Author's response to reviews

Title: Decrease in shunt volume in patients with cryptogenic stroke and patent foramen ovale

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Author's response to reviews: see over
Reviewers’ Comments

Reviewer 1 (J David Spence)

Minor essential revision
You seem to imply that the disappearance of a previously detected shunt is a good thing, as it may reduce the risk of future embolization. However, one mechanism by which a PFO may be occluded is by thrombus; thus a false negative bubble study may give false reassurance, as once the thrombus has dissolved or embolized, the shunt will re-open. Pathological photographs of this phenomenon can be seen in: Hutchinson EC, Acheson EJ. Strokes: natural history, pathology and surgical treatment. [4]. 1975. London, W.B. Saunders. Major Problems in Neurology. Editor Walton, J

A re-occlusion of a PFO by a thrombus is indeed a possibility; this is an important differential diagnosis.
In 3 of the patients with shunt reduction from curtain pattern to no evidence for right to left shunt a TEE was performed subsequently after serial ce-TCDs revealed no evidence for RLS. In these 3 patients no thrombus could be observed. Nevertheless this aspect needs to be investigated further!

These aspects were added to the discussion.

Reviewer 2 (Regina Feurer)

Tanislav et al. carried out a study on 102 stroke patients with RLS, in whom volume of RLS at the time of hospital admission for stroke was compared with the RLS volume on follow-up. The conclusions of the authors were that RLS volume decreases over time, especially in patients with cryptogenic stroke and that these temporal dynamics may influence strategies in the management of secondary stroke prevention in patients with PFO. The study is interesting and was a pleasure for me to read and review as, according to my opinion, it can be considered as a valuable contribution to the ongoing debate on how to manage secondary stroke prevention in patients with PFO.
The study design is well-structured, TEE-and c-TCD-protocolls elaborated, results are presented clearly, distinct conclusions are drawn.
I have only one remark concerning the median time between the index event TO for stroke and T1 for follow-up measurement (10 months), with seems rather short to me. It would be interesting to know what will happen to the same cohort of patients over a period of a couple of years.
Nevertheless data and presentation seemed persuasive to me, so that I do not have any major or minor revisions.

Our study is limited as for a precise determination of the time interval over which shunt size dynamics are likely to occur. The 10 mounts should be regarded as a first direction. Further studies are needed to determine the time interval more precisely!!!!

Reviewer 3 (Jill Jesurum)

Discretionary Revisions:

1. NEED TO INCLUDE PRESS RELEASE NMT CLOSURE I STUDY JUNE 2010
The press release NMT CLOSURE I was cited and added to the references.

To support our findings we mentioned the results derived from a small case series of 8 patients suffering of migraine diagnosed of PFO. In consecutive examinations (2 ceTCDs within 6 months) no changes was found.

2. ADDITION OF A CONTROL GROUP (PFO WITHOUT STROKE) USING SAME METHODOLOGY AT T(0) AND T(1) – EVEN A SMALL CONTROL WOULD BE BENEFICIAL TO SUPPORT FINDINGS
(month) no relevant changes in RLS were observed. Nevertheless we stated that this is not an appropriate control group for comparison in our study. This might support our results of a shunt reduction as a physiological process and not a variance of testing.

Moreover in 6 patients with a shunt reduction from initially curtain pattern to no evidence for RLS on T1, we repented the ce-TCD tree times in the subsequent four months. In all examinations a RLS was not detected. Furthermore in 3 of these patients a TEE was performed subsequently, as in these patients a PFO-closure was considered as a therapeutic option. This TEE remained unremarkable as well.

Therefore even based on few selected cases, these findings might definitively underline our results as accurate findings, which are not determined by methodological differences of testing procedures!!!!!

3. SEE METHODOLOGY CONCERNS DETAILED BELOW

**Minor Essential Revisions**

1. **PAPER NEEDS OVERALL EDITING**
   
   The manuscript was corrected and proved by a native speaker.

2. **TABLE 1 IS NOT USEFUL**
   
   From the neurological point of view we consider table 1 as important as it elucidate the distribution of stroke aetiologies in our cohort. And finally one of the main findings of the study is the association of shunt reduction with cryptogenic stroke.

