RESEARCH ARTICLE

No effect of tranexamic acid on platelet function and thrombin generation (ETAPlaT) in postpartum haemorrhage: a randomised placebo-controlled trial [version 1; peer review: 2 approved]

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v1 **First published:** 05 Feb 2019, **4**:21 (https://doi.org/10.12688/wellcomeopenres.14977.1)
Latest published: 05 Feb 2019, **4**:21 (https://doi.org/10.12688/wellcomeopenres.14977.1)

Abstract

Background: Postpartum hemorrhage (PPH) is a leading cause of maternal mortality and morbidity. The WOMAN trial showed that tranexamic acid (TXA) reduces death due to bleeding in women with PPH. To determine whether TXA has pro-thrombotic effects in women with PPH, we measured endogenous thrombin potential (ETP), coagulation factors V, VIII, von Willebrand (vW), fibrinogen, D-Dimers and platelet function.
Methods: We conducted a sub-study within the WOMAN trial, an international randomized, parallel-group, double blind, placebo-controlled trial. Women with primary PPH were randomly allocated to receive 1 gram of tranexamic acid or matching placebo. Baseline blood samples were collected just prior to the first dose and a follow up sample was collected 30±15 minutes afterwards. We compared before and after changes in coagulation parameters between treatment groups using repeated measurement ANOVA. Change in ETP was the primary outcome. We did an intention-to-treat analysis using ANCOVA with adjustment for baseline and the time interval between the blood samples.
Findings: A total of 187 patients were randomized to receive TXA (n=93) or matching placebo (n=94). Six patients were excluded due to incomplete data. The reduction in ETP from baseline to follow up was 43.2 nM*min (95%CI, -16.6 to 103.1) in the TXA group and 4.6 nM*min (95%CI, -51.4 to 60.6) in the placebo group. The difference was not statistically significant (95%CI, -42.9 to 120). There were no significant effects of TXA treatment on any other parameters (ADPtest, TRAPtest, coagulation factors activity, fibrinogen levels, D-Dimer level).
Conclusion: We found no evidence that tranexamic acid treatment for PPH

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 1 published 05 Feb 2019	✓ report	✓ report

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has substantial pro-coagulant effects. However, larger studies are needed to confirm or refute more modest effects.
Trial registration: ISRCTN76912190 (initially registered 10/12/2008, WOMAN-ETAPlat included on 28/10/2013) and NCT00872469 (initially registered 31/03/2009, WOMAN-ETAPlat included on 28/10/2013).

Keywords
Tranexamic Acid, Postpartum Haemorrhage, Thrombin Generation, Platelet Function

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Competing interests: No competing interests were disclosed.

Grant information: The WOMAN Trial was funded by the Department of Health (UK), grant number HICF-T2-0510-007, the Wellcome Trust, grant number WT094947, the Bill & Melinda Gates Foundation (grant number OPP1095618), and LSHTM (London, UK). An educational grant was given by Erasmus Mundus program ERAWEB [D2.12.048] and Rudolf Marx Foundation (Munich, Germany).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Dallaku K, Shakur-Still H, Beaumont D *et al.* **No effect of tranexamic acid on platelet function and thrombin generation (ETAPlaT) in postpartum haemorrhage: a randomised placebo-controlled trial [version 1; peer review: 2 approved]** Wellcome Open Research 2019, **4**:21 (https://doi.org/10.12688/wellcomeopenres.14977.1)

First published: 05 Feb 2019, **4**:21 (https://doi.org/10.12688/wellcomeopenres.14977.1)

Background

Postpartum haemorrhage (PPH) is a leading cause of maternal mortality world-wide and the incidence appears to be increasing[1]. Most deaths are in low-and-middle income countries. Immediate and appropriate management of PPH is essential to reduce mortality and morbidity. The antifibrinolytic tranexamic acid (TXA) reduces bleeding by inhibiting the enzymatic breakdown of fibrin blood clots. Plasminogen is converted into the fibrinolytic enzyme plasmin by tissue plasminogen activator. TXA is a synthetic lysine analogue which blocks the lysine binding sites on plasminogen, as a result inhibits binding of plasminogen or plasmin with fibrin and thereby inhibiting fibrin degradation. It has the half-life about two hours and is excreted mostly by the kidneys[2].

