STUDY PROTOCOL

Effect of tranexamic acid on coagulation and fibrinolysis in women with postpartum haemorrhage (WOMAN-ETAC): protocol and statistical analysis plan for a randomized controlled trial [version 1; referees: 3 approved]

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Abstract

Background: Postpartum haemorrhage (PPH) is a leading cause of maternal death. Tranexamic acid has the potential to reduce bleeding and a large randomized controlled trial of its effect on maternal health outcomes in women with PPH (The WOMAN trial) is ongoing. We will examine the effect of tranexamic acid on fibrinolysis and coagulation in a subset of WOMAN trial participants.

Methods: Adult women with clinically diagnosed primary PPH after vaginal or caesarean delivery are eligible for inclusion in the WOMAN trial. In a sub-group of trial participants, blood samples will be collected at baseline and 30 minutes after the first dose of tranexamic acid or matching placebo. Our primary objective is to evaluate the effect of tranexamic acid on fibrinolysis. Fibrinolysis will be assessed by measuring D-dimers and by rotational thromboelastometry (ROTEM). Secondary outcomes are international normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, haemoglobin and platelets. We aim to include about 180 women from the University College Hospital, Ibadan in Nigeria.

Discussion: This sub-study of WOMAN trial participants should provide information on the mechanism of action of tranexamic acid in women with postpartum haemorrhage. We present the trial protocol and statistical analysis plan. The trial protocol was registered prior to the start of patient recruitment. The statistical analysis plan was completed before un-blinding.

Trial registration: The trial was registered: ClinicalTrials.gov, Identifier

Open Peer Review

Referee Status: ✓ ✓ ✓

	Invited Referees 1	2	3
version 1 published 16 Dec 2016	✓ report	✓ report	✓ report

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NCT00872469 https://clinicaltrials.gov/ct2/show/NCT00872469; ISRCTN registry, Identifier ISRCTN76912190 http://www.isrctn.com/ISRCTN76912190 (Registration date: 22/03/2012).

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Background

Postpartum haemorrhage (PPH) is one of the most common obstetric emergencies and is a leading cause of maternal mortality world-wide[1]. Most of the deaths are in low and middle-income countries and most deaths occur soon after childbirth[2]. Severe postpartum bleeding can sometimes be managed by the administration of oxytocin and other uterotonic drugs[3]. However, if uterotonics fail to control the bleeding, surgical intervention may be required.

Tranexamic acid reduces bleeding by inhibiting the enzymatic breakdown of fibrin blood clots by plasmin[4]. A systematic review of clinical trials of tranexamic acid in surgery showed that it reduces blood loss by about one-third[5,6]. Tranexamic acid also reduces mortality in bleeding trauma patients. When given within three hours of injury, tranexamic acid reduces the risk of death due to bleeding by approximately one-third[7]. Early activation of fibrinolysis is common after trauma and worsens bleeding[8]. Trauma triggers the release of tissue plasminogen activator (TPA), the enzyme that converts plasminogen to plasmin and the resulting fibrinolysis plays a key role in the pathogenesis of trauma induced coagulopathy. Early administration of tranexamic acid in bleeding trauma patients inhibits fibrinolysis and prevents coagulopathy[9].

Increased fibrinolytic activity is also observed after childbirth[10]. Within 1 hour of delivery, the serum concentration of TPA doubles, possibly due to the trauma of childbirth[10]. Active PPH is associated with an early increase in D-dimers and plasmin-antiplasmin complexes[11]. A randomised trial conducted in obstetric centres in France found that the increase in D-dimers can be inhibited by tranexamic acid administration[11]. To examine the effect of tranexamic acid on fibrinolysis and coagulation in women at high risk of death after PPH, we aim to conduct a randomised double blind placebo controlled trial in Ibadan, Nigeria (World Maternal Antifibrinolytic Trial-Effect of Tranexamic Acid on Coagulation [WOMAN-ETAC]).

Methods

Hypothesis: We hypothesise that tranexamic acid will reduce death due to bleeding in women with PPH by inhibiting the breakdown of fibrin clots thus preventing or reducing the severity of coagulopathy. Therefore, our primary aim is to determine the effects of tranexamic acid on indicators of fibrinolysis and our secondary aim is to determine the effect of tranexamic acid on coagulation.

