) at 18-24 weeks was 14.7% (95% CI 9.4-21.3%). This was similar to the rate of 20.1% (95% CI 14.3-28.4%) at 32-36 weeks. There were no differences between responders and non-responders in gestational age at sampling, age, weight, height and BMI (Table S2, S3).

A larger proportion of participants were classified as non-responders using a VerifyNow ≥ 550 ARU compared to TXB_2 method at 18-24 weeks (25.3% 95% CI 18.2-34.2%, p=0.017) while there was no difference in non-response rates at 32-36 weeks (24.7% 95% CI 18.1-32.2%).

Based on longitudinal serum TBX_2, participants were classified into four response groups; persistent non-responders (10/124 [8.1%]), new non-responders (15/124 [12.1%]), improved responders (11/124 [8.9%]) and persistent responders (88/124 [70.9%]).

3.1.3 Adherence

Participants reported high levels of adherence to aspirin; at 18-24 weeks 90.9% participants reported taking ≥5 tablets a week compared to 88.5% at 32-36 weeks (p=0.48). There was a higher proportion of non-adherent women in the persistent non-response group compared to the persistent response group (6/10 versus 6/88, Fisher exact test p<0.001), and the new non-response group (6/10 versus 2/15, Fisher exact test p= 0.028).

3.1.4 Agreements between tests

3.1.4.1 VerifyNow and serum TXB_2

Results from both research visits were combined resulting in 289 paired TXB_2 and VerifyNow measurements (Table S4) of which 17.3% (95% CI 12.12-22.12%) had a serum TXB_2 ≥10 ng/mL. McNemar's test showed evidence of a logical difference between the proportion identifying non-response (p=0.001). Cohen's kappa showed only moderate agreement between tests (k=0.438, 95% CI 0.318-0.559). The sensitivity of the VerifyNow test for TXB_2 confirmed non-response was 70% (95%CI 55.4-82.1%) with a specificity of 82.8% (95% CI 77.5-87.4%), positive likelihood ratio (LR) of 4.08 (CI 95% 2.93-5.69) and negative LR of 0.36 (95%CI 0.24-0.56). The posterior probability of a correct result for non-response was 46% (95%CI 38-54%) while the probability of non-response when VerifyNow tested responsive was 7% (95% CI 5-10%). The area under the ROC curve for VerifyNow prediction of non-response indicated moderate test performance (0.797 95% CI 0.713-0.882, p<0.0001). The optimum threshold to detect a serum TXB_2 ≥10 ng/mL was 531 ARU (76% sensitivity, 78.2% specificity) (Figure S1). Using a lower TXB_2 threshold of < 3 ng/mL (indicating complete inhibition of platelet COX-1 (19)), VerifyNow demonstrated poor test performance (AUC

0.604 95% CI 0.539-0.668, p=0.003) with a sensitivity of 35.2% and specificity of 87.6% using the manufacturers cut-off (≥550 ARU) (figure S1).

3.1.4.2 Multiplate ASPI and serum TXB_2

In 123 paired Multiplate ASPI (Activation by Arachidonic acid) and serum TXB_2 measurements 18.7% (95% CI 12.24-26.72%) had a serum TXB_2 ≥10 ng/mL. Using a cut-off of ≥40 U, there was a difference in non-response rates between the tests (McNemar's test p=0.001). Overall agreement between the tests was poor (k=0.133, 95% CI 0.035-0.30). ROC analysis revealed an AUC of 0.667 (95% CI 0.537-0.797) with a sensitivity of 50% and specificity of 69.3%.

3.1.4.3 Urine and serum TXB_2

Analysis of 122 paired blood and urine samples demonstrated a weak positive correlation (r_s= 0.092, p=0.316 (Figure S2).

3.1.5 Predictors of non-response

VerifyNow and self-reported adherence were the only independent predictors of serum TXB_2≥10 ng/mL; for every unit increase in VerifyNow the OR for non-response was 1.017 (95% CI 1.01-1.024, p< 0.001) and for every tablet increase in weekly adherence the OR for non-response was 0.631 (95% CI 0.481-0.828, p=0.001).

