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Small cortical gray matter lesions show no persistent infarction in transient ischemic attack? A prospective cohort study

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ABSTRACT

Objectives: To find determining factors for persistent infarction signs in patients with TIA, herein initial diffusion lesion size, visibility on ADC or FLAIR and location.

Design: Prospective cohort study of patients with clinical TIA receiving 3T-MRI within 72 hours of symptom onset and at 8-week follow-up

Setting: Clinical workflow in single tertiary stroke centre between February 2012 and June 2014.

Participants: 199 candidate patients were recruited, 64 patients were excluded due to non-TIA discharge diagnosis or no 8-week MRI. 122 patients completed the study.

Primary outcome measures: The primary outcome was persistent infarction defined as 8-week FLAIR-hyperintensity or atrophy corresponding to the initial DWI lesion.

Results: 50 patients showed 84 initial DWI lesions. 29 (35%) DWI lesions did not result in infarction signs on 8-week FLAIR. 26 (90%, $p<0.0001$) reversing lesions were located in the cortical gray matter (cGM). CGM location (versus any other location) strongly predicted no 8-week infarction sign development (OR 0.02, 95% CI 0.001-0.17) or partial lesion area regression (>30% of initial DWI-area, OR 14.10, 95% CI 3.61-54.72), adjusted for FLAIR-visibility, DWI-area, ADC-confirmation and time from symptom onset to baseline MRI (TTS). Acute FLAIR-visibility was a strong predictor for persistent infarction signs (OR 64.62, 95% CI 3.41-1223.20). For cGM lesions area size was sole predictor for persistent infarction signs with a 0.31cm^2 (AUC 0.97) threshold. In 8 (16%) DWI-positive patients all lesions reversed fully.

Conclusions: 16% of DWI-positive patients and third of acute DWI lesions caused no persistent infarction signs, especially small cortical gray matter lesions were not followed by development of persistent infarction signs. Late MRI after TIA is likely to be less useful in the clinical setting, and it is dubious if the absence of old vascular lesions can be taken as evidence of no prior ischemic attacks.

Trial registration: clinicaltrials.gov NCT01531946.

Key words: Transient Ischemic Attack; cerebral cortex; brain infarction; cerebral circulation; Magnetic Resonance Imaging

Article Summary

Strengths and limitations of this study

- Prospective inclusion cohort study of patients with clinical TIA evaluated by senior consultant stroke neurologists.
- Patients received standardized baseline and 8-week follow-up MRI in clinical workflow.
- We evaluated lesions after predefined case report form in a clinical PACS-only setting.
- National electronic registers provided long-term follow-up.
- Small lesions below 3 mm were included as they are associated with increased risk of stroke and death.
- Main limitations are sequence resolution and small lesion size.

Introduction

The risk of recurrent events including devastating stroke after transient ischemic attack (TIA) remains substantial.[1,2] Recently, it was shown that the presence of a diffusion weighted imaging (DWI) lesion combined with clinical findings enhanced prediction of post-TIA stroke risk compared to clinical findings alone.[3–5] Even small vascular brain lesions below 3 mm were associated with increased risk of stroke and death.[6] In patients with TIA, DWI lesions are reported in 25% to 50%.[4,5,7–11] Also the time to baseline MRI varies from within 24 hours to 3 weeks from symptom onset.[12] A recent meta-analysis found that the up to 7-fold variation in DWI positivity rates in patients with clinical TIA remains largely unexplained despite attempts to control for factors as varying time to MRI.[12]

Pioneering studies found that patients with DWI lesion reversal had shorter symptom duration than patients with persistent infarctions[13] and noted absence of persistent infarction signs in a fraction of initially DWI-positive patients on chronic phase MRI.[13,14] Lesions with persistent infarction were larger and had lower apparent diffusion coefficient (ADC)-values.[14] A recent series of mixed TIA and minor stroke patients found only 6% of initially DWI-positive patients showed no lesion on T2-fluid attenuated inversion recovery (FLAIR) images obtained 1-3 months later.[15] Despite small numbers the absent lesions seemed primarily located in cortical gray matter,[15] suggesting that acute DWI-reversal may be related to recanalization and the presence of leptomeningeal collaterals.[16,17] The impact of the underlying vascularity is also highlighted in a perfusion study of initially DWI-negative patients with clinical time-based TIA, patients with initial focal or territorial perfusion abnormalities showed increased rates of new DWI lesions on 3-day follow-up compared with patients with no perfusion abnormalities.[18] Although the clinical significance of persistent infarction signs is unclear, it is well known that numerous cerebral infarcts gathered over time are predictive of poor outcome[6] and cognitive decline,[19] rendering it of

potential pathophysiological and clinical importance to investigate what determines the formation of persistent infarction signs. We hypothesized that lesion size and ischemic depth, and location are factors likely to predict the occurrence of infarction signs after acute ischemia in clinical TIA.

We aimed to investigate in a clinical setting which characteristics were associated with persistent infarction signs 8 weeks after DWI-positive TIA, including lesion location, size, initial ADC- and FLAIR-visibility, TIA aetiology, and clinical risk factors. The ultimate aim was to establish if no development of persistent infarction signs after DWI-positive TIA occurred with a clinically significant frequency.

Methods

We investigated a prospective patient cohort with clinical TIA included after informed consent. The study was approved by the Danish National Committee of Biomedical Research Ethics (H-1-2011-75).

Clinical methodology

TIA was defined as an episode of acute focal neurological symptoms of vascular origin with resolution within 24 hours.[20] We defined resolution as National Institutes of Health Stroke Scale (NIHSS) 0 within 24 hours. Senior consultant stroke neurologists clinically evaluated and included patients with a history and clinical findings consistent with TIA February 2012 – June 2014. Exclusion criteria were TIA (G45.9) not final clinical diagnosis, thrombolysis treatment, MRI contraindications, and severe illness likely to preclude follow-up. Definitions of clinical risk factors are presented in supplemental methodology. Risk factors of stroke, ABCD2[21], and TOAST[22] classification were recorded. All patients were treated with antithrombotics and other pharmacological secondary prevention according to guidelines.

MR imaging

We performed 3T-MRI (Siemens Magnetom Verio, Siemens, Erlangen, Germany) with a 32-channel head coil (Siemens, Erlangen, Germany) including DWI and T2-FLAIR imaging after routine stroke protocol within 72 hours from symptom onset[23] and at 8-week follow-up to visualize persistent infarction signs. The diffusion protocol was single-shot spin-echo diffusion echo planar imaging with 220-mm FOV, 25 4-mm axial 0-mm gap slices, b-value 1000 s/mm^2 along 3 orthogonal directions; TR/TE 6600/100 ms, acceleration factor R 2, matrix size 192 x 192. The T2-FLAIR protocol was 240-mm FOV, 27 4-mm axial 0-mm gap slices, TR/TE 6500/133 ms, TI 2134 ms, acceleration factor R 2, matrix size 256 x 256.

Image reading was performed as visual inspection in a clinical setting using the PACS without external software. Image analysis had two steps: first, we created an image template consisting of literature based examples of the scoring categories employed and a case report form (CRF). Two blinded certified consultant neuroradiologists (IH, AFC) tested template and CRF on 50 randomly chosen cases from the cohort, reading first independently, followed by a joined re-evaluation of the cases. A consensus-based use of the reading tools was thus achieved enabling the use of one reader for consistence and reproducibility in assessing the often small lesions. Subsequently, one reader (IH), blinded to clinical data except for the referral, systematically evaluated the cohort's scans.
In a random 10% sample (defined by date of birth 4th, 14th and 24th day in any month) we calculated Cohen's kappa for intra-observer variation using two CRF readings, except for area measurements, with 3 months' interval and observed a substantial intra-observer agreement (κ=0.80).
Definitions of post-hoc vascular findings are presented in supplemental methodology.

Definitions of lesions, size, and localization

We used co-registration marking each lesion with two perpendicular intrathecal diameters to ascertain lesion localization between sequences and baseline and 8-week MRI. Two separate lesions must not have confluent gliosis. As proxy for lesion size we used largest lesion area on one slice measured by manual ROIs.[24] On baseline MRI, we scored for presence or absence of DWI lesions, ADC-confirmation and T2-FLAIR hyperintensities, and divided lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (basal ganglia). CGM lesions may have a minor subcortical WM component.

We defined persistent infarction signs as presence of T2-FLAIR hyperintensity or atrophy in the initial DWI-lesion area on 8-week MRI. Absence of 8-week T2-FLAIR hyperintensity or atrophy in the initial DWI lesion area was defined as complete DWI-reversal (Figure 1); partial DWI-reversal was defined as 30% or more lesion area reduction. Due to small lesion size, this definition was modified from the 10% reduction used in ischemic stroke.[25] Lesion area change was graded at 30%, 50%, and 100%.

After all initial and 8-week clinical and radiological data were collected; we checked if DWI lesion site and symptoms matched – which it did in all cases - under supervision of a senior stroke consultant (HC).

Recurrence and follow-up

All patients had a scheduled 8-week telephone follow-up[26,27] by a doctor to assess self-reported functional status and recurrence of new stroke or TIA. Standard 3-month follow-up was a face-to-face interview with a trained nurse assessing functional status, changes in risk factor status, and

medication adherence. National electronic patient files provided long-term follow-up information on new vascular events.

Data Sharing Statement

Dataset is available upon publication from FigShare, DOI: 10.6084/m9.figshare.5091904.

Statistics

For categorical data, we used Fisher's exact test, and Mann-Whitney-U test for population comparison. For dichotomized outcomes, we performed a general linear model-based forced entry logistic regression analysis; p-values were calculated with the Wald test. We performed a likelihood ratio test for model fit versus an empty model. We used ROC analysis for binary classification. Recurrent cerebrovascular events were studied using Kaplan-Meier curves and Cox Proportional Hazard Model. We considered p-values less than 0.05 significant. We used R (version 3.2.0), 2015 The R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/ and SPSS (version 22.0) statistical software, IBM Corporation, Armonk, New York, USA.

Results

We included 122 patients with clinical TIA (Figure 2), median (IQR) age of 65 (54-71) years and median (IQR) ABCD2 4 (3-5) (Table 1). The median (IQR) time from ictus to initial MRI (TTS) was 31.5 (23.5-56.8) hours. Table 1 shows the patients' baseline characteristics and findings on subacute DWI and 8-week T2-FLAIR. 50 patients showed 84 DWI lesions. 32 patients had solitary DWI lesions. 33 lesions were located in white matter, 47 lesions in cortical gray matter and 4 lesions in deep gray matter. There were no statistically significant relations between DWI positivity rates and time to scan.

Initial DWI lesions located in cGM were less likely to show subsequent infarction signs on 8-week MRI ($p<0.001$, OR 0.07, 95% CI 0.01-0.27) compared to other locations. This finding persisted when including only ADC-confirmed lesions (22 with no persistent infarction, hereof 19 (86%) in cGM, $p<0.001$), or only lesions with subacute FLAIR-visibility (13 with no persistent infarction, hereof 12 (92%) in cGM, $p<0.001$, Figure 3). Lesions with persistent infarction development were significantly larger than lesions with no persistent infarction signs; DWI lesions leading to permanent infarction ranged 0.05-5.88 cm^2 with median (IQR) 0.40 (0.13-0.86) cm^2 and lesions with no permanent infarction signs ranged 0.03-1.10 cm^2 with median 0.16 (0.08-0.22) cm^2, $p<0.001$ (Supplemental table I). In multivariate analysis, cGM location (cGM vs other locations), strongly predicted no persistent infarction signs (OR 0.02, 95% CI 0.001-0.17), whereas initial FLAIR-visibility predicted subsequent infarction signs (OR 33.06, 95% CI 2.94-1432.34). Initial DWI lesion size, ADC-confirmation and TTS did not reach significance.

If the initial DWI lesion was located in cGM, no initial FLAIR- or ADC-negative lesions resulted in persistent infarction signs, but persistent infarcts were visible for 64% (21/33) of initially FLAIR-positive and 53% (21/40) of ADC-confirmed cGM lesions. Size of cGM lesions was the single predictor for persistent infarction signs with an optimal area threshold 0.31 cm^2 (AUC 0.97, Supplemental figure I) to discern between lesions with or without 8-week infarction signs.

Figure 4 shows change of lesion size between baseline and 8-week MRI stratified according to tissue type. Of the initial 84 DWI lesions, 51 (61%) lesions showed at least 30% lesion area regression and 15 (18%) showed lesion area progression of 30% or more. WM lesions had the highest progression rate 13/33 (39%) and the highest degree of progression. We found that 41/47 (87%) of the lesions located in cGM regressed and 26/47 (55%) completely vanished leaving no

persistent infarction (Figure 4). Adjusted for ADC-confirmation, initial FLAIR-visibility and DWI-lesion size cGM location (cGM vs other locations) was a strong predictor of lesion area regression (OR 14.1, 95% CI 3.61-54.72).

To assess if individual patients retain persistent infarction signs after having at least one DWI-positive lesion, we defined lesion burden as the sum of each patient's combined DWI-areas. 16% of DWI-positive patients showed no persistent infarction signs (Table 1). Only the combined sum of DWI lesion areas significantly predicted persistent infarction signs. There was a significant correlation (OR 1.20 per additional mm^2, 95% CI 1.11-1.28) between the patients' total DWI area and the probability of persistent infarction signs. There was no significant correlation between increasing ABCD2 score and the probability of persistent infarction signs (OR 1.09, 95% CI 0.82-1.46).