The CRASH 2 trial[3] showed that TXA significantly reduces death due to bleeding in trauma patients without any increase in thromboembolic events when given within 3 hours of injury. More recently, the WOMAN trial[4], showed that TXA significantly reduces death due to bleeding in women with PPH. Once again there was no evidence of any increase in thromboembolic events. On the basis of these results, TXA is recommended for the treatment of PPH and should be administered as soon as possible after onset of bleeding and within 3 hours of birth[5].

Although plasmin increases fibrin clot breakdown, it may also have effects on coagulation and platelets. Plasmin activates coagulation factors V and VIII[6,7] and increases thrombin generation[8]. Plasmin stimulates platelet aggregation, degranulation, complement activation and platelet activation[6,9,10]. If these effects are mediated via lysine binding sites then it is possible that they might be affected by TXA administration. We conducted a sub-study within the WOMAN trial to investigate the effects of TXA on coagulation and platelets.

Objective

To assess the effects of TXA treatment on endogenous thrombin potential (ETP) and platelet function. If plasmin activation increases ETP and stimulates platelet activation, we would expect TXA to reduce ETP and inhibit platelets.

Methods and study design

The full ETAPlaT protocol is available from 11.

Study design and participants

We conducted a sub-study within the WOMAN trial, an international randomized, parallel-group, double blinded, placebo-controlled trial. The study included adult women with primary PPH. The PPH diagnosis was based on the visual estimation of blood loss (>500 mL after vaginal birth or ≥1,000 mL after caesarean birth or blood loss sufficient to cause hemodynamic instability). In addition to the usual treatment for PPH, women were randomized in the study as soon as possible after informed consent had been obtained. The main criterion for eligibility was the uncertainty of clinician to use or not use TXA in a particular woman diagnosed with PPH. The study was carried out according to the guidelines of good clinical practice[12] and adhered to the regulatory requirements for Albania.

Ethical approvals for the study were obtained from the London School of Hygiene and Tropical Medicine (LSHTM) and the National Ethics Committee in Tirana, Albania. Brief information about the study was given to pregnant women. All eligible women underwent the informed consent procedure for the WOMAN trial as well as for the ETAPlat sub-study before randomization. The detailed consent procedure is reported at the ETAPlaT protocol[11]. The ETAPlaT study was carried out at the Obstetric Gynaecology University Hospital "Koço Gliozheni" in Tirana, Albania. There are approximately 4500 deliveries per year in this hospital, which offers tertiary level health care and is a referral centre for other maternity hospitals at the country.

Randomization and blinding

Women with PPH, who fulfilled the eligibility criteria and completed the consent procedures, were randomized in the study and were allocated to receive either TXA or placebo. ETAPlaT as a sub-study of WOMAN trial utilised the same randomization and blinding procedures. In summary, the trial treatment packs were identical, so both patients and healthcare workers were blinded to treatment allocation. Each box contained eight individual treatment packs, each pack contained two doses of study drugs (one dose contained: 2 vials each TXA 500mg-5 mL, or 2 vials each 5mL sodium chloride 0.9%). The packs were used in sequential order by the caregiver starting from the lowest numbered pack.

Interventions and laboratory procedures

As soon as the patient was randomized in the study, alongside with the usual treatment for PPH, the trial treatment was administered by slow intravenous injection, 1mL/minute, of 1 gram TXA or placebo. A second dose of study drugs was administered if haemorrhage did not stop after 30 minutes or restart within 24 hours of the first dose.

Baseline blood sample was collected immediately after randomization and before the first dose was administered. Three mL of venous blood was collected in hirudine (25μgr/mL) test tube - double wall (Dynabyte, Munich, Germany) for multiple electrode aggregometry (MEA) and 5 mL in tri-sodium citrate 0.106 mol/l^{-1} (S-Monovette, Sarstedt, Germany) for coagulation tests. The same procedure for blood collection was performed at 30±15 minutes after the first dose study drug administration. Follow-up blood collection procedure was performed always before administration of the second dose of study treatment, if it was needed.

Baseline and follow-up samples were analysed for platelet function (ADPtest and TRAPtest) performing MEA with Multiplate. Details of methods used for ADPtest have been previously reported[13] and for TRAPtest[14]. All material used forTRAPtest and ADPtest including Multiplate equipment, were obtained from the manufacturer (Dynabyte GmbH, Munich, Germany). The recorded platelet aggregation measured by MEA was expressed as area under curve (AUC) AU*min. The platelet function analysis using MEA was performed by the laboratory at Hospital "KoçoGliozheni" in Tirana, Albania.