**Major Compulsory Revisions**

1. **ADDRESS BASELINE GROUP DIFFERENCES (AGE, GENDER) AND HOW THIS MAY HAVE AFFECTED RESULTS OF RLS**
   
   Baseline data are illustrated in table 4. One of the noticeable findings when analysing base line data, revealed that patients showing shut reduction were younger (significant in the univariate analysis). The aspect of age was considered in the subsequent logistical regression; in this balanced analysis this factor was not longer significant. The parameter cryptogenic stroke, which is most common in young patients, remained the strongest factor associated with a change in shunt volume. In our study factors such as age and use of anticoagulants were associated with stroke type rather than change in shunt volume.
2. ADDRESS CONCERNS RE: TCD TESTING METHODOLOGY AT T(0)

Indeed this is a main limitation of the present study. Nevertheless in our department the ce-TCD is performed under strictly standardised conditions: patient’s position, the venous catheter is required in the left cubital vein, the Valsalva strain, and the insonated vessel is the proximal part of the left middle cerebral artery by a combined Duplex/Doppler method (See consensus paper: Jauss M, Zanette E (2000) Detection of right-to-left shunt with ultrasound contrast agent and transcranial Doppler sonography. Cerebrovasc Dis 10:490-496). The Echovist preparation is also strictly performed according to the manufacturer’s instructions.

We have a very detailed documentation of examinations in our department. As concerns rose after reading this review, we checked if any of our patients was treated differently when ce-TCD was performed at T0. In none of the patients any suspected documentations could be found.

Furthermore it is important to mention that patients who have not suitable conditions (such as transcranial bone window for ultrasound penetration or a sufficient vein catheter) are usually not scheduled for the procedure.

One of the points which may support the reliability of the TCD data on T0 is the remarkable shunt size concordance in comparison to TEE.

The limitations of T0 data collection and aspects which underlines their validity were added to the discussion.

3. INTER RATER RELIABILITY FOR EMBOLIC TRACK COUNTS AT T(1)

The examinations of ce-TCD were evaluated by two neurologists with expertise in transcranial Duplex/Doppler ultrasound. They were blinded from clinical data and the order of the examinations (no knowledge if analysing T0 or T1). In summary 204 examinations were re-evaluated. In 62 cases a curtain pattern was evident, in 14 cases no shunt was detected; in these 76 cases the interrater agreement was 100%. In the remaining 128 cases with countable pattern in 5 cases the evaluation revealed a different count between the two examiners. In these cases a consensus count was undertaken. The 5 patients graded differently are displayed below:
We calculated the interrater reliability for countable shunts (n=128):
Kappa=0.95; CI: 0.93-0.97; p<0.001.
For T1 alone we calculated the reliability as well, which was even higher than counting T1 and T0 together. We consider the calculated reliability in all examination in a countable range as important; this information was added to the results in the re-submitted manuscript.

Comment: As we achieved a high agreement rate between the two examiners we did not attribute high priority to mention these aspects in the first submitted manuscript. In fact the mistakes were an issue of counting failure and not of misinterpretation of microembolic signals.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rater_1</th>
<th>Rater_2</th>
<th>Consensus_read</th>
<th>T0</th>
<th>T1</th>
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</table>

We do not state, that our study DOES SUPPORT RECOMMENDATION TO “RE-EXAMINE UTILITY OR NEED OF PFO CLOSURE” BASED ON SUGGESTION THAT RLS DECREASES OVER TIME – THIS STUDY NEEDS TO BE REPEATED USING MORE STRINGENT METHODOLOGY BEFORE MAKING THIS RECOMMENDATION.

4. LAST PARAGRAPH IN DISCUSSION – AVOID TERM “HINT” WHEN REFERRING TO CLINICAL IMPORTANCE AND APPLICATION. THIS IS NO TIME TO BE SUBTLE. STATE THE IMPLICATIONS CLEARLY AND SUCCINTLY.

5. THIS STUDY DOES NOT SUPPORT RECOMMENDATION TO “RE-EXAMINE UTILITY OR NEED OF PFO CLOSURE” BASED ON SUGGESTION THAT RLS DECREASES OVER TIME – THIS STUDY NEEDS TO BE REPEATED USING MORE STRINGENT METHODOLOGY BEFORE MAKING THIS RECOMMENDATION.

In this section we mention again the main finding depicted in our study: Shut decrease in patients with cryptogenic stroke. Considering the limitations of the study we indicate further a potential impact of our results (such as a re-examination with respect to shunt persistence on follow-up), simultaneously acknowledging the need for further studies which prove our results.

We indicate the potential impact for the development of new strategies for the secondary stroke prevention in these patients. Further more we mentioned that our findings need to be verified in further studies.
1. Is the question posed by the authors well defined?

YES

2. Are the methods appropriate and well described?

METHODOLOGY REQUIRES FURTHER DEVELOPMENT

CONCERNS:

METHODS AND PROTOCOL FOR TESTING RLS AT T(0) WERE NOT CONTROLLED; FINDINGS MAY BE RELATED TO DIFFERENCES IN TESTING PROCEDURES BETWEEN T(0) AND T(1) VS ACTUAL CHANGES IN RLS

Indeed this is a main limitation of the present study. Nevertheless in our department the ce-TCD is performed under strictly standardised conditions (See consensus paper: Jauss M, Zanette E (2000) Detection of right-to-left shunt with ultrasound contrast agent and transcranial Doppler sonography. Cerebrovasc Dis 10:490-496).