Design: A randomised double blind placebo controlled trial will be conducted as a sub-study within the WOMAN trial (Figure 1). The aims and methods of the WOMAN trial are described in detail elsewhere[12,13]. Briefly, adult women with clinically diagnosed primary PPH after vaginal or caesarean delivery are eligible for inclusion. After the appropriate consent procedure has been followed, each patient is randomly allocated to receive 1 gram of tranexamic acid or matching placebo by intravenous injection. If bleeding continues after 30 minutes, or if bleeding stops and restarts within 24 h, a second dose of 1 g of tranexamic acid or placebo may be given. The trial will be conducted in accordance with versions 1 and 1.1 of the protocol.

To examine the effect of tranexamic acid on fibrinolysis and coagulation, in a sample of trial participants we will collect blood at baseline and 30 minutes after the first dose of tranexamic acid or matching placebo. Our primary objective is to evaluate the effect of tranexamic acid on fibrinolysis. Fibrinolysis will be assessed by measuring D-dimers and by rotational thromboelastometry (ROTEM). Secondary outcomes will be international normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, haemoglobin and platelets. We aim to include about 180 women from the University College Hospital, Ibadan in Nigeria.

Number of patients needed: We assume that D-dimer mean and standard deviation in the control group will be 9,000 ng/mL and 7,200 ng/mL respectively. Taking into account that we would adjust for baseline measurement and assuming a correlation between baseline and follow-up of 0.4, we estimate that a study with about 180 patients would have 90% power (two sided alpha=5%) to detect a reduction of 30% in the mean D-dimer value in the tranexamic group.

a) Blood sample collection: Immediately after we randomise a WOMAN trial participant but prior to giving the trial treatment, approximately 15 mL of venous blood will be drawn:

- Two 5 mL samples collected in 5mL vacutainer tubes containing 0.5 mL sodium citrate (0.109mol/L) for coagulation tests and thromboelastometry.
- One 5 mL sample in a 5 mL vacutainer tube containing EDTA.K3 for full blood count analysis.

Once the sample is collected, we will give the first dose of the WOMAN trial treatment according to the WOMAN trial protocol. Approximately 30 (± 15) minutes after administration of the first dose of treatment, we will collect a second blood sample in the same way.

b) Blood sample analysis: We will measure D-dimers, INR, PT, APTT and fibrinogen using the HumaClot Junior automated coagulation analyser (Human, GmBH, Germany). We will centrifuge the blood sample at 3000 g for 20 minutes before analysis. We will measure thromboelastometry parameters at 37°C using two of the four channels (EXTEM, APTEM) on the ROTEM coagulation analyser [TEM®, Munich, Germany]). The EXTEM test activates haemostasis by adding tissue factor. The result is influenced by extrinsic coagulation factors, platelets and fibrinogen. EXTEM is a screening test for the (extrinsic) haemostasis system. The APTEM test is an EXTEM based assay in which fibrinolysis is inhibited with aprotinin. A substantial improvement in clot parameters in APTEM compared to EXTEM suggests fibrinolysis. We will obtain a full blood count using a five parameter particle counter Sysmex KN analyser (Sysmex Corporation, Kobe, Japan).

c) Quality control: We will store ROTEM reagents in a refrigerator at 2–8°C and will monitor the temperature on a temperature monitoring log. Once opened, ROTEM® reagents have a limited shelf life (EXTEM, 8 days after opening; APTEM, 14 days after opening).

