RESEARCH NOTE

Statistical analysis plan for the WOMAN-ETAPlaT study: Effect of tranexamic acid on platelet function and thrombin generation [version 1; peer review: 2 approved, 2 approved with reservations]

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Abstract

Background. Postpartum haemorrhage (PPH) is a potentially life-threatening complication for women, and the leading cause of maternal mortality. Tranexamic acid (TXA) is an antifibrinolytic used worldwide to treat uterine haemorrhage and to reduce blood loss in general surgery. TXA may have effects on thrombin generation, platelet function and coagulation factors as a result of its inhibition on the plasmin.

Methods. WOMAN ETAPlaT is a sub-study of the World Maternal Antifibrinolitic trial (WOMAN trial). All adult women clinically diagnosed with PPH after a vaginal delivery or caesarean section, are eligible for inclusion in the study. Blood samples will be collected at the baseline and 30 minutes after the first dose of study treatment is given. Platelet function will be evaluated in whole blood immediately after sampling with Multiplate® tests (ADPtest and TRAPtest). Thrombin generation, fibrinogen, D-dimer, and coagulation factors vW, V and VIII will be analysed using platelet poor plasma.

Results. Recruitment to WOMAN ETAPlaT started on 04 November 2013 and closed on 13 January 2015, during this time 188 patients were recruited. The final participant follow-up was completed on 04 March 2015. This article introduces the statistical analysis plan for the study, without reference to unblinded data.
Conclusion. The data from this study will provide evidence for the effect of TXA on thrombin generation, platelet function and coagulation factors in women with PPH.

Open Peer Review

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Abbreviations

ADPtest: Test of Adenosine Di Phosphate (ADP); AUC: Area Under Curve; CI: confidence Interval; CONSORT: Consolidated Standards of Reporting Trials; ETP: Endogenous Thrombin Potential; FAS: Full Analysis Set; FV: Coagulation Factor V; FVIII: Coagulation Factor VIII; CBC: Complete Blood Count; IBE: Institute for Medical Information Sciences, Biometry and Epidemiology; Hb: Hemoglobin; Ht: Hematocrit; LT: Lag Time; PtH: Peak to Height; MPV: Mean Platelet Volume Mean Platelet Volume; PP: Per-Protocol; R: software package R (r-project.org); SAP: Statistical Analysis Plan; SD: Standard Deviations; SOP: Standard Operating Procedure; TGA: Thrombin Generation Assay; TXA: Tranexamic Acid; TRAPtest: Test of Thrombin Receptor of Thrombocyte; vWF: Coagulation Factor Von Willebrand.

Preface

The purpose of the statistical analysis plan (SAP) is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of the study data prior to hard locking the database and unblinding of the WOMAN ETAPlaT trial data. To prevent outcome bias and selective reporting, a detailed SAP is presented in order to avoid post hoc decisions that may influence the interpretation of the results and the statistical analyses of the final data.

This SAP is a technical extension of the WOMAN ETAPlaT study protocol (Version 1.1, dated August 29, 2014), which is published elsewhere (Dallaku *et al*., 2016). The SAP follows the principles of the International Conference on Harmonization (ICH) guidelines E3, E6 and E9 (ICH, 2016).

Study objectives and endpoints of WOMAN-ETAPlaT

Objectives of the study

Primary objectives: This study will assess if TXA, by inhibiting the plasmin, has an effect on decrease over time on the continuous response variable, thrombin generation assay (TGA) parameter: endogenous thrombin potential (ETP). Secondary objectives: evaluation of the effect of TXA on women with PPH, through monitoring the platelet function (multiplate analyser), plasmatic levels of fibrinogen, D-dimer, coagulation factor V (FV), coagulation factor VIII (FVIII), coagulation factor von Willebrandt (vWF) and other TGA parameters: lag time (LT), time to peak (TtP), and peak height (Ph).

Endpoints

Primary efficacy endpoint. **The primary efficacy endpoint** is the TGA parameter – ETP (nM/minute). Values of ETP will be measured in venous blood samples. Blood samples will be collected at the baseline and at 30 ±15 minutes after the first dose of study treatment is given. Analysis will be performed on processed, separated platelet poor plasma, and preserved in deep freeze, as described in the ETAPlaT protocol (Dallaku *et al*., 2016).

Secondary efficacy endpoints. Secondary outcomes will include parameters measuring the effect of TXA on other TGA parameters (LT, TtP, and Ph - we will transform variables prior to analysis if there is evidence that distributions are skewed), fibrinogen, D-dimer and coagulation factors V, VIII, vWF (measured on processed plasma samples as described on primary endpoint) and platelet function analysed by Multiplate® tests (ADPTest, TRAPTest – measured on whole blood).