The blood samples obtained in 5 mL in sodium citrate test tubes for coagulation analysis were immediately centrifuged at 3000xg for 20 min. The acquired platelet poor plasma was divided in two aliquots and preserved in deep freeze (-80°C)

until the laboratory analysis at the end of the study. The coagulation tests were performed at the Institute of Laboratory Medicine, German Heart Centre in Munich, Germany. The thrombin generation assay (TGA) was performed with Calibrated Automated Thrombogram (Stago Deutschland GmbH). The coagulation factors V, VIII, von Willebrand, Fibrinogen (Claus method) and D-Dimer were analyzed with SIEMENS BCS XP Coagulation Analyzer, using reagents FV and FVIII deficient plasma, Multifibren*U fibrinogen reagent, BC von Willebrand reagent and INNOVANCE® D-Dimer reagent, all reagents were obtained from Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany.

Outcomes

The primary outcome was the change (baseline versus follow-up) in ETP. Secondary outcomes included the change (baseline versus follow-up) in platelet function (ADPtest and TRAPtest) and coagulation factors V, VIII, von Willebrand, Fibrinogen, D-Dimer on baseline and follow-up blood samples. Thrombin generation was chosen as the primary outcome because it is a surrogate for coagulation activity[15] and factors that increase thrombin formation can potentially increase thrombotic risk[16].

Statistical analysis

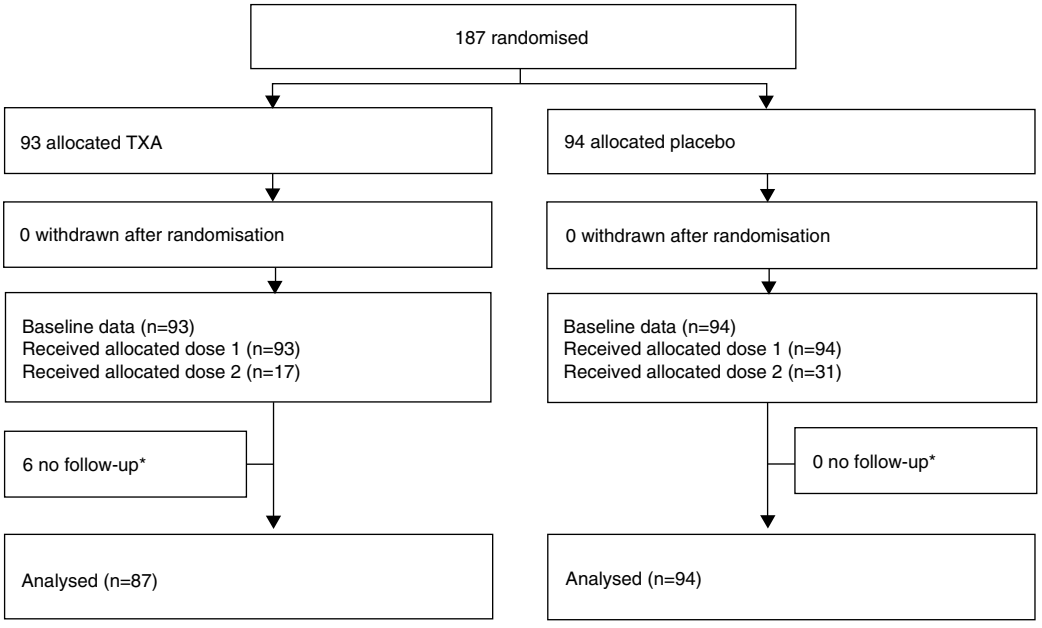
The statistical analysis plan to the ETAPlat Study was published and reviewed before database lock[17]. The study evaluates the effect of TXA compared to placebo by quantifying the change over time (baseline minus follow-up) in the primary outcome ETP and a series of secondary outcomes. The study therefore compares the changes between baseline and follow up in the TXA and placebo groups (the difference in the differences).

The sample size calculation was based on the following assumptions: ETP is normally distributed with a mean of 2410 nM*min and standard deviation (SD) of 543 nM*min[18]. We assume a decrease in ETP of 10% (241 nM*min) in the TXA group and no change in the placebo group. The calculation of the difference's standard error is based on a correlation of 0.6 between two time points and uses the standard deviation reported by McLean[18]. To detect an ETP difference in differences of 241 nM*min between groups at a 5% significance level with a power of 90%, two groups each with 88 patients are needed.