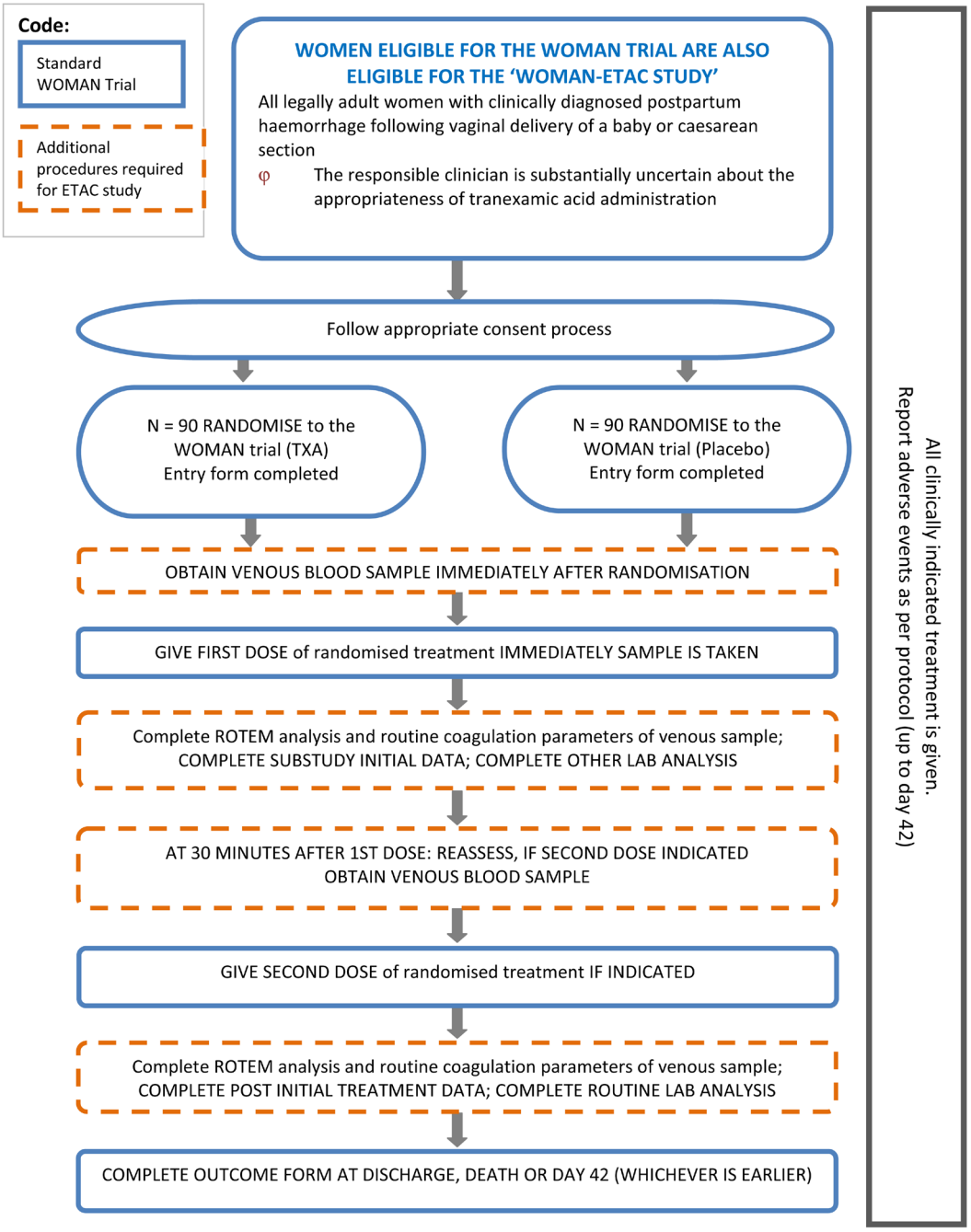


Figure 1. Study overview.

After opening a new bottle of reagent, we will write the expiry date of the reagent on the label. We will not use out of date reagents. We will conduct routine quality control (QC) analyses in accordance with the trial's standard operating procedures. Only trained personnel will make ROTEM measurements. TEM Innovations GmbH staff will train key personnel before the start of the trial. The lead investigator will train new staff members. We will store ROTEM data on the machine but will take a back-up after each analysis. These will be sent to the Trial Co-ordinating Centre in London. Quality Control of the HumaClot Junior (Human, Germany) will be as per the manufacturer's instructions . We will file QC reports at the study site so that they are available to the trial team, monitors and auditors.

d) Other data collection: We will collect patient entry and outcome data as per the WOMAN trial protocol. In addition, we will collect

the following information: time of blood samples, time trial treatment is administered, time laboratory analysis started and ended, any treatment given that may affect coagulation, adverse events, and technical problems with analysis (Supplementary material). Any untoward medical occurrence affecting a trial participant up to day 42 will be reported in line with the WOMAN trial protocol.

e) Potential risks to participants: The study involves two blood tests about 30 minutes apart which may cause pain and bruising at the venepuncture site. The results of the routine laboratory tests can be used to guide treatment in line with local procedures. However, the ROTEM tests will not be used to guide treatment and are for research purposes only.