Study methods

Study design

The **WOMAN-ETAPlaT** is a sub-study of the WOMAN trial, an international randomised, double blinded, placebo-controlled trial. The ETAPlaT sub-study has the same study design as the WOMAN trial, but includes some additional laboratory tests. Women who fulfil the eligibility criteria for the WOMAN trial, including adult women clinically diagnosed with primary PPH after a vaginal delivery (>500 ml blood loss), a caesarean delivery (>1000 mL blood loss), or enough blood loss to compromise the haemodynamic status will be randomized. Regarding exclusion criteria, will not be randomized those women with PPH for whom the physician believes there is an indication or contraindication to use TXA.

Immediately after randomization, women will receive the trial treatment by i/v administration of either TXA (1 gram) or placebo (NaCl solution 0.9%). If after 30 minutes the haemorrhage continues or if it stops and restarts within 24 hours after randomization, a second dose may be given. This second dose is not relevant for the current sub-study.

Randomisation, blinding and ethics approval

Packs with study drugs were prepared according to the randomization list, generated by an independent statistician. The active study drug (TXA) and placebo (NaCl 0.9%) will be identical in appearance, and randomization number will be used for identification of packs. Each box has eight packs of study drugs, and the lowest number pack will be used first. After completing the consent procedure, eligible patients are randomized to receive either TXA or placebo. Both the patients and the medical representatives participating in the study are masked to the treatment distribution. Ethical approval was obtained in July 11, 2013 by National Ethics Committee in Tirana, Albania (ref. 62 and 81) and in October 28, 2013 by London School of Hygiene and Tropical Medicine, UK (ref. 6518).

Study variables and study schema

Details concerning the baseline (treatment) and follow-up (post treatment) period together with the frequency and timing of relevant variables or assessments are displayed at Table 1.

Sample size

The sample size calculation is focused on the first component of the primary endpoint: change over time of ETP in women with PPH. In a previous study (McLean *et al.,* 2012) ETP in patients with term pregnancy, within each subject group, was normally distributed with a mean of 2410 nM/min and standard deviation (SD) of 543 nM/min.

Assuming a correlation of 0.6 between two time points, the standard deviation of the change can be calculated as 485 {sqrt[2×543^2×(1-0.6)]}. We assume a decrease in ETP of 10% (243 nM/min) over time in the TXA group and no change in the placebo group. To

Table 1. Datasets that will be generated from the study and will be included in a database.

Variables	Time period
MultiplateAnalyser test – ADPtest (.csv file)	Baseline data and 30 ± 15 min after study treatment
MultiplateAnalyser test – TRAPtest (.csv file)	Baseline data and 30 ± 15 min after study treatment
Thrombin generation assay (.csv file)	Baseline data and 30 ± 15 min after study treatment
Coagulation factors vW, V, VIII, Fib, and DDim (.csv file)	Baseline data and 30 ± 15 min after study treatment
Clinical data collection forms (.csv file)	Baseline data and follow-up
Full blood count (.csv file)	Baseline data and within 12 hrs after randomization

detect an ETP difference of 243 nM/min between groups at a 5% significance level with a power of 80%, two groups each with 88 patients are needed.

A correlation of 0.6 is seen as a conservative estimate as the within individual changes in TGA over time is strictly controlled, which implies a high correlation between time points.

General considerations

Timing of analyses

Participant recruitment started on 4 November 2013 and was completed on 13 January 2015. The final participant follow-up was completed on 04 March 2015.

All final analyses will be performed on the database at LSHTM (London, UK) which consists of several tables (Table 1). After documenting all data collection forms (DCF) data, data cleaning and query resolution, the following prerequisites for unblinding must be fulfilled: the resolution of all queries concerning DCF and laboratory results, and the finalisation of the SAP document.

All these processes before database locking must take place to comply with requirements.

Following data integrity checks the database will be locked after October 2016 and the statistical analysis specified in the SAP and approved by the Trial Steering Committee (TSC), will be performed in advance of the WOMAN trial database lock. In the event the TSC approves analysis before the end of the WOMAN trial, only the independent Data Monitoring Committee (DMC) statistician will be aware of each participant's treatment allocation. Each participant will be allocated a unique identifier (different from that of the WOMAN trial before unblinding). This is to ensure that the WOMAN trial blinding is not compromised in any way.

Analysis populations

Full analysis set (FAS). The primary efficacy analysis follows the principle of intention to treat (ITT), which implies that study data are analysed based on the original allocation of patients, regardless of a treatment received. Withdrawals, participants lost to follow-up and participants who did not adhere fully to the WOMAN ETAPlaT study protocol, will not be excluded from the primary efficacy analyses provided that they satisfy major entry criteria. Also, a patient will be included in the primary efficacy analysis if only the baseline measurement is available.

Per-protocol set (PP). The PP set consists of all patients who did not substantially deviate from the WOMAN ETAPlaT protocol as to be determined on a per-subject basis. The PP set of subjects defining a subset of the FAS is characterised as follows.