We compared before and after changes in coagulation parameters between treatment groups using repeated measurement ANOVA. An intention-to-treat analysis was performed by using analysis of covariance (ANCOVA) with adjustment of baseline measurement as well as adjustment of the length of time between two blood sample collection (30±15 minutes). The same analysis was carried out for secondary outcomes. Site monitoring, source data verification and trial master file review was carried out by the Sponsor and data management was performed by LSHTM using a bespoke electronic system.

Results

Recruitment to the ETAPlaT sub-study started on November 2013 and finished on January 2015, with final follow-up completed in March 2015. During this time 187 patients were randomized to receive TXA (n=93) or placebo (n=94). Of these 17 patients in TXA group and 31 in placebo group (Figure 1) received a second dose of TXA or placebo. We were unable to collect baseline or follow up blood samples as emergency situation was ongoing in six patients and these patients were excluded from the analyses.



* Patients for whom there is no information on the primary endpoint.

(2 patients had missing baseline and follow-up thrombin potential measurements, 3 patients with missing baseline thrombin potential, and 1 patient with missing follow-up thrombin potential)

Figure 1. WOMAN ETAPlaT trial Consort Flowchart.

The baseline data for TXA and placebo groups were similar (Table 1). One patient (TXA group) was known to have von Willebrand disease. The main cause of PPH in both groups was uterine atony. In the TXA group, the median (mean) time difference between both samples is 31.00 (32.98) minutes.

In the placebo group, the median (mean) time difference between both samples is 30.00 (31.74) minutes. In TXA group, the minimum (maximum) time difference between both samples is 20.00 (45.00) minutes. In placebo group, the minimum (maximum) time difference between both samples is 15.00 (63.00) minutes. There is no evidence that time differences differ between both treatment groups (Wilcoxon-Mann-Whitney-Test, p = 0.1336).

Primary outcome - results

The change in ETP (expressed in nM*min) between baseline and follow-up was 43.2 (95%CI, -16.6 to 103.1) in the TXA treated group and 4.6 (95%CI, -51.4 to 60.6) in the placebo group. The difference in differences (DiD) of 36.63 (TXA minus Placebo) was not statistically significant (p_{raw} =0.350, 95%CI_{raw}, -42.9 to 120.0). The detailed values are given in Table 2.

Table 1. Baseline characteristics.

		TXA group N=93	Placebo group N=94
Maternal Age - years	mean (SD)	27.8 (5.6)	27.0 (5.6)
Body Mass Index- kg/m^2	mean (SD)	28.5 (3.1)	29.9 (4.8)
Gestational age at birth–weeks	mean (SD)	38.3 (2.8)	38.2 (3.4)
FetalBirthweight – g.	mean (SD)	3185 (695.2)	3222 (843.8)
Hemoglobin - g/dL[+]	mean (SD)	10.8 (1.8)	11.4 (1.6)
Fibrinogen - g/L	mean (SD)	3.6 (1.1)	3.5 (1.2)
Platelet count -10^3/mm^3	mean (SD)	229.3 (80.4)	233.7 (80.1)
Blood lost mL	mean (SD)	863.2 (270.7)	893.1 (219.9)
Vaginal births Labor stages Mean (SD) [N]	- First stage(hrs) Second stage (min) Third stage (min)	6.7 (4.3) [71]* 40.5 (26.0) [67]* 10.9 (9.1) [66]*	7.8 (4.1) [68]* 48.5 (31.6) [66]* 13.5 (12.4) [65]*
Parity : N (%)	Nullipara Multipara	57 (61.3 %) 36 (38.7 %)	60 (63.8 %) 34 (36.2 %)
Type of delivery: N (%)	Vaginal Caesarean	65 (69.9%) 28 (30.1%)	65 (69.2%) 29 (30.8%)
Primary cause PPH: N (%)	Placenta previa Surgical trauma / tears Uterine atony Other Unknown	11 (11.8%) 10 (10.8%) 71 (76.3%) 1 (1.1%) 0 (0%)	14 (14.9%) 19 (20.2%) 59 (62.8%) 1 (1.1%) 1 (1.0%)
Preeclampsia: N (%)	Yes No	11 (11.8 %) 82 (88.2 %)	7 (7.4 %) 87 (92.6 %)
Chorioamnionitis: N (%)	Yes No	5 (5.4 %) 88 (94.6 %)	6 (6.4 %) 88 (93.6 %)
Placental abruption: N (%)	Yes No	9 (9.7 %) 84 (90.3 %)	5 (5.3 %) 89 (94.7 %)
Anaemia: N(%)	Yes No	34 (36.6 %) 59 (63.4 %)	23 (24.5 %) 71 (75.5 %)
Previous PPH: N (%)	Yes No	3 (3.2 %) 90 (96.8 %)	6 (6.4 %) 88 (93.6 %)
Hematologic disease N (%)	Yes No	1 (1.1 %) 92 (98.9 %)	0 (0%) 94 (100 %)
Treatment with antithrombotics N (%)	Yes No	0 (0%) 93 (100 %)	2 (2.1 %) 92 (97.9 %)