Eight weeks post-TIA we found no correlation between persistent infarction sign presence and patient-reported return to pre-TIA daily activity level (4 (33%) vs. 35 (36%), p=0.87). 14 recurrent cerebrovascular events occurred during the median (IQR) follow-up period of 817 (440-1056) days. Characteristics of patients with recurrent ischemic event are presented in Supplemental table II. Ipsilateral carotid stenosis ≥ 70% (hazard ratio [HR] 7.11, 95% CI 1.98-25.5) and several competing TOAST aetiologies (HR 3.74, 95% CI 1.13-12.4) predicted recurrent ischemic events (Supplemental figure II). Persistent 8-week infarction signs (HR 0.50, 95% CI 0.14-1.78) did not increase the risk of recurrent events.

Discussion

16% of initially DWI-positive patients and a third of identified lesions show no sign of infarction 8 weeks later; cortical gray matter location was the strongest predictor of full recovery on MRI at 8 weeks, and cGM DWI lesions smaller than 0.31cm^2 did not show persistent infarction signs. ADC-confirmation or subacute FLAIR-visibility did not in all cases result in visible long-term infarction.

Ischemic lesion reversal is documented after early intra-arterial revascularization[16] and MCA-infarctions' cortical lesion sparing was related to collateral perfusion and affected by significant stenosis of the ipsilateral anterior or posterior cerebral artery.[17] In rats early (within 1 day) post-ischemic maximum hyperperfusion indicated small final lesion size while rats with late (4 days) maximum hyperperfusion showed large lesions after transient experimental ischemia,[28] indicating rich, tightly-webbed collaterals lead to improved early reperfusion and limit tissue damage.

Literature holds few and small studies with varying populations and rates of DWI positivity and reversal. 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. Our TTS and per patient DWI positivity and reversal rates correspond to the smaller TIA study.[14] Our cohort's 31 hour median delay to imaging may explain why several lesions are FLAIR-positive representing the beginning vasogenic oedema, and the persistent ADC-reduction represents the still active cytotoxic oedema.[29] We found no association between DWI positivity rate and time to scan in line with recent metaanalysis.[12]

In TIA, DWI lesions are rarer and smaller and the TIA presumably results from smaller occlusive events compared to stroke. Changes in perfusion and underlying vascularity reflect tissue vulnerability and damage. 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. This and smaller lesion size may explain the high variation in DWI-positivity rates among TIA populations and the high rate and cortical predilection of apparent diffusion lesion reversal in this TIA study.

However, in an observational exploratory study, we cannot infer causality but hypothesize that the stronger leptomenigeal collateral circulation in cGM may prevent persistent infarction signs in small lesions.

This study has some limitations. As inclusion was based on informed consent, the study was not unselected or consecutive. 14% (12/84) of the initial lesions were small (<3 mm), and below the usual recommended cut-off level to discriminate between lacunes and perivascular spaces,[30] but we included this type of lesion as it has been associated with increased risk of stroke and death.[6] Other potential limitations are partial volume effects and the 2D-FLAIR sequence in our standard stroke MRI-protocol, though its slice thickness is similar to prior studies'.[14,15] Dedicated high-resolution 3D-FLAIR and 3D-T1 could improve differentiation between healthy tissue and post-infarction gliosis or atrophy, especially cortically.

Conclusion

CGM localization of the acute DWI lesion in TIA was a strong predictor of no persistent infarction signs after TIA. Late MRI after TIA may not detect a significant number of lesions, especially cortical lesions and no prior lesions on the MRI is not evidence of no prior ischemic events. 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.

Author Contributions: IH, HC, AC conceived and designed the study. All authors were involved in data acquisition. KÆ, JM, PM, SR, MF, HC were involved in patient inclusion. IH, CO, JD, AC, HC analysed and interpreted data. IH wrote the first draft. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Tables

Table 1. Patient characteristics

	All 8w	8w infarct	No infarct	p	OR (95% CI)
All patients	122	43	79		
Female sex, n	52 (43%)	15 (35%)	37 (47%)	*0.251	0.61 (0.28-1.31)
Age, median (IQR)	65 (54-71)	60 (53-70)	65 (55-74)	†0.228	…
Medical history:					
Prior stroke	22 (18%)	6 (14%)	16 (20%)	*0.466	0.64 (0.23-1.78)
Prior TIA	12 (10%)	7 (16%)	5 (6%)	*0.111	2.87 (0.85-9.70)
Prior MI	9 (7%)	2 (5%)	7 (9%)	*0.491	0.50 (0.10-2.53)
Atrial fibrillation	12 (10%)	7 (16%)	5 (6%)	*0.111	2.89 (0.85-9.70)
Hypertension	60 (49%)	26 (60%)	34 (43%)	*0.088	2.02 (0.95-4.31)
Diabetes	16 (13%)	8 (19%)	8 (10%)	*0.261	2.03 (0.70-5.86)
Depression	14 (11%)	7 (16%)	7 (9%)	*0.244	2.00 (0.65-6.14)
Current smoking	43 (35%)	18 (42%)	25 (32%)	*0.324	1.53 (0.71-3.30)
Alcohol overuse	12 (10%)	5 (12%)	7 (9%)	*0.754	1.32 (0.39-4.43)
Antiplatelet use	40 (33%)	13 (30%)	27 (34%)	*0.692	0.84 (0.38-1.86)
Warfarin	2 (2%)	1 (2%)	1 (1%)	*1.000	1.86 (0.11-30.5)
Index TIA:					
ABCD2	4 (3-5)	4 (3-5)	4 (3-5)	†0.485	….
Symptom duration:				*0.852	
<60 min	58	21 (36%)	37 (64%)		1.00
>60 min	64	22 (34%)	42 (66%)		0.93 (0.44-1.94)
Imaging findings:					
TTS (hours)	32 (24-57)	41 (22-66)	29 (24-50)	†0.367	….
DWI positive	50	42 (84%)	8 (16%)	*<0.0001	NA
Sum of DWI lesion area, cm^2	0.41 (0.21-1.08)	0.53 (0.31-1.22)	0.09 (0.07-0.22)	†<0.0001	….
Ipsilateral cervical carotid stenosis	5 (4%)	1 (2%)	4 (5%)	*0.656	0.45 (0.05-4.13)
Contralateral cervical carotid stenosis	6 (5%)	1 (2%)	5 (6%)	*0.522	0.35 (0.04-3.12)
Ipsilateral intracranial stenosis[a]	9 (12%)	1 (4%)	8 (17%)	*0.144	0.19 (0.02-1.63)
Contralateral intracranial stenosis[a]	1 (1%)	0 (0%)	1 (2%)	*0.450	NA
TOAST aetiology:				*0.727	
Small vessels	49	16 (33%)	33 (67%)		1.00
Large vessels	28	12 (43%)	16 (7%)		1.55 (0.59-4.03)

Cardiogenic	18	5 (28%)	13 (72%)		0.79 (0.24-2.61)
Multiple possible aetiologies	27	10 (37%)	17 (63%)		1.21 (0.45-3.24)
TTF (days)	56 (55-60)	55 (55-60)	56 (55-60)	†0.832	….

*Fishers exact test. †Mann-Whitney U test. [a]Computed tomography angiography (CTA) or transcranial Doppler (TCD) available in 75 patients, hereof 28 with 8-week infarction. TIA – transient ischemic attack. MI – myocardial infarction. NA – not applicable. TTS – time to scan. TTF – time to follow-up.

Legends

Figure 1.

Lesions with and without 8-week infarction signs. Two cortical gray matter lesions are shown on initial DWI (panel A), ADC (panel B), initial FLAIR (panel C) and 8-week FLAIR (panel D). Panel A: Both lesions are DWI-positive. Panel B: The medial lesion is ADC-confirmed, the lateral lesion shows no ADC-confirmation. Panel C: Both lesions are initially FLAIR-positive. Panel D: the medial lesion is 8-week FLAIR-positive, the lateral lesion is 8-week FLAIR-negative.

Figure 2.

STROBE diagram of patient flow, in- and exclusion. Other non-ischemic comprises patients with peripheral nerve compression (2), ophthalmological symptoms (2), trigeminal neuralgia (1), normal pressure hydrocephalus (1), hyperventilation (1), paraesthesia secondary to anaemia (1), peripheral extremity embolus (1), food poisoning (1), and secondary refusal (1).

Figure 3.

Probability of permanent infarction signs stratified by anatomical location. DWI-lesion location in the cortical gray matter was significantly less likely to show permanent infarction signs. *$p<0.001$ against other anatomical location.

Figure 4.

Regression and progression of lesion size between admission MRI and 8-week follow-up MRI for all lesions and stratified according to lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM).

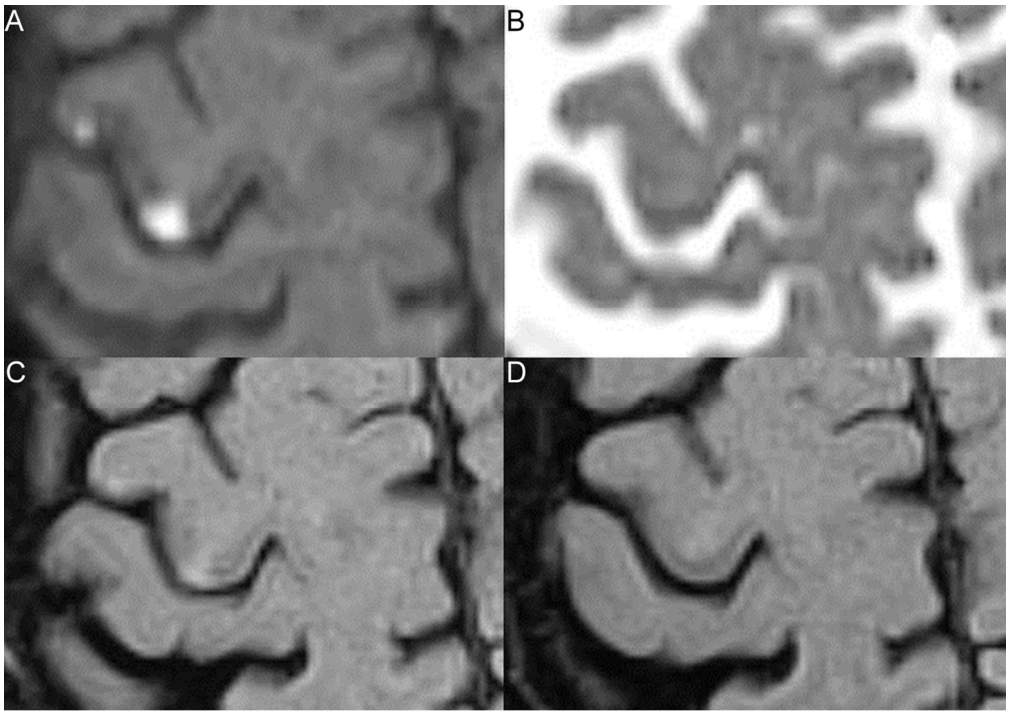


Figure 1. Lesions with and without 8-week infarction signs. Two cortical gray matter lesions are shown on initial DWI (panel A), ADC (panel B), initial FLAIR (panel C) and 8-week FLAIR (panel D). Panel A: Both lesions are DWI-positive. Panel B: The medial lesion is ADC-confirmed, the lateral lesion shows no ADC-confirmation. Panel C: Both lesions are initially FLAIR-positive. Panel D: the medial lesion is 8-week FLAIR-positive, the lateral lesion is 8-week FLAIR-negative.

56x39mm (600 x 600 DPI)

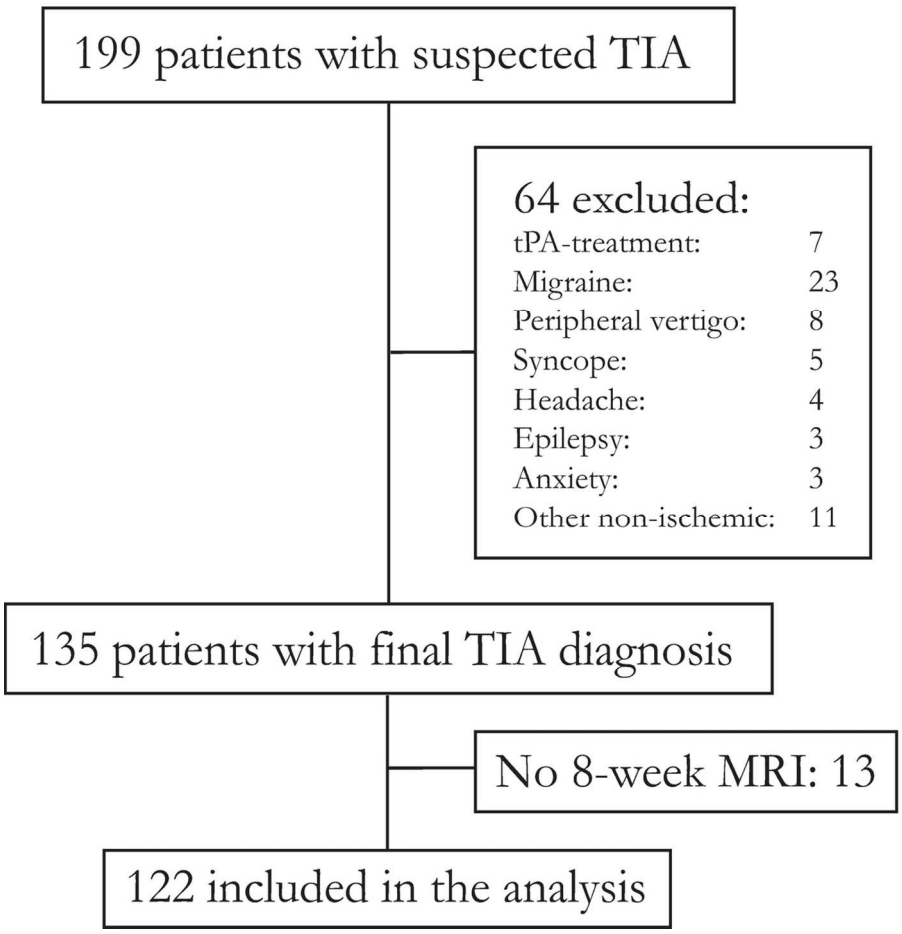


Figure 2. STROBE diagram of patient flow, in- and exclusion. Other non-ischemic comprises patients with peripheral nerve compression (2), ophthalmological symptoms (2), trigeminal neuralgia (1), normal pressure hydrocephalus (1), hyperventilation (1), paraesthesia secondary to anaemia (1), peripheral extremity embolus (1), food poisoning (1), and secondary refusal (1).