(f) Roles and responsibilities: The WOMAN-ETAC trial is sponsored by the London School of Hygiene and Tropical Medicine. The Trial Steering Committee (TSC) in place for the WOMAN trial and will be informed of this study. Decisions of the TSC may impact directly the continuation of the WOMAN-ETAC study. If required by the TSC, information about the WOMAN-ETAC study will be reported routinely.

Adverse events which are directly associated with the WOMAN-ETAC study will be reported to the Data Monitoring Committee which is in place for the WOMAN trial. Otherwise there will be no routine review of the accumulating data for the WOMAN-ETAC study.

Analysis

Exploratory analyses

We will conduct exploratory analyses to examine the association between clinical parameters (age, type of delivery, blood loss volume, cause of PPH, blood pressure, clinical signs of shock) and the presence or absence of coagulopathy as well as the presence or absence of "hyperfibrinolysis." Coagulopathy will be defined as an INR >1.2 and A5 ≤ 40mm[15,16]. Hyperfibrinolysis will be defined as ML>15% on ROTEM.

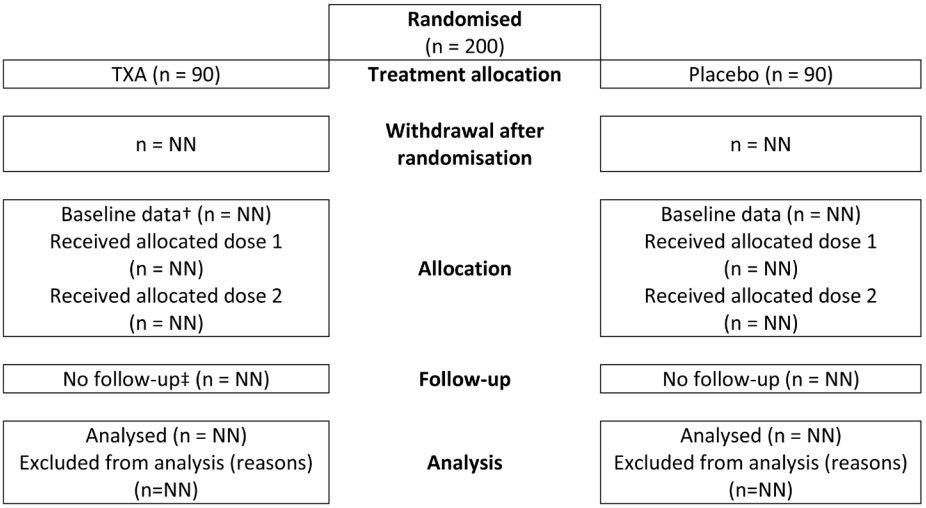
We will report the univariate odds ratios for each clinical parameter. All variables will be included in a model to assess multivariate odds ratio. Likelihood ratio tests (LRT) will be used to evaluate statistically if a variable is a risk factor.

Main analysis

We will report participant progress through the trial in a Consolidated Standards of Reporting Trials (CONSORT) flow diagram (Figure 2). We will report the number of participants randomised, allocated to each treatment, lost to follow up or excluded from the analysis (e.g. samples for which there were technical problems with processing). We will conduct per-protocol analysis that include all participants who satisfy the eligibility criteria, receive the allocated treatment, have follow up samples and at least one measurement of the primary outcome. We will not exclude outliers or impute missing data since this would be inappropriate in a study aimed at understanding the biological effects of tranexamic acid. If the coagulation analyser reports, "*no clot detected*" in lieu of a numeric result, the patient will be excluded from quantitative analysis. However, we will report the number of patients in whom there was no clot detected.