- No major protocol violations were detected:
  - Time of treatment assignment after randomization,
  - Time of blood withdrawal after study drug administration,
  - Completion of blood samples (baseline and follow-up) collection
  - Broken or damaged of the study drug vials of sequential box

Covariates and subgroups

There exists no *a priori* hypothesis of subgroup differences. Hence, no pre-planned confirmatory subgroup analyses will be performed to explore evidence for a difference in treatment effects (interaction effect).

Exploratory subgroup-specific summary statistics will be reported for main causes of PPH (uterine atony, placenta previa or abruption and genital trauma), the severity of PPH (moderate-severe PPH), platelet count, fibrinogen level, preeclampsia, chorioamionitis and prematurity (see exploratory subgroup analysis).

Summary of study data

All continuous variables will be summarised by treatment group (TXA/placebo) using the following descriptive statistics: sample size (N), mean ±standard deviation (SD), median, maximum and minimum. The absolute frequency and percentages of observed levels will be reported for all categorical variables. Summary statistics will be displayed overall and stratified by the treatment group. If considered appropriate these data are summarised by centre as well. The key baseline characteristics selected are presented in Table 2.

Baseline characteristics

In a study about the mode of anaesthesia related to PPH after caesarean deliveries, a significantly higher amount of blood loss during general anaesthesia compared to spinal anaesthesia was previously reported (Aksoy *et al.,* 2015). The reduction of blood loss in neuroaxial anaesthesia maybe was caused partly from hypotension and by fluids infused in these women (Heesen *et al.*, 2013). Another role in increased blood loss during general anaesthesia was reported from volatile anaesthetics which may decrease the myometrium contractility (Yoo *et al.*, 2006) and as result can cause uterine atony. Also, volatile anaesthetics with their inhibition effect on platelet aggregation (Yuki *et al.,* 2013) can increase the risk for PPH.

Table 2. Baseline characteristics of participants before randomization.

	TXA (n, %)	Placebo (n, %)
Patient height, weight, BMI		
Parity: nullipara (0), multipara (>1)		
Gestational age at birth: <37 or ≥37 weeks		
Any concomitant diseases of pregnancy: Preclampsia, Chorioamnionitis, Diabetes, Placental abruption, Placenta previa, Previous PPH		
Use of anaesthesia: General or spinal or none		
Labour induction/augmentation		
Duration of labour (min) 1st, 2nd and 3rd stage of labour		
Fetal birth weight: < 4000 gr or ≥ 4000 gr		
Hb level at entry of the study		
Platelet count at entry of the study		
Fibrinogen level at entry of the study		
Amount of blood loss		
Primary cause of PPH Atony, placenta, trauma, other		
Mode of delivery:vaginal- caesarean		
Additional doses of uterotonics		

Dionne *et al.* (2015) mentioned that prolonged second stage labour was associated with increased risk and severity of PPH. Other potential risk factors for PPH were null-parity, high birthweight and antepartum anaemia, where low levels of haemoglobin before delivery were associated with a low efficacy of haemostasis mechanism promoted by red cells and platelets (Biguzzi *et al.*, 2012), and as result with an increased risk for PPH.

Efficacy analyses of primary outcome

Both groups of randomised patients, those allocated to the TXA group and placebo group will be compared and analysed on an intention-to-treat basis, irrespective of treatment given. The results will be presented as appropriate effect estimates with a measure of precision (95% CI). In analysis of the primary outcome(TGA– ETP), the TXA group will be compared with the placebo group, based on analysis of covariance (ANCOVA) which adjusts for the length of time between two measurements (30±15 minutes) of the baseline and follow-up TGA - ETP value (Table 3). Primary outcome will be analysed also according to the underlying cause of PPH (uterine atony, placenta previa, placental abruption, and genital trauma) in correlation to TXA/placebo.

Effect of TXA on thrombin generation

There is no evidence about evaluation of TXA effect on thrombin generation in women with PPH. In a systematic review Valentino & Holme (2015) analysed studies about the combined administration of TXA with an anti-inhibitor coagulant complex (AICC) in haemophilia patients. The clot stability and coagulation state measured with thromboelastography and thrombin generation assay respectively, resulted with an increase of maximum clot firmness without an increase in ETP. Although it is known that AICC enhances thrombin generation, combined treatment of TXA with AICC improved the clot stability without enhancing the thrombin generation.

In patients receiving combined therapy of TXA and activated prothrombin complex concentrate (APCC), thrombin generation was lower than in healthy controls (Holmstrom *et al.*, 2012). We can assume that the main effect in decreasing the thrombin generation is by TXA, since APCC as monotherapy is known to increase thrombin generation. TXA effect to decrease the thrombin generation was confirmed also by Tran *et al.* (2014) evaluating combined therapy of APCC and TXA, which resulted without a significant increase of thrombin generation compared with healthy controls. The ETP measured in platelet poor plasma was greater in monotherapy of recombinant activated factor VII (rFVIIa) compared with combined treatment of rFVIIa and TXA. Thrombin generation was decreased by TXA also in combined treatment with rFVIIa.