119x110mm (300 x 300 DPI)

Figure 3. Probability of permanent infarction signs stratified by anatomical location. DWI-lesion location in the cortical gray matter was significantly less likely to show permanent infarction signs. *p<0.001 against other anatomical location.

125x79mm (300 x 300 DPI)

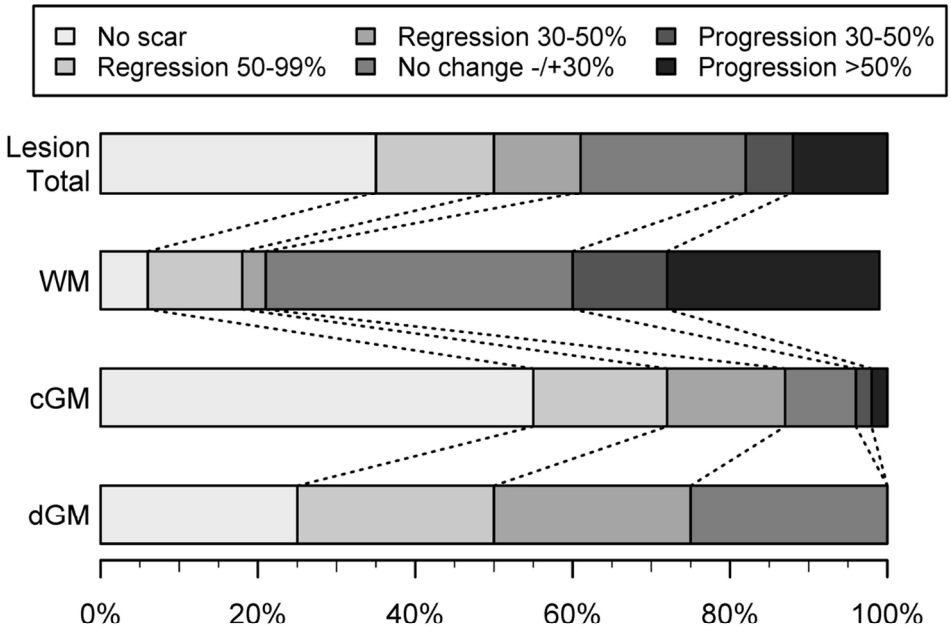


Figure 4. Regression and progression of lesion size between admission MRI and 8-week follow-up MRI for all lesions and stratified according to lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM).

114x85mm (300 x 300 DPI)

ONLINE SUPPLEMENT

Cortical gray matter lesions are associated with no persistent infarction after transient ischemic attack

Havsteen et al. 2017

Supplemental methodology:

Clinical risk factors:

Symptoms, symptom duration, vascular risk factors and ABCD2 were recorded including prior stroke, TIA or myocardial infarction (MI), angina pectoris, peripheral arterial disease, diabetes or depression. Hypertension was defined as pre-admission use of antihypertensive medication or hypertension diagnosis in our out-patient clinic. Atrial fibrillation was diagnosed by medical history, admission 12-lead ECG, in-hospital telemetry (24 - 48 hours) or subsequent out-patient cardiac follow-up. We defined hypercholestrolemia as total plasma cholesterol above 5.0 mmol/L or statin treatment. Diabetes was defined by medical history or HbA1c >6.5%. We defined smoking as present or prior smoking, and alcohol overuse as weekly alcohol consumption above 252 g for males and 168 g for females. Recorded hereditary factors were first degree relative with stroke or MI. Clinical data were collected from electronic patient files. Subsequently, clinical and radiological data were compared for consistency under supervision of a senior neurological consultant (HC).

CT-angiography or transcranial Doppler was not included in the protocol; standard carotid examination was performed by ultrasound in the department.

Definition of post-hoc vascular findings

Standard work-up was extracranial carotid Doppler ultrasound. Some patients were investigated with TCD or CTA for clinical suspicion of large vessel disease. For ultrasound we defined carotid stenosis as peak systolic velocity >230 cm/s. For CTA we defined extracranial carotid stenosis as lumen reduction >70%, posterior circulation and intracranial arteries were stenotic with lumen reduction >50%. Atherosclerosis was defined as visible plaque (ultrasound) or calcification (CTA).

Supplemental tables:

Supplemental table I: Acute lesion areas [cm^2].

	DWI	ADC	Initial FLAIR
N with visible lesion, all	84 (54%)	76 (49%)	67 (43%)
Area, range	0.03-5.88	0.03-5.07	0.04-6.03
Area, median (IQR)	0.28 (0.11-0.56)	0.25 (0.14-0.60)	0.32 (0.16-0.80)
N, scar	54 (65%)	53 (71%)	53 (80%)
Area range, persistent infarction	0.05-5.88	0.03-5.07	0.05-6.03
Area range, no persistent infarction	0.03-1.10	0.03-2.17	0.04-1.56
Area median (IQR), persistent infarction	0.40 (0.13-0.86)	0.34 (0.16-0.90)	0.37 (0.18-0.89)
Area median (IQR), no persistent infarction	0.16 (0.08-0.22)	0.17 (0.07-0.23)	0.20 (0.08-0.28)
[a]P	<0.0001	0.002	0.019
cGM area range, persistent infarction (n=21)	0.20-5.88	0.09-5.07	0.12-6.03
cGM area range, no persistent infarction (n=26)	0.03-0.38	0-0.42	0-0.91
cGM area median (IQR), persistent infarction	0.53 (0.37-1.35)	0.34 (0.22-1.22)	0.51 (0.33-1.18)
cGM area median (IQR), no persistent infarction	0.15 (0.08-0.20)	0.09 (0.04-0.22)	0 (0-0.17)
[a]P	<0.0001	<0.0001	<0.0001

122 patients with 155 events completed 8-week (8w) MRI. [a]Initial areas of lesions with and without persistent infarction development, Mann-Whitney U test.

Supplemental table II: Characterization of 122 included patients with and without recurrence

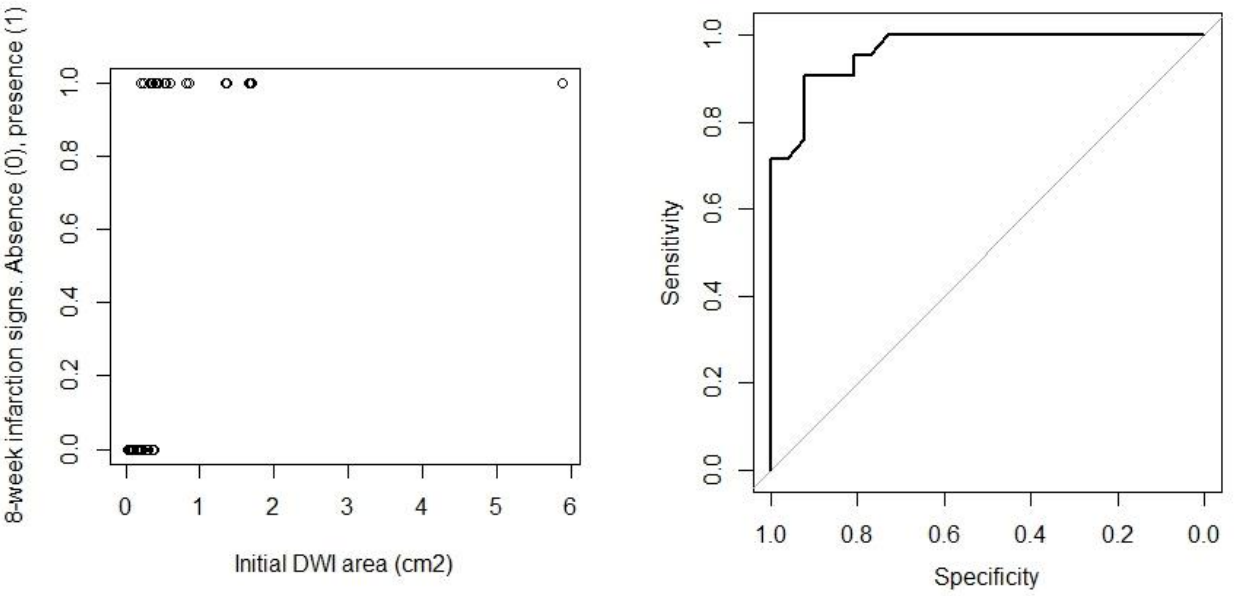
	Recurrent event	No recurrent event	P	OR (95%CI)
	n=14	n=108		-
Female sex, n(%)	5 (36%)	47 (44%)	[a]0.775	-
Median (IQR) age, y	64 (56-76))	65 (53-70)	[b]0.612	-
Microbleeds	3 (21%)	30 (28%)	[a]0.756	0.70 (0.18-2.69)
Old infarctions	8 (57%)	44 (41%)	[a]0.264	1.94 (0.63-5.98)
Small vessel disease (R)	11 (79%)	62 (57%)	[a]0.156	2.72 (0.72-10.31)
Large vessel disease (R)	8 (57%)	37 (34%)	[a]0.139	2.56 (0.83-7.93)
Cardioembolic pattern (R)	1 (7%)	13 (12%)	[a]0.703	0.56 (0.07-4.66)
DWI positive (qualifying event)	3 (21%)	47 (44%)	[a]0.152	0.35 (0.09-1.43)
Symptom duration <60 min			1.000	
<60 min	7 (50%)	51 (47%)		1.00
>60 min	7 (50%)	57 (53%)		0.90 (0.29-2.73)
Atrial fibrillation	0	12 (11%)	[a]0.356	….
Hypertension	8 (57%)	52 (48%)	[a]0.580	1.44 (0.47-4.42)
Diabetes	2 (14%)	14 (13%)	[a]1.000	1.12 (0.23-5.54)
Active smoking	8 (57%)	35 (33%)	[a]**0.083**	**2.74 (0.88-8.52)**
Alcohol overuse	1 (7%)	11 (10%)	[a]1.000	0.66 (0.08-5.58)
Ipsilateral carotid stenosis >70%	3 (21%)	2 (2%)	[a]**0.011**	**14.46 (2.18-96.0)**
Non-ipsilateral extracranial stenosis	2 (14%)	4 (4%)	[a]0.141	4.33 (0.72-26.2)
TOAST etiology (qualifying event):			0.013	
Small vessel (C+R)	4 (29%)	45 (42%)		1.00
Large vessel (C+R)	2 (14%)	26 (24%)		0.86 (0.15-5.05)
Cardiogenic (C+R)	0	18 (17%)		….

Several possible etiologies (C+R)	8 (57%)	19 (18%)		**4.74 (1.27-17.64)**
Infarction-yielding patient	3 (21%)	40 (37%)	[a]0.37	0.46 (0.12-1.76)

[a]Fisher's exact test. [b]Mann-Whitney-U test. RR=relative risk. Y=years. R=radiological. C=clinical

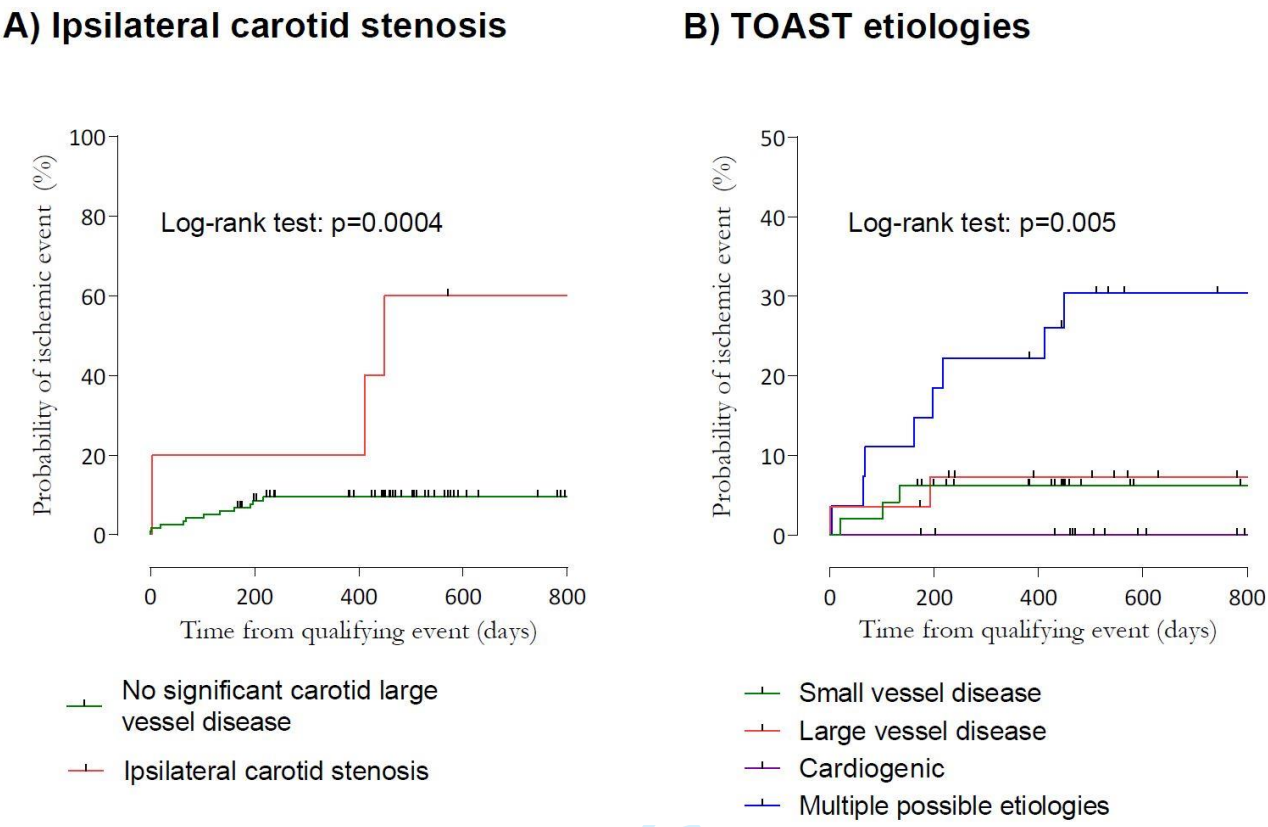
Supplemental figures:

Supplemental figure I: Association between 8-week infarction signs and cortical gray matter (cGM) lesion area.