Baseline data: We will report participant characteristics and baseline data as numbers and percentages. We will report means and standard deviations for normally distributed parameters and medians and interquartile (25% and 75%) ranges for non-normal distributions. We will test normality using the Shapiro-Wilk test. We will present dichotomous variables as number and percent.

Co-primary outcomes: We will assess the effect of tranexamic acid on fibrinolysis by examining d-dimer and maximum clot lysis (ML). D-dimer is a sensitive marker of fibrinolysis. ML (EXTEM



† Baseline data relates to those patients with information on at least one primary endpoint
‡ No follow-up relates to those patients where there is no information on both co-primary endpoints

Figure 2. Trial profile.

ROTEM®) is the percentage reduction after maximum clot formation (maximum strength of the clot firmness reached during the test) at the point of lowest clot amplitude. The time point at which ML is calculated varies, since it is calculated at the point of lowest clot amplitude during the test after maximum clot formation, which varies by participant. EXTEM ML is considered to be one of the most sensitive ROTEM® measures of fibrinolysis[15]. If tranexamic acid inhibits fibrinolysis, we would expect to see lower D-dimer measurements and ML percentages in the tranexamic acid group versus placebo. Where the primary outcomes have non-normal distributions, the data will be transformed. We will assume variances to be equal since study participants are drawn from the same population. For each co-primary outcome, we will compare the follow up results of each treatment group (t-test), and conduct a regression analysis that includes the baseline measure.

Secondary outcomes: We will assess the effect of tranexamic acid on coagulopathy by examining a range of thromboelastometry parameters using the follow up samples. These will include clotting time (CT), the interval from the start of the test until a clot firmness of 2 mm is reached; clot amplitude at 5 (A5) and ten minutes (A10); maximum clot firmness (MCF) which is the maximum clot amplitude reached during the test. Studies in bleeding trauma patients show that ROTEM A5 is a sensitive indicator of coagulopathy. When coagulopathy is defined as an INR >1.2, a threshold level of EXTEM A5 ≤ 40 mm has a sensitivity of 73%[16]. If tranexamic acid inhibits fibrinolysis and prevents coagulopathy we would expect higher values for clot amplitude A5, A10, and MCF in the tranexamic acid group compared to placebo. We will also compare the lysis index at 30 (LI30) and 60 minutes (LI60). Lysis index measures the ratio of the firmness of a clot at a given time point and MCF. We expect LI30, LI60 to be higher with tranexamic acid. Additionally, we will assess the difference between follow up results of each treatment group in the following parameters: INR, PT, APTT, fibrinogen and haemoglobin. We will analyse secondary outcomes using t-tests. We will conduct regression analysis that include the baseline measure.

Sub-group analyses

Time since delivery: In bleeding trauma patients, the effect of tranexamic acid varies according to the interval between injury and treatment. The Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage (CRASH)-2 trial showed strong evidence that tranexamic acid reduced the risk of bleeding deaths when given within 3 hours of injury but there was no reduction in those treated after 3 hours[7]. Early fibrinolysis is common after trauma, and is associated with an increase in mortality. Trauma initiates the early release of TPA from storage granules in the vascular endothelium. The release of TPA results in early fibrinolysis that exacerbates bleeding[8]. Temporal changes in fibrinolysis have also been observed after childbirth. In the first hour after delivery, there is a doubling of the serum concentration of TPA, possibly due to the trauma of childbirth. After the first hour the concentration of TPA decreases rapidly and by a large amount. At the same time, PAI-1 and PAI-2 (plasminogen activator inhibitors) increase around delivery and for several days. To examine temporal changes in fibrinolytic parameters we will report baseline data stratified by time to treatment (≤3 hours after delivery, and >3 hours after delivery). We expect fibrinolysis to be more prevalent in the first three hours. We will also examine the effect of tranexamic acid on fibrinolysis stratified by time to treatment.