Thrombin generation was increased when plasmin was added to normal plasma samples, and ETP peak height were significantly increased compared to plasmin absence (Ogiwara *et al.*, 2010). TXA administered in women diagnosed with PPH can decrease the thrombin generation by inhibiting plasmin.

Efficacy analyses of secondary outcomes

The same analyses as for primary outcome will be performed for the blood parameters which are secondary endpoint parameters of Multiplate© tests (ADPtest and TRAPtest), fibrinogen, D-dimer,

Table 3. Evaluation of TXA effect compared to placebo on TGA –ETP, in women with PPH.

	TXA (n)			Placebo (n)			95% CI p-value		
Primary outcome	Before	After*	Delta	Before	After*	Delta			
TGA – ETP (nM*min)									

* 30 minutes

coagulation factors V, VIII, vWF and other TGA parameters (lag time, time to peak, peak height) (Table 4). The results will be presented as appropriate effect estimates with a measure of precision (95% CI).

The remaining secondary outcomes will be analysed using appropriate regression models such as Cox model, Poisson or logistic regression (mortality, surgical interventions, blood transfusions, thromboembolic events) and t-tests. The influence of any coagulation disease and anticoagulation treatment/prophylaxis on the TXA effect for blood parameters under study will be assessed. A further sensitivity analysis will be done by adjusting for the amount of blood loss prior to randomisation. All statistical analyses will be conducted with the statistics package R and STATA.

Exploratory subgroup analyses

The influence of any concomitant diseases of pregnancy (preeclampsia, prematurity and chorioamnionitis), or PPH primary causes (uterine atony, placenta previa or abruption and genital tract trauma) on the TXA effect on the blood parameters under study will be assessed. Similar tables will be provided for the subgroups.

A further sensitivity analysis will be done by adjusting for severity of PPH (Table 5) and fibrinogen level or platelet count. This will be done using linear models (ANCOVA).

Table 4. Effect of tranexamic acid on coagulation factors and platelet function.

		TXA (n)			Placebo (n)			Difference 95% CIP-value
TGA parameters		Before	After*	Δ	Before	After*	Δ	
	Lag time(min)							
	Time to peak(min)							
	Peak height (nM)							
Multiplate tests								
	ADPtest(AU*min)							
	TRAPtest(AU*min)							
Coagulation tests								
	FV (%)							
	FVIII (%)							
	vWF (%)							
	D-dimer (mg/L)							
	Fibrinogen (mg/dL)							

* 30 minutes

Table 5. Efficacy (secondary endpoints) analysis (ITT) with respect to the severity of PPH in correlation with TXA/placebo and platelet function / coagulation factors.

		TXA Δ		Placebo Δ		Moderate PPH	Severe PPH
		Moderate PPH (1)	Severe PPH (2)	Moderate PPH (1)	Severe PPH (2)	Difference 95% CI P-value	Difference 95% CI P-value
TGA parameters							
Mean±SD, range	ETP (nM*min)						
	Lag time (min)						
	Time to peak (min)						
	Peak (nM)						
Multiplate tests							
Mean±SD, range	ADPtest(AU*min)						
	TRAPtest(AU*min)						
Coagulation tests							
Mean±SD, range	FV (%)						
	FVIII (%)						
	vWF (%)						
	D-dimer (mg/L)						
	Fibrinogen (mg/dL)						

(1) 500 - <1000 ml blood loss; (2) ≥1000 ml blood loss

Influence of pregnancy diseases and PPH causes on the TXA effect for study outcomes

In pregnant women diagnosed with preeclampsia, preterm labour and chorioamnionitis, was found an increased thrombin generation (Mastrolia *et al.*, 2014). Chaiworapongsa *et al.* (2002) also reported increased thrombin generation in patients diagnosed with preeclampsia and fetal growth restriction. Macey *et al.* (2010) comparing normal pregnancies with and without preeclampsia, reported an increased thrombin formation and increased platelet activation. Increased thrombin generation and platelet activity was found also in women with a history of preeclampsia (Rafik Hamad *et al.*, 2009).

There is evidence that the haemostatic impairment that occurs in haemorrhage during pregnancy and delivery is different from trauma-induced haemorrhage. The type and rate of onset of coagulopathies differ depending on the main cause of obstetric haemorrhage (Collis & Collins, 2015). Regarding haemostasis changes during obstetric haemorrhage, there is little data. The type, severity and rate of onset of the coagulopathy vary with the aetiology of bleeding.

Allard *et al.* (2014) raised the question, whether women with postpartum haemorrhage exhibit different changes from other patients who have haemorrhage during general surgery or trauma? Some of the underlying causes of postpartum haemorrhage such as uterine atony and genital tract trauma were often associated with no significant coagulopathy (Collins *et al.*, 2014). But, when the cause of PPH was placental abruption, it may be associated with rapid consumptive coagulopathy characterised with clinically severe haemostatic impairment (Thachil & Toh, 2009).