Left panel shows 47 cGM lesions' initial DWI area and presence or absence of 8-week infarction signs. Right panel shows ROC curve illustrating cGM DWI size as binary classifier for 8-week infarction with optimal threshold 0.31 cm^2, AUC 0.97.

Supplemental figure II: Kaplan-Meier curves for patients with significant risk factors of recurrent cerebrovascular event.



Patients with ipsilateral carotid stenosis (panel A) and clinically and radiologically multiple possible etiologies (panel B) have significantly higher probability of recurrent ischemic event.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract Page 1-3	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Prospective cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale Page 4-5	2	Explain the scientific background and rationale for the investigation being reported Unexplained up to 7-fold variation in DWI-positivity rates in populations of patients with TIA despite attempts to control for time to baseline MRI and subspecialty of referring physician. Small chronic lesions are also associated with increased risk of stroke and death and cumulative lesion burden has role in cognitive decline. Prior work in small or mixed populations points towards tissue type as factor for varying lesion development.
Objectives Page 5	3	State specific objectives, including any prespecified hypotheses We hypothesized that vascularity and so propensity for lesion reversal depended on lesion location, i.e. persistent infarction signs would show more frequently in end-artery dominated white matter (and deep gray matter) than in cortical gray matter with leptomeningeal collaterals. This study aims to investigate rate of lesion reversal and role of tissue type for lesion development in a prospective cohort of patients with TIA and 8-week MRI.
Methods		
Study design Page 5	4	Present key elements of study design early in the paper Observational study of prospective cohort of patients with clinical TIA for lesion evolution with standard 3T-MRI at baseline and 8-week follow-up. No intervention.
Setting Page 5-7	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Clinical workflow in tertiary stroke centre, standard TIA imaging 3T-protocol; February 2012 - June 2014.
Participants Page 5, 7-8	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Patients with clinical time-based TIA without MRI contraindications enrolled by senior consultant stroke neurologists with 8-week follow-up MRI. Standard 3T TIA imaging in clinical workflow, PACS-only assessment after predefined case report form by a neuroradiologist. 8-week follow-up MRI and telephone doctor interview. Standard clinical 3-mo follow-up. National electronic records for long-term follow-up. Blinded clinical and radiological data collection. After ended data collection, match of symptoms and lesions. *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NA

		Case-control study—For matched studies, give matching criteria and the number of controls per case
Variables Page 5,7	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Primary outcome was presence or absence of persistent infarction signs defined as 8-week FLAIR-hyperintensity or atrophy corresponding to the initial DWI lesion. Potential predictors for persistent infarction signs were lesion location (tissue type), DWI lesion size (cm^2), visibility on baseline ADC or FLAIR, time to baseline MRI scan (TTS).
Data sources/ measurement Page 6-7	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Page 6-7	9	Describe any efforts to address potential sources of bias We wanted to perform the study in a clinical workflow and as close to the real population of patients with TIA as possible. Small lesions would be challenging, below the 4-mm slice thickness of the standard TIA 3T imaging protocol in clinical workflow and similar to prior studies [14,15]. We deemed volumetric measurements unrealistic, nor do we routinely use external volumetric software. Best estimate was axial area (DWI and ADC in plane resolution was 1.1 x 1.1 mm and FLAIR 0.9 x 0.9 mm). Template, case report form (CRF), their pilot validation and CRF use are described on pages 6-7. We found 14% (12/84) of DWI lesions were < 3mm.
Study size Page 5, Figure 2	10	Explain how the study size was arrived at The study is exploratory; we aimed for a fairly robust and achievable single centre sample of 200 patients within two years.
Quantitative variables Page 7	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Lesion localization we divided into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM). We found too few dGM lesions (4) for meaningful analysis and as we expected dGM vascularity similar to WM vascularity we grouped them together, main analyses are performed as cGM-lesions versus non-cGM. Lesion reversal was analysed as dichotomous presence or absence and partial lesion reversal was analysed as area change between baseline and 8-week MRI graded at 30%, 50% and 100%.
Statistical methods Page 8	12	(*a*) Describe all statistical methods, including those used to control for confounding For categorical data, we used Fisher's exact test, and Mann-Whitney-U test for population comparison. For dichotomized outcomes, we performed a general linear model-based forced entry logistic regression analysis; p-values were calculated with the Wald test. We performed a likelihood ratio test for model fit versus an empty model. We used ROC analysis for binary classification. Recurrent cerebrovascular events were studied using Kaplan-Meier curves and Cox Proportional Hazard Model. We considered p-values less than 0.05 significant.
		(*b*) Describe any methods used to examine subgroups and interactions NA
		(*c*) Explain how missing data were addressed NA
Page 7		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed. National electronic patient records allowed long-term follow-up. *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of

	sampling strategy
	(*e*) Describe any sensitivity analyses
Page 6	Exploratory study. Intraobserver agreement quality check: In a random 10% sample (defined by date of birth 4th, 14th and 24th day in any month) we calculated Cohen's kappa for intra-observer variation using two CRF readings, except for area measurements, with 3 months' interval and observed a substantial intra-observer agreement (κ=0.80).

Continued on next page

Results		
Participants Figure 2	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Please see figure 2 for patient flow.
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram Please see figure 2 for patient flow.
Descriptive data Page 16, Supplemental data, Figure 1	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Please see Table 1 for patient characteristics, Supplemental table I for lesion areas and Supplemental table II for characteristics of patients with and without recurrence. Please also see Figure 1 for examples of lesions with and without persistent infarction signs.
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data Figures 3+4, Page 16, and Supplemental data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Please see Figure 3 for probability of permanent infarction signs and Figure 4 for lesions with full or partial area regression or progression stratified by tissue location. Please also see Table 1 for patient characteristics, Supplemental table I for lesion areas and Supplemental table II for characteristics of patients with and without recurrence.
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results Page 8-10, Figures 3+4	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included 50 patients showed 84 initial DWI lesions. 29 (35%) DWI lesions did not result in infarction signs on 8-week FLAIR. 26 (90%, p<0.0001) reversing lesions were located in the cortical gray matter (cGM). CGM location (versus any other location) strongly predicted no 8-week infarction sign development (OR 0.02, 95% CI 0.001-0.17) or partial lesion area regression (>30% of initial DWI-area, OR 14.10, 95% CI 3.61-54.72), adjusted for FLAIR-visibility, DWI-area, ADC-confirmation and time from symptom onset to baseline MRI (TTS). Acute FLAIR-visibility was a strong predictor for persistent infarction signs (OR 64.62, 95% CI 3.41-1223.20). For cGM lesions area size was sole predictor for persistent infarction signs with a 0.31cm^2 (AUC 0.97) threshold. In 8 (16%) DWI-positive patients all lesions reversed fully. There was a significant correlation (OR 1.20 per additional mm^2, 95% CI 1.11-1.28) between the patients' total DWI area and the probability of persistent infarction signs.
		(*b*) Report category boundaries when continuous variables were categorized Please see item 11 (Methods, Quantitative variables)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses Page 10	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses There was no significant correlation between increasing ABCD2 score and the probability of persistent infarction signs (OR 1.09, 95% CI 0.82-1.46).

Eight weeks post-TIA we found no correlation between persistent infarction sign presence and patient-reported return to pre-TIA daily activity level (4 (33%) vs. 35 (36%), p=0.87). 14 recurrent cerebrovascular events occurred during the median (IQR) follow-up period of 817 (440-1056) days. Characteristics of patients with recurrent ischemic event are presented in Supplemental table II. Ipsilateral carotid stenosis ≥ 70% (hazard ratio [HR] 7.11, 95% CI 1.98-25.5) and several competing TOAST aetiologies (HR 3.74, 95% CI 1.13-12.4) predicted recurrent ischemic events (Supplemental figure II). Persistent 8-week infarction signs (HR 0.50, 95% CI 0.14-1.78) did not increase the risk of recurrent events.

Discussion		
Key results Page 11	18	Summarise key results with reference to study objectives 16% of initially DWI-positive patients and a third of identified lesions show no sign of infarction 8 weeks later; cortical gray matter location was the strongest predictor for lesion reversal on MRI at 8 weeks, and cGM DWI lesions smaller than 0.31cm^2 did not show persistent infarction signs. ADC-confirmation or subacute FLAIR-visibility did not in all cases result in visible long-term infarction.
Limitations Page 12	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Inclusion was based on informed consent so the study was not unselected or consecutive. 14% (12/84) of the initial lesions were small (<3 mm), and below the usual recommended cut-off level to discriminate between lacunes and perivascular spaces, but we included this type of lesion as it has been associated with increased risk of stroke and death. Other potential limitations are partial volume effects and the 2D-FLAIR sequence in our standard stroke MRI-protocol, though its slice thickness is similar to prior studies'. Dedicated high-resolution 3D-FLAIR and 3D-T1 could improve differentiation between healthy tissue and post-infarction gliosis or atrophy, especially cortically.
Interpretation Page 11-12	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Literature holds few and small studies with varying populations and rates of DWI positivity and reversal. 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. Our TTS and per patient DWI positivity and reversal rates correspond to the smaller TIA study.[14] Our cohort's 31 hour median delay to imaging may explain why several lesions are FLAIR-positive representing the beginning vasogenic oedema, and the persistent ADC-reduction represents the still active cytotoxic oedema.[29] We found no association between DWI positivity rate and time to scan in line with recent metaanalysis.[12] In TIA, DWI lesions are rarer and smaller and the TIA presumably results from smaller occlusive events compared to stroke. Changes in perfusion and underlying vascularity reflect tissue vulnerability and damage. 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. This and smaller lesion size may explain the high variation in DWI-positivity rates among TIA populations and the high rate and cortical predilection of apparent diffusion lesion reversal in this TIA study.

However, in an observational exploratory study, we cannot infer causality but hypothesize that the stronger leptomenigeal collateral circulation in cGM may prevent persistent infarction signs in small lesions.

Generalisability Page 11	21	Discuss the generalisability (external validity) of the study results We designed the study for a clinical setting and workflow and wanted it to be as unselective and close to the real population of patients with TIA as possible. Our results are in line with a smaller reported TIA-study, but DWI positivity and reversal rates differ from a mixed TIA and minor stroke population (please see the table below). We thus think that the results are representative of populations of patients with clinical TIA.

Reference, population	DWI+ patients	[2]p	DWI reversal/DWI+ patients	[2]p
[14],[1]TIA	35% (36/103)	0.43	21% (7/33)	0.75
[15], mixed TIA and minor stroke	57% (192/337)	0.003	6% (11/192)	0.03
Our TIA study	41% (50/122)	-	16% (8/50)	-

[1]33 patients with follow-up MRI. [2]Chi squared test for independence versus our population of patients with TIA.

Other information

Funding Page 1	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based No funding as received for this study. CO holds research grants from the Velux-foundation, Bispebjerg University Hospital, University of Copenhagen, Axel Muusfeldts Foundation and Danish Medical Association. None of these were designated for this study.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Small cortical gray matter lesions show no persistent infarction in transient ischemic attack? A prospective cohort study

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Secondary Subject Heading:	Diagnostics
Keywords:	Transient Ischemic Attack, cerebral cortex, brain infarction, cerebral circulation, Magnetic resonance imaging < RADIOLOGY & IMAGING

Small cortical gray matter lesions show no persistent infarction in transient ischemic attack? A prospective cohort study

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Supplementary files: 2

Disclosures: CO holds research grants from the Velux-foundation, Bispebjerg University Hospital, University of Copenhagen, Axel Muusfeldts Foundation and Danish Medical Association. None of these were designated for this study.

Funding: No funding was received for this study.

ABSTRACT

Objectives: To find determining factors for persistent infarction signs in patients with TIA, herein initial diffusion lesion size, visibility on ADC or FLAIR and location.

Design: Prospective cohort study of patients with clinical TIA receiving 3T-MRI within 72 hours of symptom onset and at 8-week follow-up

Setting: Clinical workflow in single tertiary stroke centre between February 2012 and June 2014.

Participants: 199 candidate patients were recruited, 64 patients were excluded due to non-TIA discharge diagnosis or no 8-week MRI. 122 patients completed the study.

Primary outcome measures: The primary outcome was visible persistent infarction defined as 8-week FLAIR-hyperintensity or atrophy corresponding to the initial DWI lesion.