Type of delivery: Because a substantial proportion of all deliveries are by caesarean section and caesarean section is an established risk factor for PPH, it is important to examine whether the biological effect of tranexamic acid varies by type of delivery. Although we hypothesise that the effects of tranexamic acid on fibrinolysis and coagulation will be similar to those in trauma patients, type of delivery might not accurately reflect the extent to which delivery is traumatic. For example, a study in uncomplicated pregnancy found that D-dimer levels after instrumental vaginal delivery were higher than after spontaneous vaginal birth and similar to those after caesarean section[17]. We do not anticipate substantial heterogeneity by type of delivery.

Cause of postpartum haemorrhage: We will examine the effect of tranexamic acid by cause of bleeding (uterine atony versus all other causes) as determined at baseline. We do not anticipate substantial heterogeneity by cause of haemorrhage.

Maternal anaemia: Over fifty million pregnant women are anaemic world-wide[18]. The highest prevalence of maternal anaemia is in Africa (57%) and in South-East Asia (48%). Anaemia is a risk factor for PPH and venous thromboembolism (VTE)[19,20]. Given the high prevalence of anaemia in Africa and Asia, we will examine the effect of tranexamic acid on fibrinolysis and coagulation separately in anaemic and non-anaemic women. We will define anaemia as a haemoglobin concentration less than 110 g/L.

Declarations

Ethics approval and consent to participate: Approvals to conduct this sub-study were obtained from the Ethics Committees of London school of Hygiene and Tropical Medicine (Reference A275 5536) and the University of Ibadan & University College Hospital Ethics Committee (Reference UI/EC/09/0131). Regulatory approval was obtained from the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC). The study will be undertaken according to (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guidelines)[21]. The consent procedures will be as detailed in the WOMAN trial protocol[12]. Briefly, we will seek consent from a patient if their physical and mental capacity allows. If a patient cannot give consent, we will obtain proxy consent from a relative or representative. If a proxy is unavailable, then if local regulation allows, we will defer or waive consent. In this situation, we will inform the patient about the trial as soon as possible, and we will seek consent to use the data. The London School of Hygiene & Tropical Medicine is the sponsor.

Availability of data and material: Following publication of the primary and secondary analyses, the trial team will use the data for at least two years for further exploratory analysis. The totally anonymised data will then be made available via our data sharing website: Free Bank of Injury and emergency Research Data (freeBIRD) website (http://freebird.Lshtm.ac.uk).

Author contributions

Haleema Shakur and Ian Roberts conceived the study and contributed to the statistical analysis plan and writing of the manuscript; Haleema Shakur led the protocol development and is responsible for overseeing the trial and data management; Bukola Fawole contributed to protocol development and has overall responsibility for the study at the trial site and review of the manuscript; Oladapo Olayemi contributed to protocol development and is the site principal investigator for WOMAN trial and review of the manuscript; Modupe Kuti contributed to the protocol development and was responsible for overseeing laboratory tests, laboratory standard operating procedures and staff training and review of the manuscript; Adenike Bello contributed to protocol development and was responsible for data transfer and review of the manuscript; Olayinka Ogunbode contributed to the protocol development, development of the Standard Operating Procedures and was responsible for participant recruitment and review of the manuscript; Chris Aimakhu contributed to the protocol development and was responsible for participant recruitment and review of the manuscript; Taiwo Kotila contributed to the protocol development and was responsible for routine laboratory tests and review of the manuscript; Meghann Gregg led the development of the statistical analysis plan and review of the manuscript; Sumaya Haque contributed to the statistical analysis plan.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by the Department of Health (UK), grant number HICF-T2-0510-007 and the Wellcome Trust, grant number WT094947. The Bill & Melinda Gates Foundation (grant number OPP1095618) and Tem Innovations GmbH.

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

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Supplementary material

Data Form

Click here to access the data.

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Version 1

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This article describe the WOMAN-ETAC protocol as a substudy of the WOMAN trial.

In 180 women from the University College Hospital, Ibadan in Nigeria, fibrinolysis will be assessed at baseline and 30 minutes after the first dose of tranexamic acid or matching placebo, by measuring D-dimers and by rotational thromboelastometry (ROTEM). Secondary outcomes are international normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, haemoglobin and platelets.

The article is correctly written and the ethics, quality and administrative research controls are well configured.