Influence of PPH severity, fibrinogen and platelet count on the TXA effect for study outcomes

Low plasmatic level of fibrinogen such as 2 – 3 g/L and especially less than 2 g/L at the beginning or on-going of PPH, was associated with an increased amount of blood loss, increased need for blood transfusion and requirements for invasive procedures. Low platelet number at the diagnosis of PPH was associated with the requirement for invasive procedures (Collins *et al.*, 2014). Simon *et al.* (1997), observed an increased frequency of PPH, when in early labour platelet count is <100000/mm³ or fibrinogen level is <2.9mg/L or both. A study has reported that thrombocytopenia <80 000/mm³ was associated with an increased incidence of severe PPH (Dikman *et al.*, 2014), however, another study did not found any association between thrombocytopenia and severe PPH (Jones *et al.*, 2016).

A systematic review (Simonazzi *et al.*, 2016) confirmed that TXA can significantly decrease the incidence of PPH with an amount of blood loss >500 ml and severe PPH with blood loss > 1000ml. Consume of coagulation factors and platelets were reported in cases with severe postpartum bleeding. The decrease of fibrinogen level, antithrombin activity, and the platelet count was observed in PPH cases with blood loss >2000 ml.

We will evaluate the effect of TXA/placebo on study outcomes (TGA, coagulation factors and platelet function tests), in cases with moderate PPH (500–1000ml) and severe PPH (>1000ml) (Table 5). The effect of TXA on study outcomes regarding the PPH severity will also be analysed regarding stratified amount of blood lost (500 mL, >500–1000 mL, >1000–1500 mL, or >1500 ml blood loss). Similar analysis will also be done for the effect of TXA on

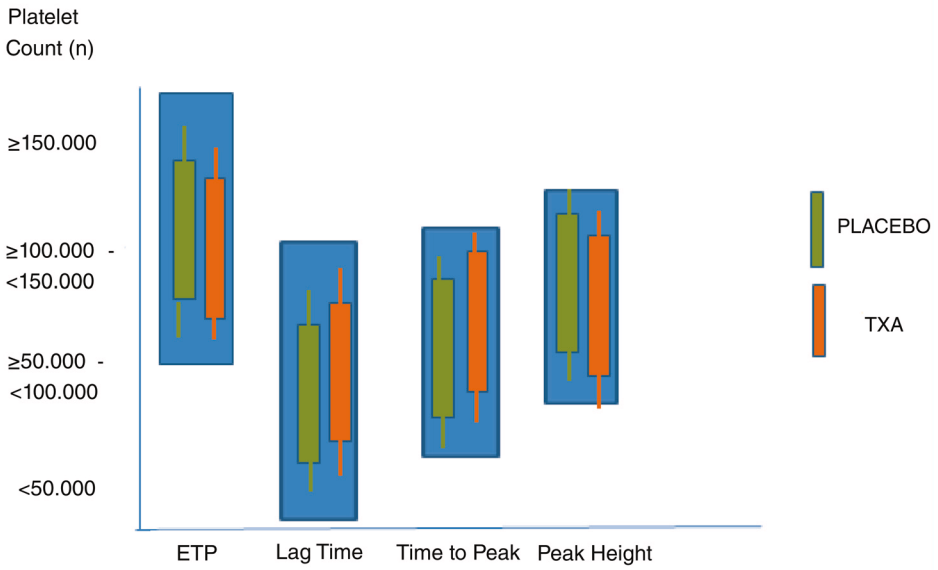

Figure 1. Effect of TXA on TGA parameters, regarding the platelet count before randomization (graph model using arbitrary data).

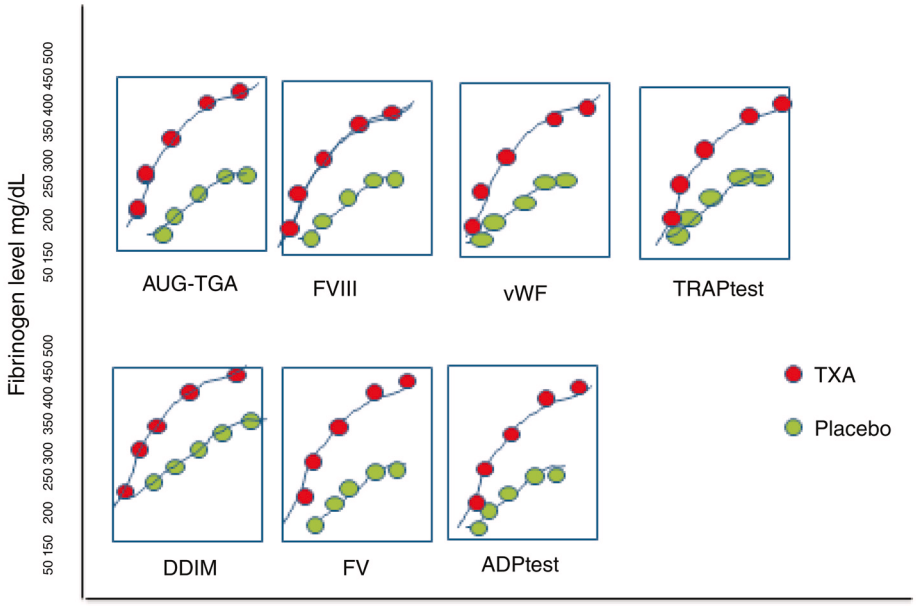

Figure 2. Effect of TXA on coagulation factors and platelet function, regarding the baseline fibrinogen level (graph model using arbitrary data).