* Patients vaginal delivery;[+] p-value Wilcoxon-Mann-Whitney-Test: 0.01

Table 2. Effect of TXA on primary and secondary endpoints.

	Baseline Mean (SD)	Follow-Up Mean (SD)	Baseline/Follow-Up Mean Difference (95% CI)	DID: TXA / Placebo groups Mean DID (95%CI) p Value
ETP(nM*min)				
TXA (N=87)	1537 (375.9)	1494 (369.1)	43.2 [-16.6; 103.1]	36.63 [-42.9; 120.0] p_{raw} = 0.350*
Placebo (N=94)	1491 (378.7)	1487 (390.5)	4.6 [-51.4; 60.6]	29.81 [-47.8; 107.4] p_{adj} = 0.453**
ADPtest(AU*min)				
TXA (N=89)	1043.0 (343.6)	964.7 (312.4)	78.0 [15.4; 140.6]	13.2 (-65.8; 92.2) p_{raw}= 0.7*
Placebo (N=91)	961.6 (339.6)	896.8 (356.8)	64.8 (15.8; 113.7)	-0.9 (-72.7; 70.9) p_{adj} = 1.0**
TRAPtest(AU*min)				
TXA (N=89)	1199 (362.3)	1093 (300.3)	106.2 (38.5; 174.0)	20.9 (-65.3; 107.1) p_{raw}= 0.6*
Placebo (N=91)	1156 (347.9)	1070 (336.8)	85.3 (31.2; 139.4)	1.5 (-72.8; 75.7) p_{adj} = 1.0**
Factor V (%)				
TXA (N=88)	103.4 (27.7)	103.4 (25.7)	0.1 (-4.6; 4.8)	-4.2 (-10.0; 1.7) p_{raw}= 0.2*
Placebo (N=94)	100.2 (28.5)	95.9 (29.2)	4.3 (0.7; 7.8)	-4.9 (-10.4; 0.7) p_{adj} = 0.1**
Factor VIII (%)				
TXA (N=88)	221.9 (102.4)	216.1 (87.4)	5.9 (-9.5; 21.2)	5.9 (-13.9; 25.6) p_{raw}= 0.6*
Placebo (N=94)	195.2 (87.0)	195.2 (87.4)	0.0 (-12.7; 12.7)	-0.9 (-18.7; 17.6) p_{adj} = 1.0**
Factor vW (%)				
TXA (N=88)	219.4 (89.8)	222.2 (89.1)	-2.8 (-15.2; 9.7)	-0.1 (-16.9; 16.7) p_{raw}= 1.0*
Placebo (N=94)	212.6 (92.6)	215.3 (92.0)	-2.7 (-14.1; 8.8)	1.3 (-15.0; 17.5) p_{adj} = 0.9**
Fibrinogen (g/L)				
TXA (N=87)	3.64 (1.09)	3.59 (1.07)	0.05 (-0.1; 0.2)	-0.08 (-0.29; 0.12) P_{raw}= 0.4*
Placebo (N=93)	3.49 (1.2)	3.36 (1.1)	0.13 (-0.01; 0.27)	-0.09 (-0.28; 0.1) p_{adj} = 0.4**
D-Dimer (mg/L)				
TXA (N=88)	7.4 (9.3)	7.8 (10.2)	-0.4 (-1.1; 0.3)	0.9 (-1.3; 3.0) p_{raw}= 0.4*
Placebo (N=94)	9.6 (24.6)	10.8 (17.7)	-1.3 (-3.4; 0.8)	1.5 (-0.2; 3.1) p_{adj} = 0.1**

Population: Patients were difference could be calculated (N); DID: Difference in Differences
Analysis: * raw (simple 95% CI of DID); ** adjusted for baseline and time between samples