Some questions and suggestions may help to improve the manuscript.

Recruitment:

- Please specify if the 180 patients are the consecutive series of the Nigerian center, allowing a blinded unselected distribution of the groups among the substudy.

P4: **Number needed to study**:

- The term "about" should be avoided. If the objective of 180 is not reached, then explain. In Figure 2 trial profile, n=200?
- The control group expected level of D-dimers is 9000±7200 ng/mL.
- Please clarify the timing of this expected control level in the course of the bleeding. How was this high level estimated?

When considering the D-dimers levels in untreated and treated haemorrhagic patients in Exadeli trial, the values were as follow (Ducloy-Bouthors *et al*. 2016), slightly less than your control group level.

Group Control untreated Haemorrhagic n=72

	Enrolment	+30 min	+2 h	+6 h	*P value
D-dimers					

(ng ml–1)	3730 [2468–8493]	6158 [3600–10000]	7495 [4400–15772]	4936 [2905–8278]	0.0001

TA treated Haemorrhagic n=72

	Enrolment	+30 min	+2 h	+6 h	*P value
D-dimers (ng ml–1)	3645 [2222–6223]	5556 [3087–7598]	3888 [2751–6123]††	2687 [1768–4502]††	0.004

This could modify the number needed to observe the TA effect on D Dimers.

P4 and 5: **ROTEM parameters.to diagnose hyperfibrinolysis:**

- Please clarify the type of ROTEM analyzer: delta or sigma.
- Concerning APTEM test, please make sure with TEM-international that the antifibrinolytic reagant used has been changed from aprotinin to tranexamic acid and precise the concentration of the APTEM reagant.
- Please clarify if the quality control Rotrol will be performed by the trained personnel every week as commonly or before each of test.
- Replace "is administered" by "administration"
- Lysis onset time LOT has been used to increase the performance of ROTEM analysis to detect and diagnose hyperfibrinolysis (Faraoni *et al. 2015*) should be also an interesting parameter to be tested

p5: **Definition of the coagulopathy**:

- The definition of the coagulopathy seems to be based on the trauma definition of the coagulopathy and hyperfibrinolysis: INR >1.2 and A5 < 40mm and ML>15% on ROTEM respectively.
- The PPH associated coagulopathy and ROTEM cutoff have been described by Huissoud (2009) then Collins (2014).
- Because the thrombin generation and plasmin generation seems very close and appears both early in the course of PPH (Ducloy-bouthors *et al.* P-011&-012, 2017), and the decrease in fibrinogen seems parallel to the increase in D-dimers, the cutoffs established by Collins to diagnose PPH induced coagulopathy should be used. Because ISTH DIC score is not sensible in PPH population and the new score has been validated (Jonard *et al.* 2016), the threshold to should be adapted

P6: **Secondary parameters:**

- Timed measure of fibrinolytic activity after childbirth needs a better description in non haemorrhagic as well as untreated and treated haemorhagic groups. The WOMAN-ETAC data will be very helpful to this better understanding and ongoing TRACES trial () will complete these data. The duration of both the hyperfibrinolytic activity and the tranexamic action is also very useful to establish with no previous data.
- The duration and intensity of the antifibrinolytic activity should be interconnected with the estimated bleeding volume and the initial estimated bleeding flow. To be aware of the estimated initial bleeding volume and flow, two methods may be used and mutually consolidated: the visual estimation (although imperfect) and the hemoglobin delta between end of pregnancy and the first

available hemoglobin (if possible before dilution due to vascular loading). The resuscitation and transfusion parameters should be considered to understand the variation of the coagulopathic and fibrinolytic process.

- Type of delivery: Delivery is not spontaneously traumatic and obstetrics coagulation activation should not be considered as a trauma induced coagulation and fibrinolysis activation. The aim of this coagulation and fibrinolysis activation is the physiological closure of the placental vascular bed after placental removal. This coagulation and fibrinolytic activation remains local and uterine-limited since the uterine tone blocks the dissemination of the large amounts of tissue factor. In trauma patients, initial shock and tissue damage are directly correlated to coagulopathy and mortality, whereas initial obstetrics coagulation and fibrinolysis activity is rarely associated with shock, even in late and severe evolution when transfusion and vascular loading are given.