Results: 50 patients showed 84 initial DWI lesions. 29 (35%) DWI lesions did not result in infarction signs on 8-week FLAIR. 26 (90%, $p<0.0001$) reversing lesions were located in the cortical gray matter (cGM). CGM location (versus any other location) strongly predicted no 8-week infarction sign development (OR 0.02, 95% CI 0.001-0.17) or partial lesion area decrease (>30% of initial DWI-area, OR 14.10, 95% CI 3.61-54.72), adjusted for FLAIR-visibility, DWI-area, ADC-confirmation and time from symptom onset to baseline MRI (TTS). Acute FLAIR-visibility was a strong associated factor for persistent infarction signs (OR 33.06, 95% CI 2.94-1432.34). For cGM lesions area size was sole associated factor for persistent infarction signs with a 0.31cm^2 (AUC 0.97) threshold. In 8 (16%) DWI-positive patients all lesions reversed fully.

Conclusions: 16% of DWI-positive patients and third of acute DWI lesions caused no persistent infarction signs, especially small cortical gray matter lesions were not followed by development of persistent infarction signs. Late MRI after TIA is likely to be less useful in the clinical setting, and it is dubious if the absence of old vascular lesions can be taken as evidence of no prior ischemic attacks.

Trial registration: clinicaltrials.gov NCT01531946.

Key words: Transient Ischemic Attack; cerebral cortex; brain infarction; cerebral circulation; Magnetic Resonance Imaging

Article Summary

Strengths and limitations of this study

- Prospective inclusion cohort study of patients with clinical TIA evaluated by senior consultant stroke neurologists.
- Patients received standardized baseline and 8-week follow-up MRI in clinical workflow.
- We evaluated lesions after predefined case report form in a clinical PACS-only setting.
- National electronic registers provided long-term follow-up.
- Small lesions below 3 mm were included as they are associated with increased risk of stroke and death.
- Main limitations are sequence resolution and small lesion size.

Introduction

The risk of recurrent events including devastating stroke after transient ischemic attack (TIA) remains substantial.[1,2] Recently, it was shown that the presence of a diffusion weighted imaging (DWI) lesion combined with clinical findings enhanced prediction of post-TIA stroke risk compared to clinical findings alone.[3–5] Even small vascular brain lesions below 3 mm were associated with increased risk of stroke and death.[6] In patients with TIA, DWI lesions are reported in 25% to 50%.[4,5,7–11] Also the time to baseline MRI varies from within 24 hours to 3 weeks from symptom onset.[12] A recent meta-analysis found that the up to 7-fold variation in DWI positivity rates in patients with clinical TIA remains largely unexplained despite attempts to control for factors as varying time to MRI.[12] 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]

Pioneering studies found that patients with apparent DWI lesion reversal had shorter symptom duration than patients with persistent infarction signs[14] and noted absence of persistent infarction signs in a fraction of initially DWI-positive patients on chronic phase MRI.[14,15] Lesions with persistent infarction signs were larger and had lower apparent diffusion coefficient (ADC)-values.[15] A recent series of mixed TIA and minor stroke patients found only 6% of initially DWI-positive patients showed no lesion on T2-fluid attenuated inversion recovery (FLAIR) images obtained 1-3 months later.[16] Despite small numbers the absent lesions seemed primarily located in cortical gray matter,[16] suggesting that acute DWI-reversal may be related to the proximity of leptomeningeal collaterals.[17,18] Cortical perfusion is higher than white matter perfusion, even though the ratio declines with age.[19] The impact of the underlying vascularity is also highlighted in a perfusion study of initially DWI-negative patients with clinical time-based TIA, patients with initial focal or territorial perfusion abnormalities showed increased rates of new DWI lesions on 3-

day follow-up compared with patients with no perfusion abnormalities.[20] 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] Although the clinical significance of persistent infarction signs is unclear, it is well known that numerous cerebral infarcts gathered over time are predictive of poor outcome[6] and cognitive decline,[23] rendering it of potential pathophysiological and clinical importance to investigate what determines the formation of persistent infarction signs. 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] We hypothesized that lesion size and ischemic depth, and location are factors likely to predict the occurrence of infarction signs after acute ischemia in clinical TIA.

We aimed to investigate in a clinical setting which characteristics were associated with persistent infarction signs 8 weeks after DWI-positive TIA, including lesion location, size, initial ADC- and FLAIR-visibility, TIA aetiology, and clinical risk factors. The ultimate aim was to establish if no development of persistent infarction signs after DWI-positive TIA occurred with a clinically significant frequency.

Methods

We investigated a prospective patient cohort with clinical TIA included after informed consent. The study was approved by the Danish National Committee of Biomedical Research Ethics (H-1-2011-75).

Clinical methodology

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] We defined resolution as National Institutes of Health Stroke Scale (NIHSS) 0 within 24 hours. Senior consultant stroke neurologists clinically evaluated and included patients with a history and clinical findings consistent with TIA February 2012 – June 2014. Exclusion criteria were TIA (G45.9) not final clinical diagnosis, thrombolysis treatment, MRI contraindications, and severe illness likely to preclude follow-up. Definitions of clinical risk factors are presented in supplemental methodology. Risk factors of stroke, ABCD2[27], and TOAST[28] classification were recorded. All patients were treated with antithrombotics and other pharmacological secondary prevention according to guidelines.

MR imaging

We performed 3T-MRI (Siemens Magnetom Verio, Siemens, Erlangen, Germany) with a 32-channel head coil (Siemens, Erlangen, Germany) including DWI and T2-FLAIR imaging after routine stroke protocol within 72 hours from symptom onset[29] and at 8-week follow-up to visualize persistent infarction signs. The diffusion protocol was single-shot spin-echo diffusion echo planar imaging with 220-mm FOV, 25 4-mm axial 0-mm gap slices, b-value 1000 s/mm^2 along 3 orthogonal directions; TR/TE 6600/100 ms, acceleration factor R 2, matrix size 192 x 192. The T2-FLAIR protocol was 240-mm FOV, 27 4-mm axial 0-mm gap slices, TR/TE 6500/133 ms, TI 2134 ms, acceleration factor R 2, matrix size 256 x 256.

Image reading was performed as visual inspection in a clinical setting using the PACS without external software. Image analysis had two steps: first, we created an image template consisting of

literature based examples of the scoring categories employed and a case report form (CRF). Two blinded certified consultant neuroradiologists (IH, AFC) tested template and CRF on 50 randomly chosen cases from the cohort, reading first independently, followed by a joined re-evaluation of the cases. A consensus-based use of the reading tools was thus achieved enabling the use of one reader for consistence and reproducibility in assessing the often small lesions. Subsequently, one reader (IH), blinded to clinical data except for the referral, systematically evaluated the cohort's scans.
In a random 10% sample (defined by date of birth 4th, 14th and 24th day in any month) we calculated Cohen's kappa for intra-observer variation using two CRF readings, except for area measurements, with 3 months' interval and observed a substantial intra-observer agreement (κ=0.80).
Definitions of post-hoc vascular findings are presented in supplemental methodology.

Definitions of lesions, size, and localization

We used co-registration marking each lesion with two perpendicular intrathecal diameters to ascertain lesion localization between sequences and baseline and 8-week MRI. Two separate lesions must not have confluent gliosis. As proxy for lesion size we used largest lesion area on one slice measured by manual ROIs.[30] On baseline MRI, we scored for presence or absence of DWI lesions, ADC-confirmation and T2-FLAIR hyperintensities, and divided lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (basal ganglia). CGM lesions may have a minor subcortical WM component.

We defined persistent infarction signs as presence of T2-FLAIR hyperintensity[24,31] or atrophy in the initial DWI-lesion area on 8-week MRI[15,16,32]. 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. Due to small

lesion size, this definition was modified from the 10% reduction used in ischemic stroke.[33] Lesion area change was graded at 30%, 50%, and 100%.

After all initial and 8-week clinical and radiological data were collected; we checked if DWI lesion site and symptoms matched – which it did in all cases - under supervision of a senior stroke consultant (HC).

Recurrence and follow-up

All patients had a scheduled 8-week telephone follow-up[34,35] by a doctor to assess self-reported functional status and recurrence of new stroke or TIA. Standard 3-month follow-up was a face-to-face interview with a trained nurse assessing functional status, changes in risk factor status, and medication adherence. National electronic patient files provided long-term follow-up information on new vascular events.

Statistics

For categorical data, we used Fisher's exact test, and Mann-Whitney-U test for population comparison. For dichotomized outcomes, we performed a general linear model-based forced entry logistic regression analysis; p-values were calculated with the Wald test. We included lesion location, DWI lesion size (cm^2), visibility on baseline ADC or FLAIR and time to baseline MRI as parameters in the multivariate analysis. We performed a likelihood ratio test for model fit versus an empty model. We used ROC analysis for binary classification. Recurrent cerebrovascular events were studied using Kaplan-Meier curves and Cox Proportional Hazard Model. We considered p-values less than 0.05 significant. We used R (version 3.2.0), 2015 The R Foundation for Statistical

Computing, Vienna, Austria. URL: http://www.R-project.org/ and SPSS (version 22.0) statistical software, IBM Corporation, Armonk, New York, USA.

Results

We included 122 patients with clinical TIA (Figure 2), median (IQR) age of 65 (54-71) years and median (IQR) ABCD2 4 (3-5) (Table 1). The median (IQR) time from ictus to initial MRI (TTS) was 31.5 (23.5-56.8) hours. Table 1 shows the patients' baseline characteristics and findings on subacute DWI and 8-week T2-FLAIR. 50 patients showed 84 DWI lesions. 32 patients had solitary DWI lesions. 33 lesions were located in white matter, 47 lesions in cortical gray matter and 4 lesions in deep gray matter. There were no statistically significant relations between DWI positivity rates and time to scan.

Initial DWI lesions located in cGM were less likely to show subsequent infarction signs on 8-week MRI ($p<0.001$, OR 0.07, 95% CI 0.01-0.27) compared to other locations. This finding persisted when including only ADC-confirmed lesions (22 with no persistent infarction signs, hereof 19 (86%) in cGM, $p<0.001$), or only lesions with subacute FLAIR-visibility (13 with no persistent infarction signs, hereof 12 (92%) in cGM, $p<0.001$, Figure 3). Lesions with persistent infarction signs were significantly larger than lesions with no persistent infarction signs; DWI lesions leading to permanent infarction signs ranged 0.05-5.88 cm^2 with median (IQR) 0.40 (0.13-0.86) cm^2 and lesions with no permanent infarction signs ranged 0.03-1.10 cm^2 with median 0.16 (0.08-0.22) cm^2, $p<0.001$ (Supplemental table I). In multivariate analysis, cGM location (cGM vs other locations), strongly predicted no persistent infarction signs (OR 0.02, 95% CI 0.001-0.17), whereas initial FLAIR-visibility predicted subsequent infarction signs (OR 33.06, 95% CI 2.94-1432.34). Initial DWI lesion size, ADC-confirmation and TTS did not reach significance.

If the initial DWI lesion was located in cGM, no initial FLAIR- or ADC-negative lesions resulted in persistent infarction signs, but persistent infarcts were visible for 64% (21/33) of initially FLAIR-positive and 53% (21/40) of ADC-confirmed cGM lesions. Size of cGM lesions was the single associated factor for persistent infarction signs with an optimal area threshold 0.31 cm^2 (AUC 0.97, Supplemental figure I) to discern between lesions with or without 8-week infarction signs.

Figure 4 shows change of lesion size between baseline and 8-week MRI stratified according to tissue type. Of the initial 84 DWI lesions, 51 (61%) lesions showed at least 30% lesion area decrease and 15 (18%) showed lesion area increase of 30% or more. WM lesions had the highest rate of area increase, 13/33 (39%), and the highest degree of increase. We found that 41/47 (87%) of the lesions located in cGM decreased in size and 26/47 (55%) completely vanished leaving no persistent infarction signs (Figure 4). Adjusted for ADC-confirmation, initial FLAIR-visibility and DWI-lesion size cGM location (cGM vs other locations) was a strong associated factor of lesion area decrease (OR 14.1, 95% CI 3.61-54.72). The distribution of lesions with area decrease or increase differed significantly between cGM and WM ($p<0.0001$, Supplemental figure II).

To assess if individual patients retain persistent infarction signs after having at least one DWI-positive lesion, we defined lesion burden as the sum of each patient's combined DWI-areas. 16% of DWI-positive patients showed no persistent infarction signs (Table 1). Only the combined sum of DWI lesion areas significantly predicted persistent infarction signs. There was a significant correlation (OR 1.20 per additional mm^2, 95% CI 1.11-1.28) between the patients' total DWI area and the probability of persistent infarction signs. There was no significant correlation between increasing ABCD2 score and the probability of persistent infarction signs (OR 1.09, 95% CI 0.82-1.46).

Eight weeks post-TIA we found no correlation between persistent infarction sign presence and patient-reported return to pre-TIA daily activity level (4 (33%) vs. 35 (36%), p=0.87). 14 recurrent cerebrovascular events occurred during the median (IQR) follow-up period of 817 (440-1056) days. Characteristics of patients with recurrent ischemic event are presented in Supplemental table II. Ipsilateral carotid stenosis ≥ 70% (hazard ratio [HR] 7.11, 95% CI 1.98-25.5) and several competing TOAST aetiologies (HR 3.74, 95% CI 1.13-12.4) predicted recurrent ischemic events (Supplemental figure III). Persistent 8-week infarction signs (HR 0.50, 95% CI 0.14-1.78) did not increase the risk of recurrent events.

Discussion

16% of initially DWI-positive patients and a third of identified lesions show no sign of infarction 8 weeks later; cortical gray matter location was the strongest associated factor of full recovery on MRI at 8 weeks, and cGM DWI lesions smaller than 0.31cm^2 did not show persistent infarction signs. ADC-confirmation or subacute FLAIR-visibility did not in all cases result in visible long-term infarction signs.

