Author’s response to reviews

Title: A pragmatic approach to sonothrombolysis in acute ischaemic stroke: The Norwegian randomised controlled Sonothrombolysis in Acute Stroke Study (NOR-SASS)

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Author’s response to reviews: see over
Author’s covering letter for initial submission

Title: A pragmatic approach to sonothrombolysis in acute ischaemic stroke: The Norwegian randomised controlled Sonothrombolysis in Acute Stroke Study (NOR-SASS)

Authors:

Version: 1 Date: 11 February 2015

Comments: see over
BMC Neurology
The Editor

Dear Madam/Sir,

We thank the reviewers for their careful reading of our paper and their constructive comments and criticism. There were a large number of questions raised, partly related to technical aspects. The reviewers also recommended a more thorough discussion on bleedings related studies like TUCSON and TRUMBI.

We have therefore refocused the paper and 1) stress the pragmatic approach to sonothrombolysis in an unselected stroke population and 2) elaborate to some extent on technical aspects and bleeding risk (TUCSON, TRUMBI).

Our responses to the reviewers’ questions/comments are included in the Reviewers’ text below.

1st Reviewer’s report
Title: The Norwegian Sonothrombolysis in Acute Stroke Study (NOR-SASS): A randomised controlled trial of contrast enhanced ultrasound treatment in acute ischaemic stroke
Version: 4 Date: 11 August 2014
Reviewer: Evan Unger

Reviewer’s report:
You have designed a multi-center clinical trial to test efficacy of sonolysis with MB in acute stroke. I think that you should do power calculations to show how many patients might be needed in the different groups to detect significant differences in the end-points making assumptions regarding efficacy.

A number of details are missing from your manuscript. Following are my specific comments.

Page 6, first paragraph, you discuss that no study has yet evaluated effect on small peripheral vessels. How will the ultrasound be applied to these vessels? In general vessels are only accessible by ultrasound through the temporal window or the foramen magnum. Then how will these other vessels be accessible? With small peripheral vessels we mean the MCA3-MCA4-segments with ultrasound application through the temporal window, partly accessible by “fanning” of the ultrasound beam (see later, Reviewer 2). In patients with a vertebrobasilar brainstem ischaemia, we apply the same “fanning” through the foramen magnum (page 5).

Page 7, second paragraph, “hypothesize” is misspelled.
Corrected.
Power calculation, page 8. I think you can take results in some of the other studies and make certain assumptions and estimate the number of patients in each treatment arm to attain statistical significance for some of the end-points. Will you perform interim analyses in your study? How will you determine how many patients to enrol. You state that the study commenced in 2012 and will finish enrolment in 2016. How many patients have been enrolled to date?

We have now performed power calculations based on results from our own NORSTROKE data base from before the initiation of the present study, published thrombolysis series in mild stroke and on the meta-analysis of sonothrombolysis studies (Saqqur 2013). The calculation is presented at page 6 An interim analysis is per protocol planned after ~2 years. Due to slow (no) recruitment of additional centres, the steering committee decided to postpone the interim analysis until Q2 2015.

Per November 2014, we have enrolled 158 patients.

Page 8, patient recruitment and inclusion criteria, you state that a pre-treatment arterial occlusion on CTA is not necessary for inclusion because you include sonolysis of the peripheral vascular bed. How do you accomplish this with the ultrasound? If there is no occlusion, how do you know where to apply the ultrasound. How do you account for acoustic attenuation through the skull.

Present page 8: The investigator judges the clinical picture and documents where she/he thinks the probable lesion/clots is located. Ultrasound is applied based on this assumption. MR examination the next day demonstrates the DWI lesion(s). The investigator thereby knows whether the initial ultrasound was aimed at the correct region of interest or not. This information will be part of our analysis.

We cannot account for or measure acoustic attenuation through the skull. As suggested by reviewer 2, we will perform measurements of skull thickness in the area of insonation and include this information in the final analysis. The assessment of skull thickness will be performed by a physician blinded to clinical information, except for the point of insonation (page 7).

General exclusion criteria, page 9. You exclude patients with mRS#3. So you are treating relatively mild strokes. The mild strokes tend to resolve on their own relatively frequently. This might make it more difficult to detect a meaningful difference between the treatment groups. You may wish to discuss this. You might cite literature where investigators have studied mild stroke.

Present page 23. We exclude patients with premorbid mRS ≥3 (table 1). In accordance with the pragmatic design of the study, we do include all patients regardless of clinical severity of the index stroke.
We acknowledge the reviewer’s important comment on mild stroke and have added a paragraph on this topic under Discussion page 14.

Sonolysis, page 11, 12, please verify that the ultrasound was continuous wave as you say. Most investigators use pulsed wave. You may wish to discuss the rationale for choosing the ultrasound parameters that you used. Please describe how ultrasound was applied to vessels. What was the footprint of the transducer?
Present page 8. The text was definitively misleading. “Continuous” referred to 60 minutes of insonation. We use pulsed wave Doppler and have changed the text accordingly.