  Thus as the authors expect, the type of delivery will not influence when considering the placental bed coagulation and fibrinolytic activation. However in the instrumental delivery as well as in caesarean section, the surgical part of the bleeding may be so severe that the hypovolemic shock could be associated. Perhaps should it be possible to analyze in clinical parameters the origin of bleeding and to confirm these data by a lactate measurement on the initial and 30 minutes blood samples.

- Maternal anemia is an interesting topic where tranexamic acid may reach an important public health objective. Goswani *et al.* (2013) already showed this favourable impact in prophylactic use during non haemorrhagic CS.

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Competing Interests: No competing interests were disclosed.

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.

Referee Report 06 March 2017

doi:10.21956/wellcomeopenres.11189.r20530

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This is a well thought-out study and will add important information to the literature. Given that the study has now finished, and the data analysis plan finalised and the study un-blinded (as per the abstract discussion) it would be inappropriate to suggest any changes to the protocol or analysis. There are however a number of ways in which the protocol could be written more clearly so as to ensure clarity for those wishing to replicate or understand the study:

1. In METHODS (b) it would be good to state exactly how long after the blood sample was taken it was analysed and whether any steps were taken to ensure that the sample did not deteriorate (eg kept on ice, or analysed within a certain time period)

2. In the same section it would be good to be explicit about how long the ROTEM protocol lasted and stating that (I assume) the ROTEM was allowed to run for the full X minutes. This is important for the assessment of lysis as that may continue to change for some time and so lysis data would be different if the ROTEM was interrupted at 10 minutes or at 20 minutes.

3. Can the authors clarify who took the samples and put them through the ROTEM analysis, and whether the results were then revealed to the clinicians? If it was, it could have the effect of unblinding those doing the clinical care and collecting the data.

4. In ANALYSIS, the definition of ML and A5 need to be clarified.

5. I assume that there are 2 of each of the ROTEM measurements for each participant with one relating to the EXTEM and the other for the APTEM. If not, then please clarify this in the script – but if there is, then each description of MCF, A5 etc should clarify which measure it comes from.

6. At the bottom of ‘Main analysis’ it states that the machine may not give data if there was 'no clot detected'. Is this correct? Surely the sodium citrate will prevent clot formation and it is only if ‘Clot Detected’ that the analyser will not work.

Competing Interests: No competing interests were disclosed.

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.

Referee Report 01 February 2017

doi:10.21956/wellcomeopenres.11189.r19830

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Title and Abstract: The title and abstract are appropriate for the content of the article.

Article content: The study design and methods are explained clearly and with sufficient detail. I have focussed on the analysis section as this is where my expertise lies.

Some consideration should be given to the possibility of under-representation of women with more severe PPH, and how that may affect the conclusions of the study. Since some time is required to take the blood samples before the first dose of study drug is administered, is there a possibility that the more severe cases of PPH will not be included in this sub-study, or may have a baseline value but no follow-up value? An analysis examining the representativeness of the women included in the sub-study versus the remainder in the main WOMAN study may help to assess this issue.

The use of a per-protocol analysis population is reasonable, given the objective of the study. With regard to the definition of this population, should this also exclude women who have received a second dose of study drug before the follow-up blood samples were taken, in the rare cases when this might happen? Have the authors considered conducting a sensitivity analysis using an intention to treat analysis population to assess the robustness of the conclusions?

The authors specify that they will use t-tests as well as regression analyses adjusting for baseline values to analyse the outcomes. They should make clear which analysis method will be considered the primary analysis. It would also be helpful to include how the outcome data will be reported (for example as adjusted mean differences), and if confidence intervals and/or p-values will be reported. The level of confidence intervals for the primary and secondary outcome results should also be specified, as well as the level to be used for statistical significance.

The rationales for each of the sub-group analyses are clearly described. A brief description of the method of analysis should also be included. Will tests of interaction be reported?

There is a minor error in the numbers reported in Figure 2. The number randomised should be 180, not 200.

Competing Interests: No competing interests were disclosed.

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.