Ischemic lesions decreased in size after early intra-arterial revascularization[17] and MCA-infarctions' cortical lesion sparing was related to collateral perfusion and affected by significant stenosis of the ipsilateral anterior or posterior cerebral artery.[18] In rats early (within 1 day) post-ischemic maximum hyperperfusion indicated small final lesion size while rats with late (4 days) maximum hyperperfusion showed large lesions after transient experimental ischemia,[36] indicating rich, tightly-webbed collaterals lead to improved early reperfusion and limit tissue damage.

Literature holds few and heterogeneous serial MRI TIA-related studies with varying populations and rates of DWI positivity and reversal. 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. Our TTS and per patient DWI positivity and reversal rates correspond to the smaller time-based TIA study.[15] Our cohort's 31 hour median delay to imaging may explain why several lesions are FLAIR-positive representing the beginning vasogenic oedema, and the persistent ADC-reduction represents the still active cytotoxic oedema.[37] We found no association between DWI positivity rate and time to scan in line with recent meta-analysis.[12]

In TIA, DWI lesions are rarer and smaller and the TIA presumably results from smaller occlusive events compared to stroke. Changes in perfusion and underlying vascularity reflect tissue vulnerability and damage. 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. This and smaller lesion size may explain the high variation in DWI-positivity rates among TIA populations and the high rate and cortical predilection of apparent diffusion lesion reversal in this TIA study.

However, in an observational exploratory study, we cannot infer causality but hypothesize that the stronger leptomenigeal collateral circulation in cGM may prevent persistent infarction signs in small lesions.

This study has some limitations. As inclusion was based on informed consent, the study was not unselected or consecutive. 14% (12/84) of the initial lesions were small (<3 mm), and below the usual recommended cut-off level to discriminate between lacunes and perivascular spaces,[38] but

we included this type of lesion as it has been associated with increased risk of stroke and death.[6] Other potential limitations are partial volume effects and the 2D-FLAIR sequence in our standard stroke MRI-protocol, though its slice thickness is similar to prior studies'.[15,16] 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]. We lost 10% (13/135) of eligible patients for 8-week MRI, this may have caused a selection bias. 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.

Conclusion

CGM localization of the acute DWI lesion in TIA was a strong associated factor of no persistent infarction signs after TIA. Late MRI after TIA may not detect a significant number of lesions, especially cortical lesions and no prior lesions on the MRI is not evidence of no prior ischemic events. 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.

Data Sharing Statement:

Dataset is available upon publication from FigShare, DOI: 10.6084/m9.figshare.5091904.

Author Contributions: IH, HC, AC conceived and designed the study. All authors were involved in data acquisition. KÆ, JM, PM, SR, MF, HC were involved in patient inclusion. LW and CO performed telephone follow-up. IH, CO, JD, AC, HC analysed and interpreted data. IH wrote the first draft. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Competing interests:

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Tables

Table 1. Patient characteristics

	All 8w	8w infarct	No infarct	p	OR (95% CI)
All patients	122	43	79		
Female sex, n	52 (43%)	15 (35%)	37 (47%)	*0.251	0.61 (0.28-1.31)
Age, median (IQR)	65 (54-71)	60 (53-70)	65 (55-74)	†0.228	…
Medical history:					
Prior stroke	22 (18%)	6 (14%)	16 (20%)	*0.466	0.64 (0.23-1.78)
Prior TIA	12 (10%)	7 (16%)	5 (6%)	*0.111	2.87 (0.85-9.70)
Prior MI	9 (7%)	2 (5%)	7 (9%)	*0.491	0.50 (0.10-2.53)
Atrial fibrillation	12 (10%)	7 (16%)	5 (6%)	*0.111	2.89 (0.85-9.70)
Hypertension	60 (49%)	26 (60%)	34 (43%)	*0.088	2.02 (0.95-4.31)
Diabetes	16 (13%)	8 (19%)	8 (10%)	*0.261	2.03 (0.70-5.86)
Depression	14 (11%)	7 (16%)	7 (9%)	*0.244	2.00 (0.65-6.14)
Current smoking	43 (35%)	18 (42%)	25 (32%)	*0.324	1.53 (0.71-3.30)
Alcohol overuse	12 (10%)	5 (12%)	7 (9%)	*0.754	1.32 (0.39-4.43)
Antiplatelet use	40 (33%)	13 (30%)	27 (34%)	*0.692	0.84 (0.38-1.86)
Warfarin	2 (2%)	1 (2%)	1 (1%)	*1.000	1.86 (0.11-30.5)
Index TIA:					
ABCD2	4 (3-5)	4 (3-5)	4 (3-5)	†0.485	….
Symptom duration:				*0.852	
<60 min	58	21 (36%)	37 (64%)		1.00
>60 min	64	22 (34%)	42 (66%)		0.93 (0.44-1.94)
Imaging findings:					
TTS (hours)	32 (24-57)	41 (22-66)	29 (24-50)	†0.367	….
DWI positive	50	42 (84%)	8 (16%)	*<0.0001	NA
Sum of DWI lesion area, cm^2	0.41 (0.21-1.08)	0.53 (0.31-1.22)	0.09 (0.07-0.22)	†<0.0001	….
Ipsilateral cervical carotid stenosis	5 (4%)	1 (2%)	4 (5%)	*0.656	0.45 (0.05-4.13)
Contralateral cervical carotid stenosis	6 (5%)	1 (2%)	5 (6%)	*0.522	0.35 (0.04-3.12)
Ipsilateral intracranial stenosis[a]	9 (12%)	1 (4%)	8 (17%)	*0.144	0.19 (0.02-1.63)
Contralateral intracranial stenosis[a]	1 (1%)	0 (0%)	1 (2%)	*0.450	NA
TOAST aetiology:				*0.727	
Small vessels	49	16 (33%)	33 (67%)		1.00
Large vessels	28	12 (43%)	16 (7%)		1.55 (0.59-4.03)

Cardiogenic	18	5 (28%)	13 (72%)		0.79 (0.24-2.61)
Multiple possible aetiologies	27	10 (37%)	17 (63%)		1.21 (0.45-3.24)
TTF (days)	56 (55-60)	55 (55-60)	56 (55-60)	†0.832	….

*Fishers exact test. †Mann-Whitney U test. [a]Computed tomography angiography (CTA) or transcranial Doppler (TCD) available in 75 patients, hereof 28 with 8-week infarction signs. TIA – transient ischemic attack. MI – myocardial infarction. NA – not applicable. TTS – time to scan. TTF – time to follow-up.

Legends

Figure 1.

Lesions with and without 8-week infarction signs. Two cortical gray matter lesions are shown on initial DWI (panel A), ADC (panel B), initial FLAIR (panel C) and 8-week FLAIR (panel D). Panel A: Both lesions are DWI-positive. Panel B: The medial lesion is ADC-confirmed, the lateral lesion shows no ADC-confirmation. Panel C: Both lesions are initially FLAIR-positive. Panel D: the medial lesion is 8-week FLAIR-positive, the lateral lesion is 8-week FLAIR-negative.

Figure 2.

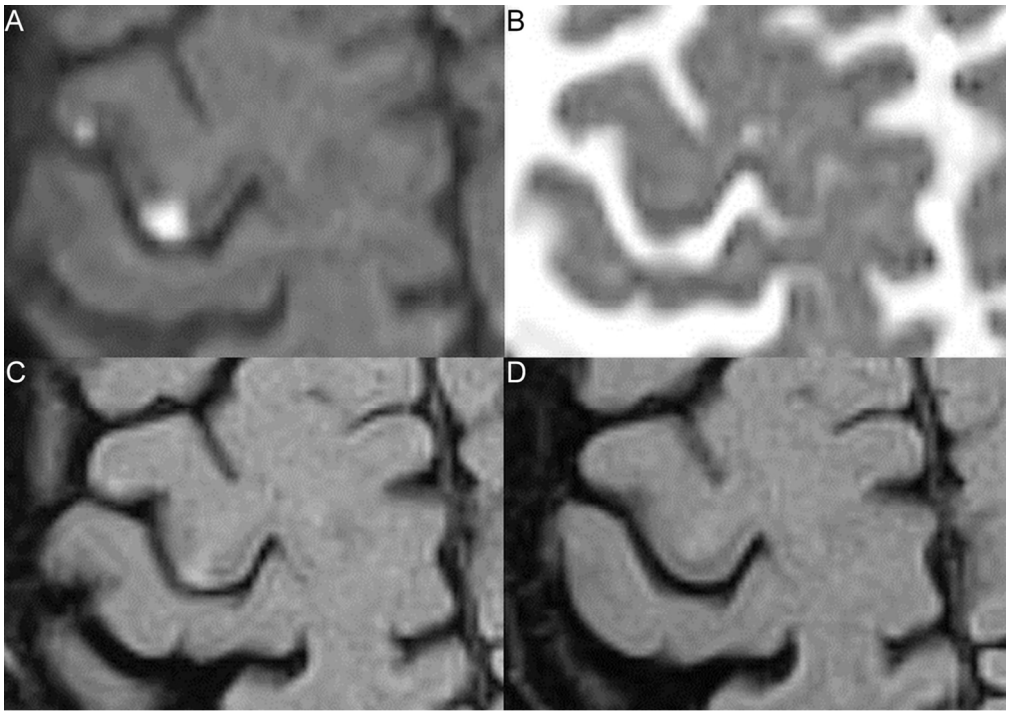
STROBE diagram of patient flow, in- and exclusion. Other non-ischemic comprises patients with peripheral nerve compression (2), ophthalmological symptoms (2), trigeminal neuralgia (1), normal pressure hydrocephalus (1), hyperventilation (1), paraesthesia secondary to anaemia (1), peripheral extremity embolus (1), food poisoning (1), and secondary refusal (1).

Figure 3.

Probability of permanent infarction signs stratified by anatomical location. DWI-lesion location in the cortical gray matter was significantly less likely to show permanent infarction signs. *$p<0.001$ against other anatomical location.

Figure 4.

Decrease and increase of lesion size between admission MRI and 8-week follow-up MRI for all lesions and stratified according to lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM).



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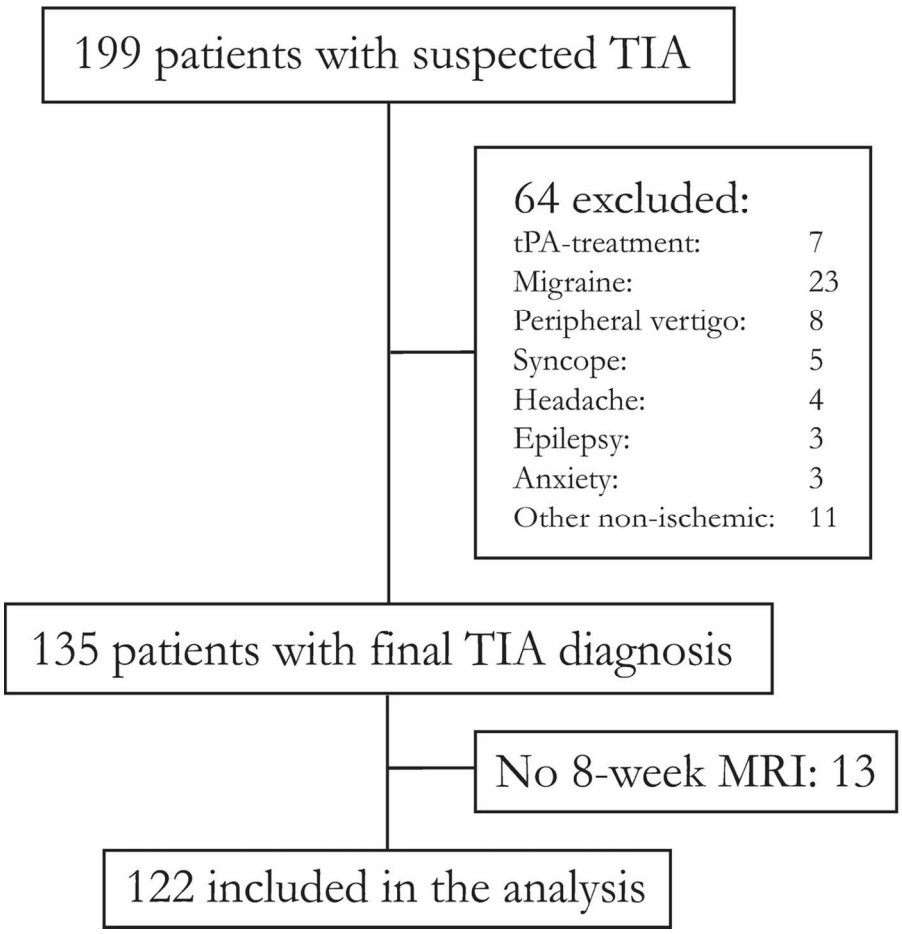
56x39mm (600 x 600 DPI)



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119x110mm (300 x 300 DPI)

Figure 3. Probability of permanent infarction signs stratified by anatomical location. DWI-lesion location in the cortical gray matter was significantly less likely to show permanent infarction signs. *p<0.001 against other anatomical location.