Were the temporal windows or foramen magnum used? Was the ultrasound applied across the skull irrespective of the window? If so did you do acoustic measurements to assess the effect on attenuation? You also might explain how the presumed location for insonation was determined in the absence of a proven occlusion on CTA.
Present page 8. The temporal window and the foramen magnum were both used. We have now specified the approach in the text.
We are not able to assess the effect of attenuation and have added this information under Discussion (page 15). As mentioned above, we will perform measurements of skull thickness in the area of insonation as a surrogate marker of skull penetration and include this information in the final analysis. The investigator judges the clinical picture in cooperation with the treating physician and decides where she/he thinks the probable lesion/clot/ischemia may be. Ultrasound is applied based on this assumption. MR examination the next day demonstrates the DWI lesion(s). The investigator thereby knows whether the initial ultrasound was aimed at the correct region of interest or not. This information will be part of our analysis (page 8, 10).

Vascular outcome (recanalization) – was the reviewer(s) blinded to clinical data? How many reviewers were used? If multiple readers, was it consensus read or independent reads?
Present page 9. Recanalisation at 1 hour is by necessity performed unblinded by the investigator. MR/CT-recanalisation at 24 hours will be performed by independent, blinded reviewers. For a limited number of patients, multiple readers will test consensus. The final analysis plan will be set up prior to the closure of the database and the statistical analyses.

Discussion, page 17, you should include discussion of the TUCSON trial and perhaps the TRUMBI trial where there was hemorrhage in both of these
We agree that this is an important topic and have included TUCSON, TRUMBI and Eggers 2008 in Background (page 3) and Discussion (page 12-13).
2nd Reviewer's report

Title: The Norwegian Sonothrombolysis in Acute Stroke Study (NOR-SASS): A randomised controlled trial of contrast enhanced ultrasound treatment in acute ischaemic stroke

Version: 4 Date: 18 August 2014

Reviewer: Stephen Meairs

Reviewer's report:

There is no doubt that a large multicenter trial is needed to investigate the efficacy of an adjunct therapy of ultrasound and microbubbles combined with rt-PA in ischemic stroke patients. Although recent meta-analyses of previous trials of sonothrombolysis suggest safety and efficacy, it should be kept in mind that meta-analyses that are performed by advocates of a specific therapeutic strategy are notoriously biased. It is therefore very important that more solid data is acquired to translate this promising approach into routine clinical practice. As a whole, the NOR-SASS study has been carefully designed and should help in contributing data for evaluation of sonothrombolysis in ischemic stroke. My further comments will be directed towards possible enhancement of the quality of the proposed NOR-SASS study to meet this goal.

First, let us apply what we may have learned from previous trials of ultrasound and microbubbles for stroke therapy. Mistakes that were made in the TUCSON study, which was unfortunately discontinued prematurely, should not be duplicated. An excellent message to this extent was published by Patrick Lyden in Annals of Neurology entitled “Premature Closure of the TUCSON Trial: Stroke Research Is Not for the Faint of Heart” (Annals of Neurology Vol 66 No 1 July 2009). In his letter he emphasized that “a statistical analysis should have been done to logically and rigorously define a stated sample size needed to detect elevated hemorrhage rates. Such a calculation would have yielded a sample size that would have avoided the imbalances seen in this trial, which led to the apparent elevation of the hemorrhage rate in the second dose tier.” Thus, trials must be rigorous, with appropriate blinding, randomization, and most importantly adequate power. I do not agree with the reasons presented by the study initiators to refrain from providing sample size for their study. The comments regarding the TUCSON trial are likewise relevant for safety monitoring in the NOR-SASS study.

We have given iv thrombolysis as routine treatment since 1998 and agree with Dr. Lyden that Stroke Research Is Not for the Faint of Heart. We also basically agree with the reviewer and therefore have included a power/sample size calculation based on 1) our own patients receiving thrombolysis before the start of NOR-SASS (NORSTROKE registry; n=~250), 2) published thrombolysis series in mild
stroke and 3) data from published meta-analysis of sonothrombolysis, albeit with the in-built uncertainties and possible bias (page 6). We have used NIHSS score improvement during the first 7 days as variable for the power calculation.

The well-known concomitant risk factors for sICH - age, baseline severity, blood pressure, perhaps elevated glucose - do impact all thrombolytic trials. Rigorous trial design is the only insurance against random occurrence of more hemorrhages in a group early in the trial. We carefully monitor and treat hypertension and hyperglycemia during the treatment period and thereafter, include all patients regardless of age or baseline severity and do believe that NOR-SASS has a rigorous design which will cope with this (page 10-11).

The study stresses the novelty of adding ultrasound and microbubbles to thrombolytic treatment with tenecteplase. Although such data could be interesting, to my knowledge there is not even one preclinical study supporting such a therapeutic strategy. Moreover, a safety study has not been performed with tenecteplase, ultrasound and microbubbles. Thus, this combination therapy is definitely not qualified to enter a phase III trial as the authors suggest – “NOR-SASS is conducted as phase III trial without prior power calculation”. But in the statistical analysis, NOR-SASS A, B and NOR-SASS C are characterized as explorative pilot-studies and will not be compared with each other. These discrepancies are confusing and should be clarified.

