PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Small cortical gray matter lesions show no persistent infarction in transient ischemic attack? A prospective cohort study
AUTHORS	Havsteen, Inger; Ovesen, Christian; Willer, Lasse; Nybing, Janus; Ægidius, Karen; Marstrand, Jacob; Meden, Per; Rosenbaum, Sverre; Folke, Marie; Christensen, Hanne; Christensen, Anders

VERSION 1 – REVIEW

REVIEWER	PD Dr. Mohamed Al-Khaled 1-Department of Neurology,AlAhli hospital, Doha, Qatar 2- Department of Neurology, University of Lübeck, Germany
REVIEW RETURNED	18-Jul-2017

GENERAL COMMENTS	the study by Havsteen et al. investigated the persistence of DWI lesion among TIA patient 8 weeks after event. the study is very interesting and has direct implications to the clinical work up of TIA. The study can be improved if the authors addressed some points: 1- the definition of TIA from time based to tissue based should be addressed clearly in the manuscript and the title could include TIA and minor stroke, the manuscript should include also TIA and minor stroke. The definition used for TIA is old (1990), there are new definitions and approsal. 2- 64 patient was excluded due to the lack of follow MRI: this leads to bias selection, this should be stated clearly in the limitations sections. 3- the statistics section should provide the parameters included in the multivariate analysis. 4- I do not agree the terms predictor, because the lack of validation group, I suggest associated factors, and OR of 64 (3.4-1223) should be corrected for more clarity. all parameters with P<0.1 should be entered in the multivariate analysis. 5. the association between evidence of DWI-lesions and MRI-time should be discussed to the known literature. 6- the sentence in the discussion: "Literature holds few and small studies..." this is not correct, the literature holds studies with a cohort>1000 patients. 7- the last sentence in the conclusion is not accurate. Please remove.

REVIEWER	Philip Barber Calgary Stroke Program, University of Calgary, Canada
REVIEW RETURNED	04-Oct-2017

GENERAL COMMENTS	This study in patients with clinically defined transient ischemic attack were recruited to determine factors that influence persistent infarction and visibility of lesion on DWI and FLAIR. Persistent infarction was determined by 8-week FLAIR hyperintensity or atrophy corresponding to the initial DWI lesion. 35% of DWI lesions did not result in infarction signs on the 8-week FLAIR image. They found that most of these lesions were found in the cortical gray matter. Lesions didn't show persistent FLAIR lesions were smaller than those that did and these smaller ranged at a median size of .16 cm2. The major limitation of this study is that 2-month FLAIR can sensitivity detect infarction. Salient background to support this in the study methodology would be important. In addition, some of the terminology that was used was difficult to understand, for instance, the use of "regression" and "progression" of lesion size. In addition to these comments, I have some specific comments. 1. The authors refer to DWI lesions where there is no hyperintense lesion on FLAIR imaging at two months. In the strictest sense this is not DWI reversibility and this has not been demonstrated. 2. It has been previously shown that MRI can be insensitive to stroke damage using both DWI and FLAIR imaging. I refer the authors to Dennis et al JNMP 2011. For FLAIR hyperintensity there was also literature to support that T2 hyperintensities in the deep white matter can also disappear on FLAIR imaging (Wardlaw et al). Persistence of T2 hyperintensities on FLAIR imaging might relate to depth of ischemia, type of injury, size and location. 3. The authors refer to infarction on FLAIR imaging. Are they referring to pan-necrosis or any type of neuronal ischemia injury such as selective injury? I very much doubt the absolute sensitivity of FLAIR for detecting infarction. Standard clinical MRI is not capable of detecting selective neuronal injury. 4. There are several technical issues where the DWI lesion may not be detectable on FLAIR at two months. These include the size of the lesion, slice thickness, volume averaging, change intensity of the lesion, brain atrophy, and collapse/cavitation of the ischemic region. The authors should comment on these potential confounders to the persistently visible lesion. The example, Figure 1, is proposed to supportive of their thesis that the hyperintense DWI lesion does not persistent on FLAIR imaging at two months yet there is a vague hyperintensity at two months, more in the superficial white matter than the gray matter but still present. The authors should refer to Biessels G. et al with respect to detection of microinfarcts in the cortical gray matter. 5. In the abstract and discussion the authors refer to the involvement of pial collaterals yet this study has not measured such collaterals. I would question whether the large artery conduits are responsible for the small lesions that are detected in the cortical gray matter. These are likely related to involvement of the small penetrating end arterioles that originate from the surface arteries that are beyond the resolution of MRI at 3T.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: PD Dr. Mohamed Al-Khaled
Institution and Country: 1-Department of Neurology, AlAhli hospital, Doha, Qatar; 2- Department of Neurology, University of Lübeck, Germany
Competing Interests: No conflict of interests

The study by Havsteen et al. investigated the persistence of DWI lesion among TIA patient 8 weeks after event.
the study is very interesting and has direct implications to the clinical work up of TIA.
The study can be improved if the authors addressed some points:

1- the definition of TIA from time based to tissue based should be addressed clearly in the manuscript and the title could include TIA and minor stroke, the manuscript should include also TIA and minor stroke. The definition used for TIA is old (1990), there are new definitions and approsal.