125x79mm (300 x 300 DPI)

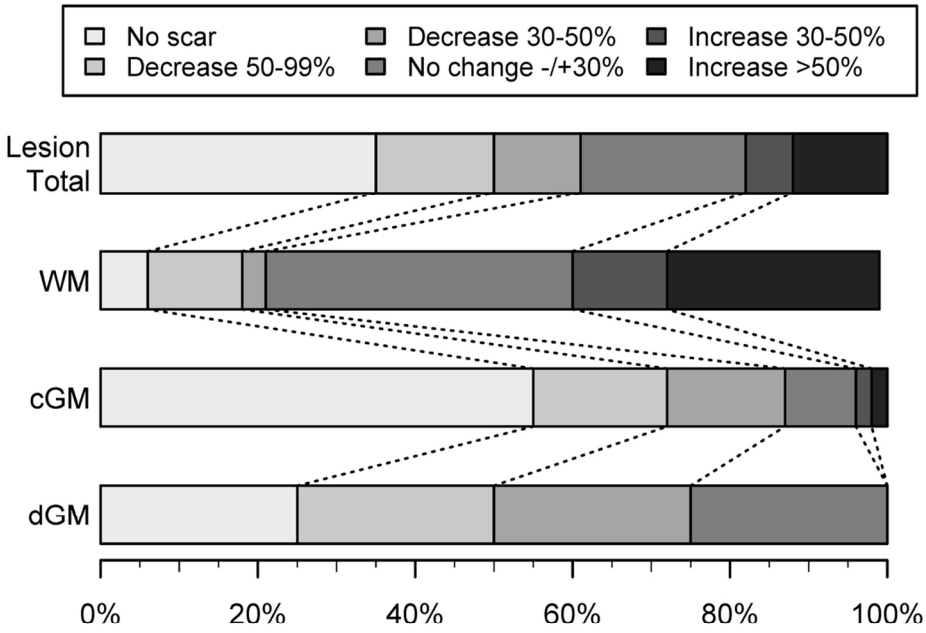


Figure 4.
Decrease and increase of lesion size between admission MRI and 8-week follow-up MRI for all lesions and stratified according to lesion localization into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM).

114x85mm (300 x 300 DPI)

ONLINE SUPPLEMENT

Cortical gray matter lesions are associated with no persistent infarction after transient ischemic attack

Havsteen et al. 2017

Supplemental methodology:

Clinical risk factors:

Symptoms, symptom duration, vascular risk factors and ABCD2 were recorded including prior stroke, TIA or myocardial infarction (MI), angina pectoris, peripheral arterial disease, diabetes or depression. Hypertension was defined as pre-admission use of antihypertensive medication or hypertension diagnosis in our out-patient clinic. Atrial fibrillation was diagnosed by medical history, admission 12-lead ECG, in-hospital telemetry (24 - 48 hours) or subsequent out-patient cardiac follow-up. We defined hypercholestrolemia as total plasma cholesterol above 5.0 mmol/L or statin treatment. Diabetes was defined by medical history or HbA1c >6.5%. We defined smoking as present or prior smoking, and alcohol overuse as weekly alcohol consumption above 252 g for males and 168 g for females. Recorded hereditary factors were first degree relative with stroke or MI. Clinical data were collected from electronic patient files. Subsequently, clinical and radiological data were compared for consistency under supervision of a senior neurological consultant (HC).

CT-angiography or transcranial Doppler was not included in the protocol; standard carotid examination was performed by ultrasound in the department.

Definition of post-hoc vascular findings

Standard work-up was extracranial carotid Doppler ultrasound. Some patients were investigated with TCD or CTA for clinical suspicion of large vessel disease. For ultrasound we defined carotid stenosis as peak systolic velocity >230 cm/s. For CTA we defined extracranial carotid stenosis as lumen reduction >70%, posterior circulation and intracranial arteries were stenotic with lumen reduction >50%. Atherosclerosis was defined as visible plaque (ultrasound) or calcification (CTA).

Supplemental tables:

Supplemental table I: Acute lesion areas [cm^2].

	DWI	ADC	Initial FLAIR
N with visible lesion, all	84 (54%)	76 (49%)	67 (43%)
Area, range	0.03-5.88	0.03-5.07	0.04-6.03
Area, median (IQR)	0.28 (0.11-0.56)	0.25 (0.14-0.60)	0.32 (0.16-0.80)
N, scar	54 (65%)	53 (71%)	53 (80%)
Area range, persistent infarction	0.05-5.88	0.03-5.07	0.05-6.03
Area range, no persistent infarction	0.03-1.10	0.03-2.17	0.04-1.56
Area median (IQR), persistent infarction	0.40 (0.13-0.86)	0.34 (0.16-0.90)	0.37 (0.18-0.89)
Area median (IQR), no persistent infarction	0.16 (0.08-0.22)	0.17 (0.07-0.23)	0.20 (0.08-0.28)
[a]P	<0.0001	0.002	0.019
cGM area range, persistent infarction (n=21)	0.20-5.88	0.09-5.07	0.12-6.03
cGM area range, no persistent infarction (n=26)	0.03-0.38	0-0.42	0-0.91
cGM area median (IQR), persistent infarction	0.53 (0.37-1.35)	0.34 (0.22-1.22)	0.51 (0.33-1.18)
cGM area median (IQR), no persistent infarction	0.15 (0.08-0.20)	0.09 (0.04-0.22)	0 (0-0.17)
[a]P	<0.0001	<0.0001	<0.0001

122 patients with 155 events completed 8-week (8w) MRI. [a]Initial areas of lesions with and without persistent infarction development, Mann-Whitney U test.

Supplemental table II: Characterization of 122 included patients with and without recurrence

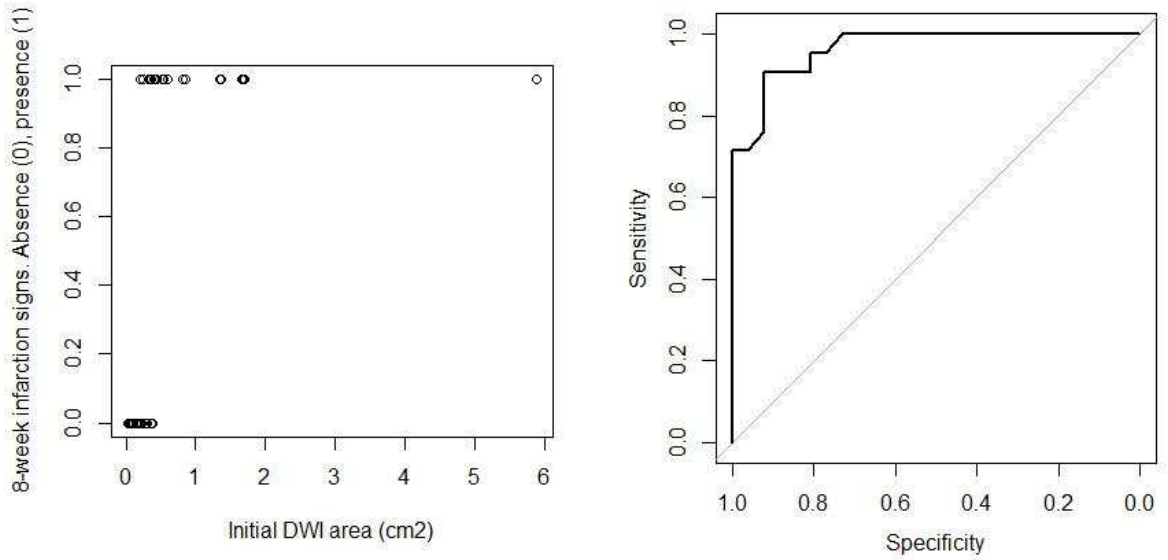
	Recurrent event	No recurrent event	P	OR (95%CI)
	n=14	n=108		-
Female sex, n(%)	5 (36%)	47 (44%)	[a]0.775	-
Median (IQR) age, y	64 (56-76))	65 (53-70)	[b]0.612	-
Microbleeds	3 (21%)	30 (28%)	[a]0.756	0.70 (0.18-2.69)
Old infarctions	8 (57%)	44 (41%)	[a]0.264	1.94 (0.63-5.98)
Small vessel disease (R)	11 (79%)	62 (57%)	[a]0.156	2.72 (0.72-10.31)
Large vessel disease (R)	8 (57%)	37 (34%)	[a]0.139	2.56 (0.83-7.93)
Cardioembolic pattern (R)	1 (7%)	13 (12%)	[a]0.703	0.56 (0.07-4.66)
DWI positive (qualifying event)	3 (21%)	47 (44%)	[a]0.152	0.35 (0.09-1.43)
Symptom duration <60 min			1.000	
<60 min	7 (50%)	51 (47%)		1.00
>60 min	7 (50%)	57 (53%)		0.90 (0.29-2.73)
Atrial fibrillation	0	12 (11%)	[a]0.356	….
Hypertension	8 (57%)	52 (48%)	[a]0.580	1.44 (0.47-4.42)
Diabetes	2 (14%)	14 (13%)	[a]1.000	1.12 (0.23-5.54)
Active smoking	8 (57%)	35 (33%)	[a]**0.083**	**2.74 (0.88-8.52)**
Alcohol overuse	1 (7%)	11 (10%)	[a]1.000	0.66 (0.08-5.58)
Ipsilateral carotid stenosis >70%	3 (21%)	2 (2%)	[a]**0.011**	**14.46 (2.18-96.0)**
Non-ipsilateral extracranial stenosis	2 (14%)	4 (4%)	[a]0.141	4.33 (0.72-26.2)
TOAST etiology (qualifying event):			0.013	
Small vessel (C+R)	4 (29%)	45 (42%)		1.00
Large vessel (C+R)	2 (14%)	26 (24%)		0.86 (0.15-5.05)
Cardiogenic (C+R)	0	18 (17%)		….

Several possible etiologies (C+R)	8 (57%)	19 (18%)		**4.74 (1.27-17.64)**
Infarction-yielding patient	3 (21%)	40 (37%)	[a]0.37	0.46 (0.12-1.76)

[a]Fisher's exact test. [b]Mann-Whitney-U test. RR=relative risk. Y=years. R=radiological. C=clinical

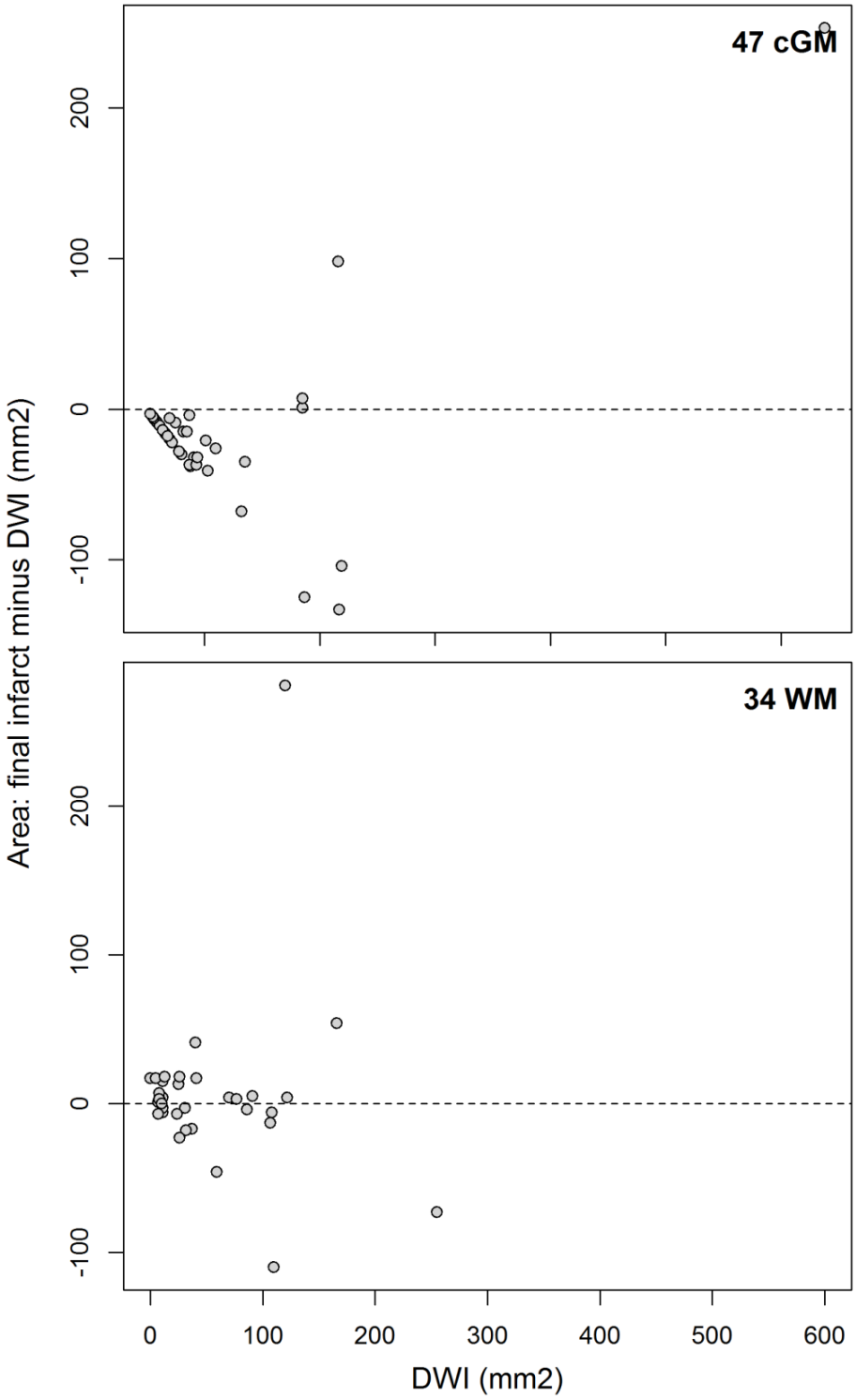
Supplemental figures:

Supplemental figure I: Association between 8-week infarction signs and cortical gray matter (cGM) lesion area.