One of the points which may support the reliability of the TCD data on T0 is the remarkable shunt size concordance in comparison to TEE. And what the analysis for shunt changes is concerned, the examiners were blinded from the order the ce-TCD were performed. They analysed only ce-TDC-examination without knowledge of T0 or T1 or any other data.

After collecting the first experiences with shunt dynamic in PFO we also ask if our results may be caused by differences between testing procedures. For proving these doubts in 6 patients with a shunt reduction from initially curtain pattern to no evidence for RLS on T1, we repented the ce-TCD three times in the subsequent four months. In all examinations a RLS was not detected. Even a TEE performed in 3 of these patients no further RLS was evident.

CONTRAST INJECTION SITE FOR T(0)?

See comment Point 2. in Major Compulsory Revisions

CLARIFY WHAT CEREBRAL ARTERIES WERE INSONATED?

See comment Point 2. in Major Compulsory Revisions

BILATERAL OR UNILATERAL MONITORING?

See comment Point 2. in Major Compulsory Revisions

WAS A CALIBRATED VALSALVA MANEUVER USED FOR T(1)?

See comment Point 2. in Major Compulsory Revisions

WAS INTER-RATER RELIABILITY TESTED IN COUNT OF EMBOLIC TRACKS AT T(1)?

See comment Point 2. in Major Compulsory Revisions

STUDY WOULD BE SIGNIFICANTLY IMPROVED IF THERE WAS A CONTROL GROUP (PFO WITHOUT STROKE) FOR COMPARISON – HIGHLY

We mentioned the results derived from a small case series of 8 patients suffering of migraine diagnosed of PFO. In consecutive examinations (ce-TCD within 6 month) we found...
RECOMMEND THIS APPROACH USING SAME METHODOLOGY AT T(0) AND T(1)

(2 ceTCDs within 6 month) no relevant changes in RLS were observed. Nevertheless we stated that this is not an appropriate control group in our study. This might support our results of a shunt reduction as a physiological process and not a variance of testing.

Additionally in 6 patients with a shunt reduction from initially curtain pattern to no evidence for RLS on T1, we repented the ce-TCD tree times in the subsequent four months. In all examinations a RLS was not detected.

Furthermore in 3 of these patients a TEE was performed subsequently, as in these patients a PFO-closure was considered as a therapeutic option. This remained unremarkable as well. Even based on few selected cases, these findings might definitively underline our results as accurate findings, which are not determined by methodological differences of testing procedures.

IN MY EXPERIENCE, RLS CAN BE SIGNIFICANTLY INFLUENCED BY HYDRATION STATUS, MEDICATIONS, INJECTION SITE – THESE CONDITIONS MAY HAVE DIFFERED BETWEEN T(0) AND T(1)

At T0 in ce-TCD as well as in TEE the PFO was evident (high shunt size concordance). Especially in patients with CS a shunt reduction was observed. In 6 patients with a shunt reduction from initially curtain pattern to no evidence for RLS on T1, the subsequent ce-TCDs (3 ceTCDs within 4 month) revealed the same result (no shunt). In this context it appears unlikely that factors such as hydration may cause our results.

In three of these patients even a TEE revealed no RLS on follow-up.

REPORT INTER RATER RELIABILITY OF EMBOLIC COUNT DETECTION

3. Are the data sound?

DATA COLLECTION PROCEDURES FOR TCD AND RLS MEASUREMENT AT T(0) CAN NOT BE ASSURED – MAJOR LIMITATION; STUDY SHOULD BE REPEATED AS A PROSPECTIVE STUDY USING SAME METHODS AT ALL TIME POINTS

Indeed the data collection on T0 is a major limitation in this study. Nevertheless we believe these data are robust data, as ce-TCD is a standardized procedure in our department (See comment point 2. in Major Compulsory Revisions). A high reliability of shunts assessed on T0 is also supported by the remarkable shunt size concordance as compared to RLS assessments on TEE.

See here also point 3. in Major Compulsory Revisions.
The main reason for large confidence intervals is the relatively small numbers of investigated patients. Even our study includes a small number of patients the results are distinct in indicating an increased likelihood for shunt reduction in patients with PFO and CS. Even regarding the lower range of the large confidence interval, a sixfolded probability to experience a shunt reduction in case of PFO and CS is a remarkable result.