thrombin generation and platelet function regarding complete blood count parameters such as baseline levels of haemoglobin (anaemia), platelets and their difference within 12 hours of randomization.

A series of graphs will present each TGA parameter (ETP, LT, TtP, Ph) as a panel of 4 boxplots which show the measured values for the treatment groups split with respect to baseline platelet count categorized as follows: <50000, 50000–100000, 100000–150000, ≥ 150000 (Figure 1). Similar graphs will display the coagulation factors (FV, FVIII, vWF, fibrinogen and D-dimer) and Multiplatetests (ADPtest and TRAPtest) measured values, with respect to platelet count for both treatment groups. Differences between the TXA/placebo groups will be tested using the non-parametric Kruskall-Wallis test.

Baseline fibrinogen level and a panel of 7 dot-plots showing the corresponding individual measurements for each individual patient with respect to TGA-ETP, FV, FVIII, vWF, D-dimer, ADPtest and TRAPtest for both treatment groups TXA/placebo (Figure 2).

The dots will be color-coded with respect to the treatment groups, a smoother will be presented in the corresponding colour, the Spearman correlation will be calculated and a 95% CI for each treatment group will be presented. Also, the difference between the correlation coefficients of the treatment groups will be calculated and a 95% confidence interval will be presented. The confidence intervals related to the Spearman correlation coefficient will be calculated by bootstraping.

Two panels of graphs will present the ADPtest parameters as well as the TRAPtest parameters. They will show individual measurements for aggregation, velocity, and AUC of TXA/placebo groups correlated with individual platelet count. These graphs (similar to Figure 2) will be produced for baseline, after treatment and change values.

Per-protocol analyses

All analyses as described in of the section 'Efficacy analysis for primary and secondary outcomes' will be repeated using the PP analysis set.

Secondary endpoints measured

The difference between study groups in absolute change between baseline and 30±15 minutes after study drug administration will be analysed in a descriptive manner. The comparison of the treatment groups for all secondary endpoints will be performed using a Mann-Whitney U test for quantitative measures and a chi-square test for frequencies. Continuous variables are expressed as mean±SD and stratified by treatment group, if normally distributed overall; otherwise these variables are expressed as median and IQR.

Conclusions

This article presents the principles of statistical analysis of the WOMAN ETAPlaT study, in order to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of the study data. The detailed SAP presented will help to prevent outcome bias, selective reporting and to avoid post hoc decisions that may influence the interpretation of the results and the statistical analyses of the final data. The data from this study will provide evidence for the effect of TXA on thrombin generation, platelet function and coagulation factors in women with PPH.

Author contributions

KD, UM, HS, IR, PE, SH, DB and MD have contributed to the drafting of the statistical analysis plan and have reviewed it critically. All authors approved the final version of the statistical analysis plan.

Competing interests

No competing interests were disclosed.

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Open Peer Review

Current Peer Review Status: ✔ ? ✔ ?

Version 1

Reviewer Report 06 April 2017

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Manisha Nair
National Perinatal Epidemiology Unit (NPEU), Nuffield Department of Population Health (NDPH), University of Oxford, Oxford, UK
Louise Linsell
National Perinatal Epidemiology Unit, University of Oxford, Oxford, UK

This is an interesting study. The authors may want to consider the following points:

Comments from Manisha Nair

1. I understand that the primary aim of this study was to generate evidence about the effect of TXA on thrombin generation, platelet function and coagulation factors in women with PPH. However, this study could also provide answers to why TXA had or did not have the desired effect. In this context it would be important to analyse how the TXA effect is modified by baseline haemoglobin, platelets and coagulation factors, especially considering a high prevalence of anaemia during pregnancy in LMICs. I am surprised that the authors did not pre-specify this, but intend to conduct this only as an exploratory analysis. Clearly, these sub-group analyses will be underpowered to derive any meaningful results.

   In the second paragraph under sub-heading “Exploratory subgroup analyses” (page-6), authors write, “A further sensitivity analysis will be done by adjusting for severity of PPH (Table 5) and fibrinogen level or platelet count” – I presume this is fibrinogen or platelet count at baseline?