Secondary outcomes - results

Table 2 also summarizes the exploratory results for the secondary outcomes. The change in platelet activity (expressed in AU*min) in the ADPtest was larger with TXA (mean change 78.0, 95%CI, 15.4 to 140.6) compared to placebo group (mean change 64.8, 95%CI, 15.8 to 113.7), but with no significant difference in difference (DiD 13.2, 95%CI, -65.8 to 92.2). The mean difference of the TRAPtest for the TXA group was 106.2, (95%CI, 38.5 to 174.0) compared to the placebo group 85.3, (95%CI, 31.2 to 139.4). The difference in difference was not significant (DiD -20.9, 95%CI, - 65.3 to 107.1). There was no significant DiD between treatment groups for coagulation factors activity (expressed in % of the norm). The results are as follows: factors V DiD -4.2, 95%CI, -10.0 to 1.7, factor VIII DiD 5.9, 95%CI, -13.9 to 25.6, and von Willebrand factor DiD -0.1, 95%CI, -16.9 to; 16.7. No significant difference in difference was observed for Fibrinogen, expressed in g/L, (DiD -0.08 with 95%CI, -0.29 to 0.12) or D-Dimer, expressed in mg/L, (DiD 0.9 with 95%CI, -1.3 to 3.0). Detailed changes in the single treatment groups are presented in Table 2.

Discussion

We found no evidence that tranexamic acid (TXA) has large effects on thrombin generation or platelet function. However, we cannot exclude the possibility of more modest effects. Thrombin plays a crucial role in coagulation[15] and increased thrombin generation is associated with an increased risk of thrombosis[19]. Plasmin has been shown to increase thrombin formation in the blood of healthy volunteers *in vitro*[8]. An increase in thrombin generation by plasmin was also reported during treatment with tissue plasminogen activators[20]. ETP was decreased about 30% after administration of very effective anticoagulant agents such as low-molecular-weight heparin, in postpartum period soon after caesarean delivery[21] and also during pregnancy at an *in-vitro* study[22]. In our study there was a small decrease (3%) in ETP with TXA administration (DiD 36.63, 95% CI, -120; 42.8) that was

not statistically different to that seen in the placebo group. This study provides no evidence TXA has a pro-thrombotic effect.

Plasmin has multifactorial pro-coagulant effects on platelet activation[10,23]. Activation of platelets may contribute to thrombus formation[24]. The evaluation of platelet activity using MEA with ADPtest and TRAPtest provides information about the thrombotic risk. The reported range for healthy volunteers of ADPtest was 483 to 1173 AU*min. The reported range for the TRAPtest was 897 to 1469 AU*min.[25]. The observations of our study are comparable within both treatment groups and in a good fit with the results of Rubak[25]. They show a modest decrease in platelet activity in both tests in TXA group compared to placebo, but the difference was not statistically significant. Once again, these results provide no evidence that TXA has pro-thrombotic effects.

In the last trimester of pregnancy, plasma levels of plasminogen and fibrinogen increase by about 50% whilst levels of plasminogen activator inhibitors 1 and 2 increase 3-fold and 25-fold, respectively[7] . Immediately following delivery there is early fibrinolytic activation and this can be inhibited by TXA[26]. The inhibition of fibrinolysis with TXA has the potential to increase thrombotic risk[27]. By reducing fibrinolysis, TXA can help to maintain fibrinogen levels. In our study, the drop in fibrinogen (DiD -0.08, 95%CI -0.29; to 0.12) was smaller in the TXA group but again the difference was not statistically significant.

Study limitations

The study was designed to prove a difference (more relevant changes with the use of TXA compared to placebo) and was not planned to establish therapeutic equivalence. There are no predefined therapeutic equivalence bounds which would allow an objective comparison between derived DiD confidence intervals. The study uses a large series of secondary endpoints and multiple testing performed in an explorative setting. In the ETAPlaT study, although we did not measured plasmin directly but evaluated thrombin generation and platelet function as an indirect effect of TXA on plasmin inhibition. Some post-randomization exclusions were performed, because of the emergency situation of PPH it was difficult to collect the baseline or follow up or both blood samples.

Conclusion

Although the inhibition of fibrinolysis with TXA has the potential to increase thrombotic risk, we found no increase in thrombin generation and no increase in platelet activity with TXA.