We acknowledge that tenecteplase has not been studied with sonothrombolysis and agree that the tenecteplase combination therapy cannot be considered a phase III trial. The main aim of NOR-SASS is, however, to study sonothrombolysis in patients receiving “any kind of thrombolysis” and we present this as a phase III trial. The number of patients receiving tenecteplase will probably be too low to yield definite effect results, but may be seen as a smaller phase II safety sub-study. We agree that the discrepancy stated is unfortunate and confusing. We have clarified this by deleting the term “explorative pilot-studies” in the article and specified the mix of phase II and phase III (page 14). NOR-SASS A+B contains patients with “any kind of thrombolysis”, whereas NOR-SASS C contains patients without thrombolysis. These two groups will not be compared with each other.

Adding tenecteplase to the study protocol adds another arm to the study with a need to enroll many extra patients. Here again the number of patients required is not mentioned. Is this portion of the study rather a safety, dose de-escalation study? Indeed, the study protocol calls for a decrease in the dosage of tenecteplase in case of an increased number of hemorrhages. I would suggest that the study group cancel this combination and focus on the primary goals of the trial.

We agree that the NOR-SASS set-up is complex. NOR-SASS A+B contains patients with “any kind of thrombolysis” and tenecteplase is thus not a separate arm. We have specified under Design that NOR-
SASS is superimposed on the randomised alteplase-tenecteplase trial NOR-TEST (page 6). NOR-TEST is running and cannot be stopped through NOR-SASS. The statement that increased bleeding rate in NOR-SASS will be followed by a dose reduction of tenecteplase is unfortunate and incorrect. We have corrected the stopping rules on page 11.

There are several issues, which could be clarified in the study protocol.
1) Patients with unsuitable bone windows should not be entered into the study. The criteria for poor bone windows should be defined in the protocol. For pragmatic reasons, patients with low quality bone windows are included in the study, similar to the approach in CLOTBUST-Hands Free (Barreto et al. 2013), since this approach probably will be the way patients are handled in “real life”. These patients are registered as having a surrogate marker (bone thickness) for reduced ultrasound effect through the skull. This aspect will be analysed separately. We have added a definition for a good bone window on page 8.

2) Administration of ultrasound therapy is performed with either a hand-held TCD probe or a probe in a fixation head-band. There will be “fanning” per hand, but not with device. The purpose of “fanning” will be to increase the therapeutic volume of insonation to the brain. How can the two administration techniques be compared? This will create problems. Sometime the use of a head band is difficult and time consuming. In these cases a hand-held approach is accepted for real-life pragmatic reasons. A “fanning” is performed both in the head-band and the hand-held approach. This is now specified on page 8.

3) A critical issue involves the actual dose of ultrasound to the intended site of therapy. For safety, efficacy, and reproducibility of treatment, it would be ideal to evaluate the cavitation state (moderate oscillations, stable cavitation, and inertial cavitation) and activity level in and around a treatment area. Indeed, a number of approaches for achieving this type of monitoring are currently being tested. I doubt that such devices can be implemented in this clinical trial. However, it would be helpful to at least have an estimate of the bone window thickness for semi-quantification of the applied ultrasound therapy (see recent article by Alexandrov group). We agree with the reviewer. We can, however, neither assess the cavitation state nor measure acoustic attenuation through the skull. As the reviewer suggests, we will perform measurements of bone window thickness in the area of insonation and include this information in the final analysis. The assessment of bone window thickness will be performed by a physician blinded to clinical information, except for the point of insonation. We have included this analysis in the text under Assessment methods (page 7) and Discussion (page 15).
4) Both bolus and infusion of microbubbles are used. This will certainly present significant problems for data assessment. I would suggest to use one or the other method, but not both. We agree. We have so far only used pump infusion of microbubbles and have now deleted the option of bolus administration (page 8).

5) The protocol describes “In patients with presumed occlusion of perforating arteries (lacunar infarct), insonation is performed with the sample volume at the depth of the proximal MCA1-segment at 50 mm. In patients with presumed occlusion of other arteries, the insonation is performed with the sample volume at the depth of the presumed artery.” It is highly doubtful that ultrasound can be directed to lacunar infarctions in a meaningful and consistent manner in this study. Also the idea that ultrasound can be applied to more distally occluded arteries by adjusting the sample volume to a specific depth is not in agreement with transcranial Doppler physics. Indeed, the recent development of new operator-independent systems for sonothrombolysis with multiple transducers (see recent article by Barreto et al Stroke. 2013 Dec;44(12):3376-81) aims at addressing exactly such questions, because conventional transcranial Doppler is not suitable for such treatments. We acknowledge with some embarrassment that the text is misleading and not in agreement with Doppler physics. The original idea was to place the TCD sample volume at a specific depth to assess any arterial flow changes every 15 minutes during the insonation period. The protocol was later changed to assessment of recanalisation with TCCS at 60 minutes, but without changing the whole text accordingly. We have now deleted the text on specific depth settings for the sample volume (page ca. 8).

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.