2. In Table 2, authors have grouped induction and augmentation of labour. These are two different interventions and would probably be better to report them separately.
3. Will there be any information about the management of third stage of labour for those who underwent vaginal delivery?
4. The participants will be recruited at a point when they are diagnosed with PPH, so some women particularly in LMICs are likely to be rushed into the hospital and may not have records of antenatal care. Will it be possible to have complete information on preclampsia, chorioamnionitis, diabetes, placental abruption, placenta praevia, previous PPH and other baseline parameters? Therefore, will it not be difficult to ascertain the cause of PPH at least for some women? The authors do not comment on these issues and resultant missing data and how this will be handled in the analyses.

5. My concern is that the authors propose a lot of tests with a small sample. Although these are important, I am not sure if the results will be meaningful. While we will know if TXA had an effect on three coagulation parameters, but we will not know in which population groups this drug works well and in which it may not.

Comments from Louise Linsell

Page 3 Study design, 1st paragraph
Add that this sub-study is in a single centre.
Last sentence doesn't make sense. Suggest, "Women with PPH for whom the physician believes there is an indication or contraindication to use TXA will be excluded."

Page 4 Full analysis dataset, 1st paragraph
Please clarify that women who have withdrawn consent to use their data will be excluded from the ITT analysis. Will women with a follow-up measure but no baseline measure be included in the ITT analysis?

Page 4 Per protocol set, 1st paragraph
The bullet points after "No major protocol violations were detected" are not clear and do not make sense. In addition to completion of blood samples add "with measurements of outcome available"

Page 4 Covariates and subgroups
Please clarify all pre-specified subgroup analysis and methods of analysis, e.g. test for interaction and tie up with paragraph on **Page 6 Exploratory subgroup analyses** and the **last paragraph on page 7**. Provide detail on the coding of the sub group variables, and which outcomes will be included for which subgroup variables all in one place. Will subgroup analysis be conducted on the ITT set, PP set or both?

Page 4 Summary of study data, 1st paragraph
Remove sentence, "If considered appropriate these data are summarised by centre as well." as there is only one centre.

Page 5 Efficacy analyses of primary outcome
The last sentence refers to a subgroup analysis - this should go in the section detailing subgroup analyses.

Page 5 Effect of TXA on thrombin generation, 1st and 2nd paragraph
Not sure why this text is included in the SAP at this point - it belongs either in the protocol or the background.

Page 6 Efficacy analyses of secondary outcomes, 2nd paragraph
"The remaining secondary outcomes" - these are not listed in the protocol?

Figure 1
Please provide labels or specify what the measurements in the boxplots represent, i.e. are the maximum, minimum values represented by the end of the line, or the end of the box?

General comments
Subgroup and exploratory analyses
The text related to subgroup analyses is scattered and confusing. It is stated that these are exploratory, however if they are pre-specified and statistical tests are conducted, then the results should be reported

in full as part of the main trial report. There are also blocks of text reporting evidence from other studies that detract attention and disrupt the flow of the SAP. Perhaps it would be clearer to have one SAP for the analysis and reporting of the main trial results and any pre-specified subgroup analysis in which all results are reported in full and a second SAP outlining any further exploratory analysis investigating the pathology/epidemiology.

CONSORT Flowchart
Please include a CONSORT flowchart

Adherence
It would be useful to report measures of compliance and other useful information by trial arm, e.g. number of damaged, misallocated packs, time from randomisation to drug administration, time from baseline to 2nd measurement, number of 2nd doses administered.

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 29 May 2017

Kastriot Dallaku, Ludwig-Maximilian University, Munich, Germany

We would like to thank you for helpful and constructive feedback.

Below you find our answers regarding comments of **Manisha Nair**:

1. We approach the search for factors at baseline which modify the effect of TXA on thrombin generation (etc.) by the formal analysis of an interaction between the treatment and the corresponding baseline parameter on the specific outcome. As mentioned in your comment, we did not power the study to detect specific interactions.
   Furthermore, we did not prespecify subgroup analyses to assess possible effect modifications of baseline parameters on the treatment.
   In the section “Covariates and Subgroups” we addressed this issue. According to your comments we tried to make this point more obvious.We see now the task to reduce the reporting of post hoc subgroup analyses.

2. Since we have grouped the patient with induced and augmentation of labour in data collection form, it is difficult to separate, so we will remove these data from the SAP.

3. There are no data concerning management of third stage of labour.

4. There are no missing values regarding concomitant and preexisting diseases of pregnancy.

5. The total sample size is small and the subgroup analyses run the risk of false positive. We will react by removing the exploratory subgroup analysis.

Below you find our answers regarding comments of **Louise Linsell**:

1. Page 3 Study design, 1st paragraph: Many thanks for your comment. We changed the sentence in the SAP.

2. Page 4 Full analysis dataset, 1st paragraph:Thanks, we address this issue now in the revised SAP version. Missing values will be imputed.

3. Page 4 Per protocol set, 1st paragraph: We clarified this formulation in the revised SAP.

4. Page 4 Covariates and subgroups: The total sample size is small and the subgroup analyses run the risk of false positive. We removed the exploratory subgroup analysis.