Response: Thank you for this suggestion. We have changed the first sentence in Methods, subsection Clinical methodology from “TIA was defined as an episode of acute focal neurological symptoms of vascular origin with resolution within 24 hours” to “We included patients with TIA or minor stroke defined as an episode of acute focal neurological symptoms of vascular origin[25] with resolution within 24 hours.[26]”

In the Discussion we have changed the sentences “The mixed TIA and minor stroke study reported a 6% DWI-lesion reversal with imaging performed at a median delay of 13 hours after symptom onset.[15] Our cohort only consists of patients with TIA, which may explain our larger 16% rate of patients with lesion reversal” to “The mixed “high risk TIA”[16] and minor stroke study reported a 6% DWI-lesion reversal with imaging performed at a median delay of 13 hours after symptom onset. Our cohort only consists of patients with symptom duration of less than 24 hours, which may explain our larger 16% rate of patients with no persistent infarction signs.”

2- 64 patient was excluded due to the lack of follow MRI: this leads to bias selection, this should be stated clearly in the limitations sections.

Response: We have added the sentence to limitations: “We lost 10% (13/135) of eligible patients for 8-week MRI, this may have caused a selection bias.” We excluded 64 patients as they were discharged with non-TIA diagnoses as tPA-treatment (7 patients), migraine (23), peripheral vertigo (8), syncope (5), headache (4), epilepsy (3), anxiety (3), and other non-ischemic discharge diagnoses (11) as detailed in the STROBE diagram in Figure 2.

3- the statistics section should provide the parameters included in the multivariate analysis.

Response: In the statistics section we have added the sentence “We included lesion location, DWI lesion size (cm2), visibility on baseline ADC or FLAIR and time to baseline MRI as parameters in the multivariate analysis.”

4- I do not agree the terms predictor, because the lack of validation group, I suggest associated factors, and OR of 64 (3.4-1223) should be corrected for more clarity. all parameters with $P<0.1$ should be entered in the multivariate analysis.

Response: Thank you for pointing this out. The abstract's OR ratio is from Fisher's exact test (and identifies the initial FLAIR parameter as having p<<0.1 and eligible for regression). The OR in section Results is derived from the regression, where the initial FLAIR is one among several parameters.

We have explored this a little further with stepwise addition of the parameters into the regression: The regression's parameters lesion location, DWI lesion size (area, cm2) and initial FLAIR visibility are independent as they do not influence on each other's OR when present or absent in the regression analysis. Initial ADC visibility seems correlated to DWI area as its presence influences on DWI area's OR, even though ADC visibility has a non-significant p-value in the regression. TTS has a non-significant p-value of 0.37 (Mann-Whitney U, table 1), but we have tested the regression with and without TTS and find that TTS does not reach significance, but is an independent factor, as its presence shows no influence on the other factor's OR.

We agree with the reviewer and have for clarity changed the abstract's FLAIR OR to the regressions inFLAIR OR 33.06, 95% CI 2.94-1432.34, as this holds most information.
All parameters with P<0.1 have been entered into the multivariate analysis and we have changed "predictor" to "associated factor" throughout the text.

5. the association between evidence of DWI-lesions and MRI-time should be discussed to the known literature.

Response: In our study with 31.5 hours median time to scan (TTS) we find no statistical correlation between DWI positivity rate and time to baseline MRI, this is in accordance with a large recent meta-analysis by Brazzelli, et al, as described in the Discussion.
A third of stroke patients are DWI negative yet their long-term outcome and recurrence risk did not differ from DWI positive patients (Makin, et al. Stroke 2015). Imaging time point, territory and anatomy versus susceptibility artefacts from bone have been proposed as possible factors in the association between DWI lesion occurrence and time to MRI.
A new study investigating the optimal timing of DWI after TIA symptom onset found that TTS<2 hours held a risk of false negative DWI (Shono, et al. Stroke 2017). For vertebrobasilar strokes false negative DWI has been described as late as 24 hours after ictus (Oppenheim, et al, AJNR 2000). In the posterior fossa and brainstem this may relate to anatomy with small territories and often very small lesions and imaging limitations due to susceptibility artefacts from bone (Sylaja, et al. Stroke 2008). Also patients with initial perfusion deficts have been shown with DWI lesions at the hypoperfused site days later and the finding was interpreted as lesion development rather than de novo lesions (Asdaghi, et al. Stroke 2011). Thus, this relation seems complicated. Our study's imaging point is beyond the hyperacute window and we had no territorial exclusion criteria, under these circumstances we find no correlation.