This aspect was added in the discussion.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

SEE SPENCER VASCULAR WEBSITE FOR PUBLISHED PROTOCOL FOR MEASUREMENT OF RLS/PFO USING TCD

5. Are the discussion and conclusions well balanced and adequately supported by the data?

PFO PREVALENCE IS HIGHER IN YOUNGER PATIENTS THAN IN ELDERLY PATIENT – AUTOPSY DATA HAVE BEEN REPORTED. AGE WAS SIGNIFICANTLY DIFFERENT BETWEEN GROUPS.

Indeed in the univariate analysis a shunt reduction was associated with younger age. This aspect was considered in the subsequent logistical regression; in this balanced analysis this factor was not longer significant. The parameter cryptogenic stroke, which is most common in young patients, remained the strongest factor associated with a change in shunt volume.

In conclusion age was associated with stroke type (CS) rather than change in shunt volume.

This aspect is clearly stated in the discussion!!!

ASSUMPTION THAT RELIABILITY HAS BEEN ESTABLISHED WITH USE OF TCD TO GRADE/MEASURE RLS AND IT HAS NOT. AUTHORS NEED TO ADDRESS THE RELIABILITY OF TCD IN MEASURING RLS OVER TIME – I HAVE NOT SEEN A PUBLISHED PAPER ADDRESSING THIS ISSUE. IN MY EXTENSIVE EXPERIENCE WITH PM-TCD IN PATIENTS WITH PFO AND FOLLOWING PFO CLOSURE, I HAVE NOTED A SIGNIFICANT VARIABILITY IN RLS GRADE/EMBOLIC TRACKS AND FREQUENTLY HAVE WONDERED IF THIS WAS A FACTOR OF THE MODALITY/INSTRUMENTATION VS A PHYSIOLOGICAL CHANGE.

We are pleased to hear you made more or less the same experience as we did. After we investigated the hypothesis of shunt dynamic over time in certain systematic way, we think this is a real “PHYSIOLOGICAL CHANGE” rather than caused by “MODALITY/INSTRUMENTATION”

Changes in RLS across a PFO have been indirectly addressed by other investigators, while focusing on other aspects. For instance Anzola et al. investigated predictors for recurrent stroke in patients with PFO and RLS (1). When starting the observation period the shunt volume was re-evaluated using ce-TCD. In 8% of the patients a RLS was no longer detected on re-examination.

6. Are limitations of the work clearly stated?

RETROSPECTIVE T(0) TCD MEASUREMENT OF RLS GRADE WAS NOT PERFORMED UNDER PROTOCOL THEREFORE INTERNAL VALIDITY IS COMPROMISED

Indeed this is a limitation of the present study. Nevertheless in our department the ce-TCD is performed under strictly standardised conditions: patient’s position, the venous catheter is required in the left cubital vein, the Valsalva strain, and the insonated vessel is the proximal part of the left middle cerebral artery by a combined Duplex/Doppler method. Of course the Echovist preparation is also strictly prepared according to the manufacturers instructions.

We have a very detailed documentation of documentation of examinations in our department. As concerns rose after reading this review, we checked if any of our patients was treated differently when ce-TCD was performed at T0. In none of the patients any suspected documentations could be found.

And the examinations at T1 were definitely performed in the manner mentioned above, and in case on condition was violated patients were not included in the study.

One of the points which may support the reliability of the TCD data on T0 is the remarkable shunt size concordance in comparison to TEE. And what the analysis for shunt changes is concerned, the examiners were blinded as the order in they were performed is concerned. They analysed only ce-TDC-examination without knowledge of T0 or T1 or any other data!!!!!!!

DID NOT ADDRESS POSSIBILITY OF SECONDARY SOURCE OF RLS COEXISTING WITH PFO AS A POSSIBLE FACTOR AFFECTING DATA – SEE PUBLISHED LITERATURE ON THIS TOPIC

There is true, the evidence for RLS in ceTCD does not automatically mean there is a PFO (see Lochner P, Tezzon F, Nardone R, Tanislav C (2009) Paradoxical brain embolism caused by an arterial-venous fistula: a diagnostic pitfall. Neurol Sci).

At T0 the PFO was evident in ce-TCD as well as in TEE (high shunt size concordance). In this case it was proved that the RLS was caused by PFO, as well as the shunt size measured by ce-TCD was confirmed by TEE (high shunt size concordance). In this contest context additional coexisting shunts are unlikely.

7. Do the authors clearly acknowledge any work upon which they are building, both
published and unpublished?

NEED TO INCLUDE PRESS RELEASE NMT CLOSURE I STUDY JUNE 2010

The press release NMT CLOSURE I was cited and added to the references.