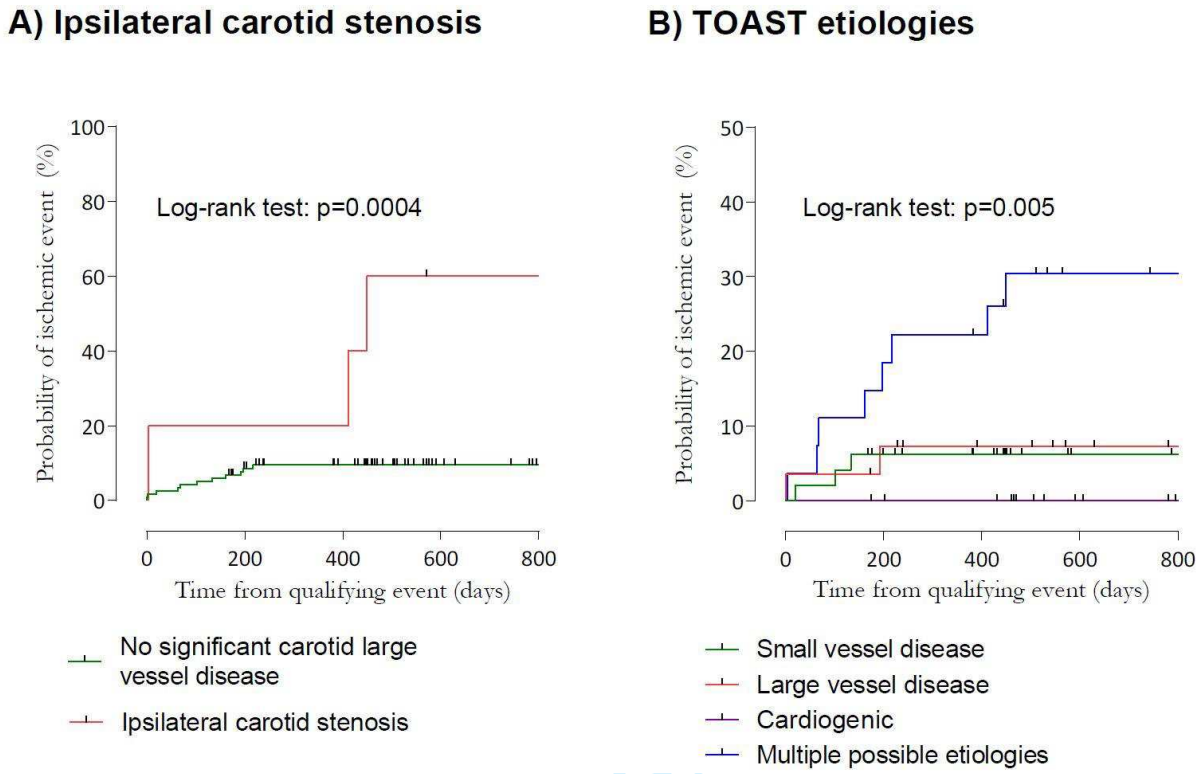


Left panel shows 47 cGM lesions' initial DWI area and presence or absence of 8-week infarction signs. Right panel shows ROC curve illustrating cGM DWI size as binary classifier for 8-week infarction with optimal threshold 0.31 cm^2, AUC 0.97.

Supplemental figure II: Lesion area decrease or increase between ictus and 8-week follow-up for 47 cortical gray matter (cGM) lesions (upper panel) and 34 white matter lesions (lower panel).



Supplemental figure III: Kaplan-Meier curves for patients with significant risk factors of recurrent cerebrovascular event.



Patients with ipsilateral carotid stenosis (panel A) and clinically and radiologically multiple possible etiologies (panel B) have significantly higher probability of recurrent ischemic event.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract Page 1-3	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Prospective cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale Page 4-5	2	Explain the scientific background and rationale for the investigation being reported Unexplained up to 7-fold variation in DWI-positivity rates in populations of patients with TIA despite attempts to control for time to baseline MRI and subspecialty of referring physician. Small chronic lesions are also associated with increased risk of stroke and death and cumulative lesion burden has role in cognitive decline. Prior work in small or mixed populations points towards tissue type as factor for varying lesion development.
Objectives Page 5	3	State specific objectives, including any prespecified hypotheses We hypothesized that vascularity and so propensity for lesion reversal depended on lesion location, i.e. persistent infarction signs would show more frequently in end-artery dominated white matter (and deep gray matter) than in cortical gray matter with leptomeningeal collaterals. This study aims to investigate rate of lesion reversal and role of tissue type for lesion development in a prospective cohort of patients with TIA and 8-week MRI.
Methods		
Study design Page 5	4	Present key elements of study design early in the paper Observational study of prospective cohort of patients with clinical TIA for lesion evolution with standard 3T-MRI at baseline and 8-week follow-up. No intervention.
Setting Page 5-7	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Clinical workflow in tertiary stroke centre, standard TIA imaging 3T-protocol; February 2012 - June 2014.
Participants Page 5, 7-8	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Patients with clinical time-based TIA without MRI contraindications enrolled by senior consultant stroke neurologists with 8-week follow-up MRI. Standard 3T TIA imaging in clinical workflow, PACS-only assessment after predefined case report form by a neuroradiologist. 8-week follow-up MRI and telephone doctor interview. Standard clinical 3-mo follow-up. National electronic records for long-term follow-up. Blinded clinical and radiological data collection. After ended data collection, match of symptoms and lesions. *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NA

		Case-control study—For matched studies, give matching criteria and the number of controls per case
Variables Page 5,7	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Primary outcome was presence or absence of persistent infarction signs defined as 8-week FLAIR-hyperintensity or atrophy corresponding to the initial DWI lesion. Potential predictors for persistent infarction signs were lesion location (tissue type), DWI lesion size (cm^2), visibility on baseline ADC or FLAIR, time to baseline MRI scan (TTS).
Data sources/ measurement Page 6-7	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Page 6-7	9	Describe any efforts to address potential sources of bias We wanted to perform the study in a clinical workflow and as close to the real population of patients with TIA as possible. Small lesions would be challenging, below the 4-mm slice thickness of the standard TIA 3T imaging protocol in clinical workflow and similar to prior studies [14,15]. We deemed volumetric measurements unrealistic, nor do we routinely use external volumetric software. Best estimate was axial area (DWI and ADC in plane resolution was 1.1 x 1.1 mm and FLAIR 0.9 x 0.9 mm). Template, case report form (CRF), their pilot validation and CRF use are described on pages 6-7. We found 14% (12/84) of DWI lesions were < 3mm.
Study size Page 5, Figure 2	10	Explain how the study size was arrived at The study is exploratory; we aimed for a fairly robust and achievable single centre sample of 200 patients within two years.
Quantitative variables Page 7	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Lesion localization we divided into white matter (WM), cortical gray matter (cGM) and deep gray matter (dGM). We found too few dGM lesions (4) for meaningful analysis and as we expected dGM vascularity similar to WM vascularity we grouped them together, main analyses are performed as cGM-lesions versus non-cGM. Lesion reversal was analysed as dichotomous presence or absence and partial lesion reversal was analysed as area change between baseline and 8-week MRI graded at 30%, 50% and 100%.
Statistical methods Page 8	12	(*a*) Describe all statistical methods, including those used to control for confounding For categorical data, we used Fisher's exact test, and Mann-Whitney-U test for population comparison. For dichotomized outcomes, we performed a general linear model-based forced entry logistic regression analysis; p-values were calculated with the Wald test. We performed a likelihood ratio test for model fit versus an empty model. We used ROC analysis for binary classification. Recurrent cerebrovascular events were studied using Kaplan-Meier curves and Cox Proportional Hazard Model. We considered p-values less than 0.05 significant.
		(*b*) Describe any methods used to examine subgroups and interactions NA
		(*c*) Explain how missing data were addressed NA
Page 7		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed. National electronic patient records allowed long-term follow-up. *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of

	sampling strategy
	(*e*) Describe any sensitivity analyses
Page 6	Exploratory study. Intraobserver agreement quality check: In a random 10% sample (defined by date of birth 4th, 14th and 24th day in any month) we calculated Cohen's kappa for intra-observer variation using two CRF readings, except for area measurements, with 3 months' interval and observed a substantial intra-observer agreement (κ=0.80).

Continued on next page

Results		
Participants Figure 2	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Please see figure 2 for patient flow.
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram Please see figure 2 for patient flow.
Descriptive data Page 16, Supplemental data, Figure 1	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Please see Table 1 for patient characteristics, Supplemental table I for lesion areas and Supplemental table II for characteristics of patients with and without recurrence. Please also see Figure 1 for examples of lesions with and without persistent infarction signs.
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data Figures 3+4, Page 16, and Supplemental data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Please see Figure 3 for probability of permanent infarction signs and Figure 4 for lesions with full or partial area regression or progression stratified by tissue location. Please also see Table 1 for patient characteristics, Supplemental table I for lesion areas and Supplemental table II for characteristics of patients with and without recurrence.
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results Page 8-10, Figures 3+4	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included 50 patients showed 84 initial DWI lesions. 29 (35%) DWI lesions did not result in infarction signs on 8-week FLAIR. 26 (90%, p<0.0001) reversing lesions were located in the cortical gray matter (cGM). CGM location (versus any other location) strongly predicted no 8-week infarction sign development (OR 0.02, 95% CI 0.001-0.17) or partial lesion area regression (>30% of initial DWI-area, OR 14.10, 95% CI 3.61-54.72), adjusted for FLAIR-visibility, DWI-area, ADC-confirmation and time from symptom onset to baseline MRI (TTS). Acute FLAIR-visibility was a strong predictor for persistent infarction signs (OR 64.62, 95% CI 3.41-1223.20). For cGM lesions area size was sole predictor for persistent infarction signs with a 0.31cm^2 (AUC 0.97) threshold. In 8 (16%) DWI-positive patients all lesions reversed fully. There was a significant correlation (OR 1.20 per additional mm^2, 95% CI 1.11-1.28) between the patients' total DWI area and the probability of persistent infarction signs.
		(*b*) Report category boundaries when continuous variables were categorized Please see item 11 (Methods, Quantitative variables)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses Page 10	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses There was no significant correlation between increasing ABCD2 score and the probability of persistent infarction signs (OR 1.09, 95% CI 0.82-1.46).

Eight weeks post-TIA we found no correlation between persistent infarction sign presence and patient-reported return to pre-TIA daily activity level (4 (33%) vs. 35 (36%), p=0.87). 14 recurrent cerebrovascular events occurred during the median (IQR) follow-up period of 817 (440-1056) days. Characteristics of patients with recurrent ischemic event are presented in Supplemental table II. Ipsilateral carotid stenosis ≥ 70% (hazard ratio [HR] 7.11, 95% CI 1.98-25.5) and several competing TOAST aetiologies (HR 3.74, 95% CI 1.13-12.4) predicted recurrent ischemic events (Supplemental figure II). Persistent 8-week infarction signs (HR 0.50, 95% CI 0.14-1.78) did not increase the risk of recurrent events.

Discussion		
Key results Page 11	18	Summarise key results with reference to study objectives 16% of initially DWI-positive patients and a third of identified lesions show no sign of infarction 8 weeks later; cortical gray matter location was the strongest predictor for lesion reversal on MRI at 8 weeks, and cGM DWI lesions smaller than 0.31cm^2 did not show persistent infarction signs. ADC-confirmation or subacute FLAIR-visibility did not in all cases result in visible long-term infarction.
Limitations Page 12	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Inclusion was based on informed consent so the study was not unselected or consecutive. 14% (12/84) of the initial lesions were small (<3 mm), and below the usual recommended cut-off level to discriminate between lacunes and perivascular spaces, but we included this type of lesion as it has been associated with increased risk of stroke and death. Other potential limitations are partial volume effects and the 2D-FLAIR sequence in our standard stroke MRI-protocol, though its slice thickness is similar to prior studies'. Dedicated high-resolution 3D-FLAIR and 3D-T1 could improve differentiation between healthy tissue and post-infarction gliosis or atrophy, especially cortically.
Interpretation Page 11-12	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Literature holds few and small studies with varying populations and rates of DWI positivity and reversal. 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. Our TTS and per patient DWI positivity and reversal rates correspond to the smaller TIA study.[14] Our cohort's 31 hour median delay to imaging may explain why several lesions are FLAIR-positive representing the beginning vasogenic oedema, and the persistent ADC-reduction represents the still active cytotoxic oedema.[29] We found no association between DWI positivity rate and time to scan in line with recent metaanalysis.[12] In TIA, DWI lesions are rarer and smaller and the TIA presumably results from smaller occlusive events compared to stroke. Changes in perfusion and underlying vascularity reflect tissue vulnerability and damage. 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. This and smaller lesion size may explain the high variation in DWI-positivity rates among TIA populations and the high rate and cortical predilection of apparent diffusion lesion reversal in this TIA study.

However, in an observational exploratory study, we cannot infer causality but hypothesize that the stronger leptomenigeal collateral circulation in cGM may prevent persistent infarction signs in small lesions.

Generalisability Page 11	21	Discuss the generalisability (external validity) of the study results We designed the study for a clinical setting and workflow and wanted it to be as unselective and close to the real population of patients with TIA as possible. Our results are in line with a smaller reported TIA-study, but DWI positivity and reversal rates differ from a mixed TIA and minor stroke population (please see the table below). We thus think that the results are representative of populations of patients with clinical TIA.

Reference, population	DWI+ patients	[2]p	DWI reversal/DWI+ patients	[2]p
[14],[1]TIA	35% (36/103)	0.43	21% (7/33)	0.75
[15], mixed TIA and minor stroke	57% (192/337)	0.003	6% (11/192)	0.03
Our TIA study	41% (50/122)	-	16% (8/50)	-

[1]33 patients with follow-up MRI. [2]Chi squared test for independence versus our population of patients with TIA.

Other information

Funding Page 1	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based No funding as received for this study. CO holds research grants from the Velux-foundation, Bispebjerg University Hospital, University of Copenhagen, Axel Muusfeldts Foundation and Danish Medical Association. None of these were designated for this study.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.