6- the sentence in the discussion: "Literature holds few and small studies..." this is not correct, the literature holds studies with a cohort>1000 patients.

Response: Thank you for raising this point. To our knowledge there are only few TIA-related studies with serial MRI, besides case reports the main are Kidwell, et al 1999, Oppenheim, et al 2006 and Asdaghi, et al 2014. The included populations vary, e.g. Asdaghi et al's population was a mixed "high risk" TIA and minor stroke population and not restricted to symptom duration < 24 hours, and the two newest studies used MRI for follow-up only but still their DWI positivity rates (35-57%) and rate of DWI reversal among DWI positive patients (6-21%) varied notably. The studies' percentage of females (30-52%), mean or median age (60-72 years), and field strength (1.5T, 3T) also varied. Only Asdaghi, et al.' study had scheduled follow-up MRI, and still they lost almost 20% to follow-up. We have clarified the mentioned sentence, it now runs "Literature holds few and heterogeneous serial MRI TIA-related studies…"

7- the last sentence in the conclusion is not accurate. Please remove.

Response: Thank you for advancing this. We have clarified the sentence changing it from “It is yet to be determined if the apparent full recovery of brain tissue is related to the clinical course including risk of recurrence and sequels of TIA including vascular dementia, fatigue and depression” to “It is yet to be determined if the apparent full resolution of brain lesions is related to the clinical course including risk of recurrence and sequels of TIA including vascular dementia, fatigue and depression.”

Reviewer: 2
Reviewer Name: Philip Barber
Institution and Country: Calgary Stroke Program, University of Calgary, Canada
Competing Interests: None

Comment: This study in patients with clinically defined transient ischemic attack were recruited to determine factors that influence persistent infarction and visibility of lesion on DWI and FLAIR. Persistent infarction was determined by 8-week FLAIR hyperintensity or atrophy corresponding to the initial DWI lesion. 35% of DWI lesions did not result in infarction signs on the 8-week FLAIR image. They found that most of these lesions were found in the cortical gray matter. Lesions didn’t show persistent FLAIR lesions were smaller than those that did and these smaller ranged at a median size of .16 cm2.
The major limitation of this study is that 2-month FLAIR can sensitivity detect infarction. Salient background to support this in the study methodology would be important.

Response: Thank you for your insightful comments and raising this important point that may explain the well-described difference between clinical deficits and imaging findings (Makin, et al, Stroke 2015) . Our study is an imaging study and has no histological correlate. As in prior similar studies (Oppenheim, et al, AJNR 2006, Asdaghi, et al, AJNR 2014), our infarction definition is therefore 8-week T2-FLAIR hyperintensity or atrophy corresponding to the initial DWI lesion. This would most likely correspond to pan-necrosis. We do not believe that selective neuronal injury with otherwise mostly intact tissue architecture would show on FLAIR. In rats with baseline MRI-changes after filament-MCAO one may in subcortical lesions see 10-week T1- and T2-signal normalization while histology shows selective neuronal loss and some gliosis (Wegener, et al J Cereb Blood Flow Metab 2006). We do not expect our 8-week T2-FLAIR to pick up subtle changes as selective neuronal loss and this may also partly explain why we see no difference in functional outcomes in patients with or with no persistent infarction signs, other issues are our small sample size and the relatively small deficits associated with TIA.

Regarding the gliosis we think of it as a spectrum where FLAIR would pick up larger changes ranging towards pan-necrosis.
A recent study (Bernbaum, et al. J Cereb Blood Flow Metab 2015) in a TIA/minor stroke population with baseline and 18-month MRI showed that normal appearing white matter areas with baseline hypoperfusion were associated with development of new 18-month white matter hyperintensities (WMH). Their FLAIR sequence had slightly thinner slices of 3.5 mm versus our 4 mm.
A recent study (Harston, et al. Ann Clin Transl Neurol 2017) in ischemic stroke patients with baseline DWI and 1-week and 1-month FLAIR used for infarct definition manual infarct delineation (i.e. visual) on DWI and FLAIR and thresholded ADC. Also Galinovic, et al. (Stroke 2014) found that ROIs comparing diseased and contralateral normal tissue did not improve interrater agreement for detection of subtle early (edematous) FLAIR hyperintensities compared to visual assessment.