Ethics approval and consent to participate

Ethical approval for WOMAN ETAPlaT protocol was obtained from the London School of Hygiene and Tropical Medicine Ethics Committee, United Kingdom, and by the National Ethics Committee in Tirana, Albania. ETAPlaT study was undertaken according to local regulatory requirements, and adhered the ICH-GCP guidelines. The consent procedure was approved by each Ethics Committee and is detailed in the previously published WOMAN trial and ETAPLaT protocols[4,11]. Briefly, consent was obtained from a woman if her physical and mental capacity allowed (as judged by the treating clinician). If a woman was unable to give consent, proxy consent was obtained from a relative or representative (who was not involved in the trial and was approved by the hospital). If a proxy was unavailable, then as permitted by local ethics approval, consent was deferred. When consent was deferred or given by a proxy, the woman was informed about the trial as soon as possible, and consent was obtained for ongoing data collection, if needed.

Data availability

The anonymised data used for this publication is available from the freeBIRD data portal at https://freebird.lshtm.ac.uk/index.php/data-sharing/downloads/etaplat/ following free registration: http://www.doi.org/10.17037/DATA.00000970[28]. Data are available under an Open Data Commons Attribution License (ODC-By) licence.

Reporting guidelines

This study is compliant with CONSORT guideline recommendations[29].

Grant information

The WOMAN Trial was funded by the Department of Health (UK), grant number HICF-T2-0510-007, the Wellcome Trust, grant number WT094947, the Bill & Melinda Gates Foundation (grant number OPP1095618), and LSHTM (London, UK). An educational grant was given by Erasmus Mundus program ERAWEB [D2.12.048] and Rudolf Marx Foundation (Munich, Germany).

The Sponsor (LSHTM) and the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 04 June 2019

https://doi.org/10.21956/wellcomeopenres.16336.r35666

✔ **Homa K. Ahmadzia**
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Summary:
This article by Dallaku *et al*. is a subgroup analysis of the WOMAN trial, in which women with diagnosed PPH were randomly assigned to receive placebo or TXA (1g IV) for treatment. The objective was to assess effects of TXA on thrombin generation and platelet function, testing the hypothesis that TXA would reduce endogenous thrombin potential (ETP) and inhibit platelets.

'Baseline' blood samples were drawn prior to initial drug administration and 'follow up' samples approximately 30 min after first drug administration. A majority of patients received 1g TXA but a third or less received a second 1g due to continued bleeding.

The results showed that there was a slight decrease in ETP, but certainly no increase after TXA administration *in vivo*. In addition, in the TXA group comparing post drug to pre drug levels there was no increase in platelet activation or other coagulation markers/assays (d-dimer, factors V/VIII/vW and fibrinogen).

General Comment:
Overall important contribution to the literature since it adds new information about drug pharmacodynamics in the setting of treatment for PPH, and supports that TXA does not increase hypercoaguable profile in the peripartum period.

Suggestions for improvement:
Although this was a novel approach to use thrombin generation assay (TGA) in the peripartum period, the results focused solely on ETP. The conclusion would be more robust if similar findings were true for other parameters on TGA (i.e. include analysis on comparisons of lag time and time to peak pre/post drug, perhaps as supplement if not main table/figure).

Also a figure (with individual data points) to show distribution of the ETP values in pre/post drug groups would be helpful as well to illustrate the data.

I am not sure the 'difference in difference' statistical approach is the clearest way to represent the analysis, especially when the post minus pre drug levels were what was focused on as the key findings.

I agree with Reviewer #1 that a separate analysis of the women who received 2 grams would be of interest to see if any difference was seen from post drug dose #1 to post drug dose #2 and the difference in baseline from the dose #2. That would make the data more robust if there was no difference seen in that ETP as well, even if not powered to look at that as primary outcome.

Consideration for limitation/discussion:
I also found the estimated blood loss to be on lower end for PPH, possibly due to the fact that more than 2/3 of patients in both groups were vaginal deliveries. I would include in the limitations that this is a selective group of PPH and some groups may not define as such with more recent definitions (US ACOG Revitalize which is >=1000 mL regardless of mode of delivery). However, this could also be seen as a strength to the study given if these findings could support preliminary safety data using TXA for 'mild' PPH cases, which is clinically more useful to intervene rather than severe PPH when other coagulopathies can result simply from high volume blood loss and when in lower resource settings blood transfusion is not readily available.