3.1.6 Aspirin dose change

At 18-24 weeks 25.3% (42/166) had a VerifyNow ≥550 ARU and were advised to increase aspirin dose to 150 mg. Seven of these 42 participants disclosed incomplete adherence with >2 doses missed in the week preceding 18-24 week visit. At the 32-36 week follow-up visit, two participants were still taking 75 mg of aspirin, 7 reported missing >2 doses in the preceding week and one failed to attend. Thus paired data from 25 participants with a VerifyNow ≥550 ARU at 18-24 weeks and self-reported adherence with the increased dose were available for analysis; in this group VerifyNow reduced from 598 (95% CI 550-665) ARU at 20 weeks to

509 (95% CI 350-667) at 32-36 weeks (Wilcoxon signed-rank test one tailed, p<0.0001) with 10 (40%) participants having a persistent VerifyNow ≥550 ARU. Twenty of the 25 participants had paired TXB_2 results available for analysis; mean TXB_2 reduced from 18.94 ng/ml (95% CI 0.04-84.93) at 18-24 weeks to 14.1 ng/ml (95% CI 0-111), p= 0.032, with 4 (20%) persistent non-responders i.e. TXB_2 ≥10 ng/ml Ffigure S3).

3.1.7 Clinical outcomes

There were no differences in prevalence of clinical outcomes in the four response groups (Table S5).

4 Discussion

4.1.1 Main findings

Understanding response to LDA is important in reducing the incidence of PE and/or other adverse clinical outcomes. This study has shown that at least 15% of participants prescribed 75 mg aspirin were non-responsive to LDA as assessed by a failure to inhibit serum TXB_2. Although overall adherence was high; this was not the case in persistently non-responsive women. Despite the potential advantages of point-of-care testing for aspirin response, the VerifyNow test showed limited agreement with serum TXB_2 at the optimum threshold of 531 ARU. VerifyNow and self-reported adherence independently predicted non-response. Finally, the study provides some evidence that increasing the dose of aspirin to 150 mg in non-responsive women improves response.

Non-response is likely to contribute to aspirin failing to prevent PE in some high-risk women. The rates of non-response reported in the present study are lower than those reported by Navaratnam et al in the second and third trimesters (36% and 26% respectively) (15). This may reflect the different methods used to assess inhibition of the COX-1 pathway; Navaratnam et al measured urinary levels of 11-dehydrothromboxane B_2 (15). However, based on VerifyNow results, response rates were similar in the two studies (25% versus 28%) (15). In contrast, a

study of 71 high-risk pregnant women prescribed LDA reported no non-responsive participants using Platelet Function Testing (PFA100) amongst women adherent to aspirin therapy (23). Rates of aspirin non-response in obstetric populations are broadly consistent with the summary rate of 28% reported in a systematic review and meta-analysis of aspirin 'resistance' and risk of cardiovascular morbidity in non-pregnant populations (24); in this review 'resistance' was defined using a variety of tests (PFA-100, Light Transmittance Aggregometry (LTA), and bleeding time).

There is no agreement on the definition of drug adherence nor on the optimal method of assessment of adherence (25). Direct methods such as drug or metabolite levels or indirect methods such as questionnaires, diaries or pill count can be used (26). Although inexpensive, both questionnaires and pill count methods are not reliable relying on participants memory and honesty (26). No obstetric studies have measured aspirin metabolites as an objective measure of adherence nor established critical levels of adherence based on biochemical response. We defined adherence based on historical pill adherence in the week preceding biochemical testing based on studies of platelet recovery time after aspirin withdrawal (21). In contrast, in the ASPRE trial optimal adherence was defined as >85% of pills taken as determined by pill count and interviews (27), while in the CLASP trial optimal adherence was defined as adherence to the study treatment for at least 80% of the trial based on postal questionnaires (28). The value of direct (objective) testing was recently shown in the TEST study (29); while adherence based on patient-reported diary cards and tablet counts was 95-96%, 16% of women had an increase in urinary TXB_2 after starting aspirin and a further 12% had less than a 40% fall.