5. Page 4 Summary of study data, 1st paragraph: Done

6. Page 5 Efficacy analyses of primary outcome: Done

7. Page 5 Effect of TXA on thrombin generation, 1st and 2nd paragraph: We removed the paragraph, because we have provided evidence about this in ETAPlaT protocol paper.

8. Page 6 Efficacy analyses of secondary outcomes, 2nd paragraph: We removed this section.

9. Figure 1: Here we follow standards which define the boxplot. The specific definitions are now added to the SAP.

10. General comments: We follow your advice already given regarding page 4 and 5.

11. CONSORT Flowchart: Good point: Will be added to the SAP

12. Adherence: We added a section adherence to the revised SAP.

Competing Interests: No competing interests were disclosed.

Reviewer Report 04 April 2017

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Christel Weiß
Department of Biometry and Statistics, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

The sample size has been assessed assuming a 1-sided 2 sample t test which will be performed in order to compare the changes. This should be mentioned (1-sided). Furthermore, patients will be included in the primary efficacy analysis if only the baseline measurement is available. If a rather big number of

patients will not have the 30-minute-measurement then the sample size off 88 per group may be too small.

Competing Interests: No competing interests were disclosed.

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

Author Response 29 May 2017

Kastriot Dallaku, Ludwig-Maximilian University, Munich, Germany

Thank you for your comments. We will impute missing values of baseline measurements and modified this issue in our revised SAP. It was clear from the performance in the single center that the problem of missing baseline measurements will not be severe.

Competing Interests: No competing interests were disclosed.

Reviewer Report 31 March 2017

https://doi.org/10.21956/wellcomeopenres.10886.r20785

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Stephen J. Senn
Competence Centre for Methodology and Statistics, Luxembourg Institute of Health, Strassen, Luxembourg

I have approved this report with reservations because my opinion is that the plan is generally sound and prepared in sufficient detail. Nevertheless, I consider that there is one aspect that is puzzling and might be improved.

As part of the sample size determination, the authors calculate the variance of the change from baseline measure of ETP This might be fair enough for the purpose of sample size determination. However, it is well known that this is an inefficient use of baselines and that one can do better by including the baseline as a covariate [1]. The section headed **Efficacy analyses of primary outcome** states:

In analysis of the primary outcome(TGA– ETP), the TXA group will be compared with the placebo group, based on analysis of covariance (ANCOVA) which adjusts for the length of time between two measurements (30±15 minutes) of the baseline and follow-up TGA - ETP value

This does not, therefore, appear to adjust for the baseline value (apart for the crude and inefficient adjustment through subtraction). On the other hand, there is an adjustment for the length of time between the two measurements. This implies that this length of time is itself considered to be predictive. That is to say it is supposed that two women given identical treatment would be expected to have different values of TGA according to the length of time it takes to measure TGA. This may well be reasonable (if some

secular trend in TGA is expected) but in that case I would have expected some discussion of the point.

In short, I consider that the plan shows evidence of careful thought on the part of the authors but still leaves room for some doubt as regards the analysis. I must thus conclude that either the analysis proposed is not optimal or that the authors have failed to provide sufficient clarity to entirely convince at least one reader of its appropriateness.

References

1. Senn S: An unreasonable prejudice against modelling?. *Pharmaceutical Statistics*. 2005; **4** (2): 87-89
Publisher Full Text

Competing Interests: As far as I am aware I have no competing interests. However I was once involved in designing a trial that used tranexamic acid (as a control treatment) in hereditary angioedema (HAE) and I have consulted for various companies on the subject of HAE. I maintain a full declaration of interest here: http://www.senns.demon.co.uk/Declaration_Interest.htm

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 May 2017

Kastriot Dallaku, Ludwig-Maximilian University, Munich, Germany

Many thanks for this comment. We will add the baseline adjustment to the linear model used to quantify treatment effects.

Competing Interests: No competing interests were disclosed.

Reviewer Report 06 February 2017

https://doi.org/10.21956/wellcomeopenres.10886.r18944

Anne-Mette Hvas
Department of Clinical Biochemistry, Centre for Haemophilia and Thrombosis, Aarhus University Hospital, Aarhus, Denmark

The title is appropriate.
The abstract provides an adequate summary of the manuscript.

The presented study is scientifically sound; The study is well-designed, it seems well conducted and the laboratory methods used to investigate the research questions are relevant. However, the statement on whether a possible effect of TXA is due to inhibition of plasmin cannot be investigated (the authors don't

investigate the mechanisms of the TXA-effect), so that is a theoretical deduction. The statistical approach is clearly and comprehensively stated.

Competing Interests: No competing interests were disclosed.

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

Author Response 29 May 2017

Kastriot Dallaku, Ludwig-Maximilian University, Munich, Germany

Thanks for this comment and the clear formulation of the limitation given by our approach. This comment will be relevant when discussing the results of the trial.

Competing Interests: No competing interests were disclosed.