For final infarct size description T2-FLAIR has higher subacute lesion conspicuity (Ricci, et al. AJNR 1999) and higher interrater agreement (Neumann, et al. Stroke 2009) than T2, and is commonly used for assessment of WMH and final infarct demarcation.
Overall, we think that brain ischemia may occur below the imaging detection threshold in both acute and chronic stage. Yet our methodology is comparable with other imaging studies and with that used in clinical practice. We agree that imaging can confirm but not rule out ischemia.

We have added these sentences to the Introduction:
“Among clinical ischemic stroke patients a third is DWI-negative, but their long-term outcome and recurrence rates did not differ from DWI-positive patients.[13]”

“Animal imaging studies of transient ischemia with subcortical DWI-lesion have shown apparent 10-week T1- and T2-signal normalization while histology showed selective neuronal loss and gliosis.[21] Persistent signal changes most likely correspond to pannecrosis. A recent 7T in vivo human study showed the presence of likely cortical microinfarcts with similar MRI-appearance compared to microinfarcts on ex vivo brain slices with histopathological correlate.[22]”

“Clinical MRI may not be able to detect all acute or chronic ischemic changes[13]; yet DWI and T2-FLAIR are the most commonly used tools for ischemia assessment.[24]”

In Methods in subsection “Definitions of lesions, size and localization” we have added references and clarified the text regarding DWI-reversal. It now runs “Absence of 8-week T2-FLAIR hyperintensity or atrophy in the initial DWI lesion area was defined as no persistent infarction signs (Figure 1); lesion area decrease was defined as 30% or more lesion area reduction.“

Comment: In addition, some of the terminology that was used was difficult to understand, for instance, the use of “regression” and “progression” of lesion size.

Response: We have replaced “regression” with “decrease” and “progression” with increase” in lesion size.

In addition to these comments, I have some specific comments.
1. The authors refer to DWI lesions where there is no hyperintense lesion on FLAIR imaging at two months. In the strictest sense this is not DWI reversibility and this has not been demonstrated.

Response: We agree with the reviewer that ‘reversibility’ is inaccurate; we have changed “DWI-reversal” to “no persistent infarction signs”, “partial DWI-reversal” and “lesion regression” to “lesion area decrease” and “lesion progression” to “lesion area increase”. In the Discussion we have changed the sentence “Ischemic lesion reversal is documented after early intra-arterial revascularization” to “Ischemic lesions decreased in size after early intra-arterial revascularization”.

2. It has been previously shown that MRI can be insensitive to stroke damage using both DWI and FLAIR imaging. I refer the authors to Dennis et al JNMP 2011. For FLAIR hyperintensity there was also literature to support that T2 hyperintensities in the deep white matter can also disappear on FLAIR imaging (Wardlaw et al). Persistence of T2 hyperintensities on FLAIR imaging might relate to depth of ischemia, type of injury, size and location.

Response: We agree. What strikes us though is the difference in lesion development between locations. In our study we found that few white matter lesions disappeared or became smaller, most remained roughly the same size or enlarged. This distribution differed greatly from cortical gray matter lesions, where most disappeared or became smaller. This finding is consistent with a previous study (Asdaghi, et al 2014), although they had only 11 patients with disappearing lesions, hereof were 8 cortical, 2 in subcortical white matter and 1 cortical and subcortical. Oppenheim, et al show an image example of a cortical lesion disappearing at follow-up, but do not detail lesion location for their 7 patients with 14 lesions with no persistent infarction signs. Both prior studies report that lesions that disappear on follow-up FLAIR are smaller than lesions with persistent infarction signs, which is consistent with our findings. On a larger scale also apparent cortical sparing in relation to MCA infarcts is observed (e.g. Cho, et al. JNNP 2010).
In the online supplement we have added a figure, Supplemental figure II, and in Results we have added the sentence, “The distribution of lesions with area decrease or increase differed significantly between cGM and WM ($p<0.0001$, Supplemental figure II).”