We found a high non-adherence rate in the group of women with persistent non-response. The reported levels of non-adherence are likely to be underestimated due to the use of self–reported methods of assessment (20, 30). Further adherence to a drug by motivated participants in a clinical trial is likely to be higher than in the general obstetric population; van Montfort et al

recently reported adherence to aspirin in a non-research setting was only 25% with some high-risk women not ever recalling any discussions regarding aspirin treatment (31). In the ASPRE trial, there was a relationship between aspirin adherence and PE; the OR for preterm PE when adherence was 90% or more (based on a pill count) was 0.24 (95% CI 0.09-0.65) compared to 0.37 (95% CI 0.17-0.82) when adherence was less than 90% of pills (32).

Reliable point-of-care testing could allow identification of non-responsive women during their antenatal visit, allowing clinicians to communicate and act on test results. The potential of point-of-care testing to improve medication compliance, as well as health and economic outcomes, is recognised (33); women may respond to unsatisfactory results and clinicians can use test results as an effective behaviour change technique (34). We focused on VerifyNow because it is easy to use and, compared to other point-of-care tests, has the highest reproducibility and closest correlation with TXB_2 inhibition (35). However, we unable to confirm the high sensitivity (100%) and specificity (91.4%) reported by the manufacturer in non-obstetric populations using a cut-off of ≥550 ARU (17, 36). Our results confirm the poor correlation (r=0.32) between the two tests reported by Nielsen et al (37) in a non-obstetric population. The Multiplate assay is known to have poorer reproducibility than light LTA methods (38) and studies comparing VerifyNow and Multiplate assays in non-pregnant populations have shown very variable agreement (39-41). In this study we found the performance of the VerifyNow test to be superior to Multiplate analyser in detection of suboptimal aspirin response.

New National Institute for Health and Care Excellence (NICE) guidance (6) recommends aspirin at a dose of 75-150 mg, leaving it up to clinicians to decide what dose should be used. Although there is evidence to support the effectiveness of 150 mg of aspirin (27), there are no large trials comparing the effectiveness and acceptability of 150 mg versus 75 mg of aspirin. A higher dose of aspirin may increase maternal concern about fetal effects, especially given

recent evidence that aspirin doubled the rate of vaginal bleeding (15.1% versus 7.9%, OR 2.1 [95% CI 1.2-3.6] (29). Dose increase in non-responsive women merits consideration, given the increase in plasma volume and platelet turnover during pregnancy (42). While conclusions are limited, we provide evidence that increasing the dose of aspirin to 150 mg in non-responsive women improves platelet response in some.

4.2 Limitations

Biochemical outcomes were not available for all women and it is possible that adherence and non-response rates might be higher in those who failed to attend for study follow-up visits. The serum TBX level we used to define non-response was based on non-pregnant data (11, 19) and consensus (43, 44) and there is no evidence supporting the clinical impact of this cut-off in pregnant women. Further, we used a simple self-reported method to determine short-term adherence prior to laboratory testing. The relationship between adherence and platelet response in pregnant women needs further study to identify relevant cut-offs. Finally, the study was not large enough to assess clinical outcomes of key significance.

5 Conclusions

Based on serum TXB_2, this study has shown that non-response to LDA is common in high-risk women and adherence is a key factor in response. Although dose change regimens based on point-of-care testing may increase response rates, the present results do not support this strategy given the important contribution of non-adherence to non-response and the poor reliability of the point-of-care tests. Further research is required to assess the pharmacokinetics of different aspirin dose regimens in high-risk obstetric women to establish an objective test to assess non-adherence.

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7 List of tables and figures supplied

Table S1: Participants characteristics at the study entrance

Table S2: Demographics and clinical outcomes incidence of non-response (as defined by serum thromboxane below threshold) at 18- 24 wk.

Table S3: Demographic details and clinical outcomes incidence of non-response (as defined by serum thromboxane below threshold) at 32- 36 wk. groups

Table S4: Frequencies of response using VerifyNow and serum TXB_2

Table S5: Clinical outcome by response to aspirin

Figure 1: Study recruitment diagram

Figure S1: Receiver operating characteristic curve for performance of VerifyNow test to detect non-response to aspirin using two thresholds.

Figure S2 - Correlations between Urinary and Serum TXB_2

Figure S3- Change in levels of serum TXB_2 and VerifyNow from 18-24 to 32-36 wk with increase in aspirin dose.