Minor comments:
- Table 2 - ETP DID TXA should be 38.6 and not 36.6.
- Page 7 - typo to remove 'd' in 'study limitations:...'we did not measure[d] plasmin directly'.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Maternal-Fetal Medicine, postpartum hemorrhage, tranexamic acid translational research.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 February 2019

https://doi.org/10.21956/wellcomeopenres.16336.r34770

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Tournoys Antoine
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We thank the authors for the quality of their trial and scientific data, the obstetric and anesthesia and intensive care community may benefit from this article.

The authors analyzed the effects of tranexamic acid (TXA) administration on endogenous thrombin potential (ETP) and other coagulation factors and markers compared to placebo.

The study is a WOMAN Trial sub study. The tubes were sampled out of the patients included in this international randomized double blind trial. One Albanian center was the site where samples were taken and biological products were centrifuged and frozen. Biological results were obtained by laboratory tests in a German lab in Munich.

The authors showed no impact of TXA administration compared to placebo on ETP and platelet function. According to the authors, the stability of endogenous thrombin potential and platelet activation tests suggests that TXA has no pro-thrombotic effect. WOMAN trial clinical and ETAPlat biological data support the safety of TXA use in obstetric hemorrhage.

My major comment is that the selected population is a mild haemorrhage population developing minor or no coagulopathy as suggested by:

- minor blood loss baseline in both groups : 863.2 (270.7) and 893.1 (219.9)mL;
- baseline plasma fibrinogen level above 3g /L predicting a moderate thrombin generation and moderate final severity and
- moderate decrease in baseline to follow-up fibrinogen plasma level and platelet count decrease;
- minor fibrinolysis as showed by the moderate decrease of baseline factor V and moderate increase of baseline D Dimers.

Thus, to consider this population data, it would be of interest to clarify the conditions of substudy recruitment regarding in one part the center's recruitment and on the other part WOMAN trial's global population:

- Was the series of sampled patients a consecutive series of all patients included during the substudy period?
- The substudy period was shorter than the study period. Did the center continue its recruitment to complete clinical part of the study or did the center stop after the time when substudy recruitment was completed?

It would be of interest to describe the final volume of PPH in order to give information about the proportion of severe patients.

My second comment concerns the mild ETP level reached in the study:

Thrombin generation analysis was the primary criteria.

The pre-supposed normal value of thrombin generation that was used to calculate the sample size of the sub study was 2410 +/- 543 nM*min". Joly et al had observed a similar reference value during the third trimester of pregnancy 2123 ± 335 (nM.min) (CAT Thermoscientific Labsystems assay)[1] as well as Macey et al in their pregnant control at 38 Weeks of pregnancy 2515 [2289 – 2726]nMIIa (CAT STAGO assay)[2].
It is important to clarify if these reference values had been given by the reagent's supplier or tested by the involved laboratory and if the population recruited to measure these reference values were both gender or only women of child-bearing age or only normal pregnant women or normal immediate postpartum women.

- Both baseline and follow-up ETP observed in the ETAPlat substudy groups were lower than the reference value, see here.
- There was no significant decrease from ETP baseline to follow-up in both groups. Follow up was sampled 30 minutes after baseline: maybe too early to observe the changes in thrombin generation as the maximal PPH-induced coagulation changes were observed 2 hours after the beginning of PPH[3].
- Regarding the therapeutic effect of TXA focused on plasmin generation decrease, the authors based their hypothesis on the indirect impact of thrombin potential reduction due to plasmin generation reduction. It had been previously observed that TXA inhibits D-Dimers increase with no effect on fibrinogen and factor II decreases (substrate and proenzyme of thrombin)(3). As suggested by the TRACES trial pilot study[4], if this mechanism exists, it would be better to select coagulopathic patients and higher doses of TXA. Following this suggestion, it could be of interest to analyze the thrombin generation and fibrinogen and platelets data from the 18 % and 33 % of patients receiving 2 allocated doses. Although the number of these patients is limited, the second dose's administration shows that PPH is ongoing and thus concerns the more severe ones with higher thrombin and plasmin generation and TXA dose effect than the 1g population. Pharmacobiological TRACES trial is aimed to provide more detailed data in this setting[4].

Finally platelet activation assays were potentially useful to better understand the evolution of primary hemostasis in PPH.

Conclusion : regarding my comments concerning the selected population and the TA dose, I would ask the authors to change their title and conclusion by introducing the two important details: "1g TXA" and " mild postpartum haemorrhage".

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Obstetric anesthesia and intensive care. Hemostasis and pregnancy. Massive bleeding.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.