FLAIR is only a surrogate marker for tissue fate and has limitations, but it is the most commonly used. The study is in clinical context, at 3T and with sequences similar to prior studies.
In the chronic stage lesions, especially those mainly characterized by atrophy, may only show small signal changes on T2-, T2-FLAIR or T1, especially near CSF, and may only be visible on a subset of sequences (Oppenheim, et al AJNR 2006), and may be hard to detect even when compared to initial DWI lesion location (Rovira, et al, Neuroradiol 2002). Higher gradient strength and sub-millimeter resolution (Veluw, et al. J Cereb Blood Flow Metab 2013) would further improve detection, but are beyond the scope of this study.
We have added this in the Discussion: “Artifacts on T2-FLAIR caused by magnetic susceptibility, pulsatile CSF flow or no nulling of the CSF signal are common[39] and may have masked small lesions. Other potential confounders are change and variation in FLAIR signal intensity of the lesion[40] and our lesion definition includes atrophy corresponding to the initial DWI lesions although this can be difficult to visualize, even when aided by T1 and T2, and may explain why lesions are not persistently visible[15,32]”

3. The authors refer to infarction on FLAIR imaging. Are they referring to pan-necrosis or any type of neuronal ischemia injury such as selective injury? I very much doubt the absolute sensitivity of FLAIR for detecting infarction. Standard clinical MRI is not capable of detecting selective neuronal injury.

Response: Thank you for emphasizing this point, we agree. We see that the three main prior studies similar to ours, Kidwell 1999, Oppenheim 2006 and Asdaghi 2014, do not use consistent terminology for presence or absence of radiological signs related to permanent ischemic injury (i.e. chronic phase T2-FLAIR hyperintensities or atrophy corresponding to the baseline DWI-lesion). In the text we mostly used the operational expression ‘infarction signs’, we have now consequently changed the word ‘infarction’ to ‘infarction signs’.

4. There are several technical issues where the DWI lesion may not be detectable on FLAIR at two months. These include the size of the lesion, slice thickness, volume averaging, change intensity of the lesion, brain atrophy, and collapse/cavitation of the ischemic region. The authors should comment on these potential confounders to the persistently visible lesion.

Response: We agree with the reviewer. Please see point (3) above.

Comment: The example, Figure 1, is proposed to supportive of their thesis that the hyperintense DWI lesion does not persistent on FLAIR imaging at two months yet there is a vague hyperintensity at two months, more in the superficial white matter than the gray matter but still present. The authors should refer to Biessels G. et al with respect to detection of microinfarcts in the cortical gray matter.

Response: We have looked up the patient again in the PACS and find that the 8-week FLAIR signal at the location of the lateral lesion does not differ from the slightly heterogeneous signal in many other areas, especially at borders. This assessment is of course visual and an assessment.
We are very grateful for the 7T-study reference and have changed end of the Discussion from ”Dedicated high-resolution 3D-FLAIR and 3D-T1 could improve differentiation between healthy tissue and post-infarction gliosis or atrophy, especially cortically.” to “At 3T dedicated high-resolution 3D-FLAIR and 3D-T1 could improve differentiation between healthy tissue and post-infarction gliosis or atrophy, especially cortically. While probably not in clinical reach soon, higher field strength has shown promising results for the in vivo identification of cortical microinfarcts[22] and visualized a hereto unseen but probably quite common structural ischemic burden.”
We have also added a new Supplemental figure II that shows that distributions of lesion area changes significantly differ between lesions in cortical gray matter and white matter. Please see also point (2) above.

5. In the abstract and discussion the authors refer to the involvement of pial collaterals yet this study has not measured such collaterals. I would question whether the large artery conduits are responsible for the small lesions that are detected in the cortical gray matter. These are likely related to involvement of the small penetrating end arterioles that originate from the surface arteries that are beyond the resolution of MRI at 3T.

Response: Thank you for pointing this out. We agree that the involved vessels are below 3T MRI resolution. We have made the following clarifications:
In the Introduction, we changed “suggesting that acute DWI-reversal may be related to recanalization and the presence of leptomeningeal collaterals.[16,17]” to “suggesting that acute DWI-reversal may be related to the proximity of leptomeningeal collaterals.[16,17]” and added “Cortical perfusion is higher than white matter perfusion, even though the ratio declines with age.[19]”
In the Discussion, we changed “This study indicates that ischemic tissue damage in TIA is heterogeneous and differs with lesion localization, which may mirror the inherent difference between end-artery dominated white matter and cortical gray matter with leptomeningeal collaterals.” to “This study indicates that ischemic tissue damage in TIA is heterogeneous and differs with lesion localization, which may mirror the inherent difference between end-artery dominated white matter and cortical gray matter with proximity to leptomeningeal collaterals.”
We found no reference hereto in the abstract.

VERSION 2 – REVIEW

REVIEWER	Dr. Mohamed Al-Khaled Department of Neurology, University of Lubbock, Germany
REVIEW RETURNED	07-Nov-2017

GENERAL COMMENTS	The authors have answered my questions. thanks

REVIEWER	Philip Barber University of Calgary, Canada
REVIEW RETURNED	14-Nov-2017

GENERAL COMMENTS	All my comments have been addressed satisfactorily.