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RESEARCH PAPER

# Extensive cerebral microbleeds predict parenchymal haemorrhage and poor outcome after intravenous thrombolysis

Shenqiang Yan,[1] Xinchun Jin,[1] Xuting Zhang,[1] Sheng Zhang,[1] David S Liebeskind,[2] Min Lou[1]

[1]Department of Neurology, The 2nd Affiliated Hospital of Zhejiang University, School of Medicine, Hangzhou, China
[2]University of California-Los Angeles Stroke Center, Los Angeles, California, USA

**Correspondence to**
Dr Min Lou, Department of Neurology, the 2nd Affiliated Hospital of Zhejiang University, School of Medicine, #88 Jiefang Road, Hangzhou 310009, China;
loumingxc@vip.sina.com

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## ABSTRACT

**Purpose** Thrombolysis-related haemorrhagic transformation (HT) subtypes may have different prognostic implications. We aimed to analyse the impact of cerebral microbleeds (CMBs) burden on HT subtypes and outcome after intravenous thrombolysis.

**Methods** We retrospectively examined clinical and radiological data from 333 consecutive patients with acute ischaemic stroke who underwent susceptibility-weighted imaging before intravenous thrombolysis. Logistic regression analysis was used to determine the impact of CMBs on HT subtypes and neurological outcome.

**Results** We observed 596 CMBs in 119 (39.7%) patients on initial gradient-recalled echo scans. HT occurred in 88 (29.3%) patients, among which 62 were haemorrhagic infarction and 26 were parenchymal haemorrhage (PH). Logistic regression analysis indicated that the presence of extensive (≥3) CMBs was independently associated with PH (OR 6.704; 95% CI 2.054 to 21.883; p=0.002) and poor clinical outcome (OR 2.281; 95% CI 1.022 to 5.093; p=0.044).

**Conclusions** The presence of extensive (≥3) CMBs increased the risk of PH 24 h after intravenous thrombolysis, and predicted poor clinical outcome independently.

## INTRODUCTION

Intravenous thrombolysis is currently the most promising treatment for acute ischaemic stroke.[1] One of the most important complications of thrombolytic therapy is bleeding, especially intracranial haemorrhage (ICH). Determining risk factors for post-thrombolysis ICH in individual patients is an essential step in weighing the risks and benefits.

Cerebral microbleeds (CMBs), defined as small, rounded or circular hypointense lesions within the brain parenchyma with clear margins on gradient-recalled echo (GRE) T2*-weighted MRI of the brain,[2] appear to be a potential risk factor for ICH,[3] especially in Asian cohorts.[4] They can be detected in healthy populations (especially in elderly individuals) and commonly in patients with stroke.[5 6] A previous MRI–pathological correlation study has confirmed focal accumulation of hemosiderin-containing macrophages in areas with signal loss on MRI.[7]

However, it has been questioned whether the presence of CMBs increases the risk of ICH after intravenous thrombolysis.[8 9] Across the studies that addressed the potential risk of pre-existing CMBs on post-thrombolysis bleeding risk, the reported CMBs prevalence differed markedly, ranging from 12.2% to 38.5%, possibly through differences in the MRI sequences used.[10–14] Moreover, thrombolysis-related haemorrhagic transformation (HT) subtypes were reported to have different prognostic implications.[15] We thus deemed it worthy to detect the CMBs with a contiguous, thin-slice (2-mm), three-dimension (3D) multiecho GRE sequence, that is, the sequence of susceptibility-weighted imaging (SWI), performed with a 3 T MRI unit. On the basis of such measurements, we aim to analyse the impact of CMBs burden on HT subtypes and clinical outcome after intravenous thrombolysis.

## METHODS

### Study subjects

We retrospectively reviewed our prospectively collected database for consecutive patients with acute ischaemic stroke received thrombolytic therapy between June 2009 and February 2014. We then enrolled patients who (1) had a diagnosis of acute ischaemic stroke confirmed by diffusion-weighted imaging (DWI); (2) received intravenous recombinant tissue-type plasminogen activator (rtPA); (3) underwent SWI before rtPA infusion; (4) underwent follow-up SWI or a CT scan 24 h after rtPA infusion. We excluded patients who were treated with combined endovascular and rtPA therapy, and whose image quality was poor due to motion artefacts. Intravenous rtPA (Alteplase 0.9 mg/kg up to a maximum of 90 mg) was used with 10% of the total dosage as a bolus and the rest over 1 h. No patient received antithrombotic agents within 24 h after rtPA infusion.

We retrieved demographic, clinical, laboratory and imaging data including age and gender; comorbid conditions such as history of hypertension, diabetes mellitus, hyperlipidaemia and atrial fibrillation; previous use of aspirin and warfarin; time interval from stroke onset to rtPA infusion; the National Institutes of Health stroke scale (NIHSS) score and serum platelet level before rtPA infusion; presence, number and grade of baseline CMBs, infarct volume before rtPA infusion, HT 24 h after rtPA infusion, and modified Rankin Scale (mRS) score after 3 months.

### MRI parameters

All patients underwent MRI on a 3.0T system (General Electric Medical System, Milwaukee,

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Wisconsin, USA) equipped with an eight-channel phased array head coil. Foam pads were inserted into the space between the patient's head and the MRI head coil to minimise head motion. The 3D multiecho GRE sequence was in an axial orientation parallel to the anterior commissure to posterior commissure (AC–PC) line and covered the whole brain, using 11 equally spaced echoes: TE=4.5 ms (first echo); interecho spacing=4.5 ms; TR=58 ms; FOV=24×24 $cm^2$; matrix size=256×256; flip angle=20°; slice thickness=2.0 mm with no gap between slices, and in-plane spatial resolution of 0.4688×0.4688 mm/pixel. Flow compensation was applied. Magnitude images were acquired and used in further analysis. DWI sequence was used to measure the infarct volume before rtPA infusion (TR=4000 ms; TE=69.3 ms; b value=1000 s/$mm^2$; FOV=24×24 $cm^2$; matrix size=160×160; slice thickness=5.0 mm; interslice gap=1.0 mm and in-plane spatial resolution of 0.9375×0.9375 mm/pixel). The axial T2 fluid attenuated inversion recovery (FLAIR) sequence was used to measure the leukoaraiosis volume (TR=10 000 ms; TE=150 ms; FOV=24×24 $cm^2$; matrix size=256×256; inversion time=2500 ms; slice thickness=5.0 mm with no gap between slices, and in-plane spatial resolution of 0.4688×0.4688 mm/pixel).

### Detection criteria of CMBs

We identified a CMB according to a field guide of CMBs detection and interpretation.[2] Briefly, the signal of a CMB should be black or very hypointense on T2*-weighted MRI, round or ovoid with a size of ≤10 mm, blooming, devoid of T1-weighted or T2-weighted hyperintensity, and at least half surrounded by brain parenchyma. Mineralisation of the basal ganglia or diffuse axonal injury was excluded based on appearance or clinical history.

We described the locations and counted the numbers of CMBs on baseline SWI. CMBs were classified as absent (grade 1), mild (grade 2; total number of CMBs, 1–2) and extensive (grade 3; total number of CMBs, ≥3). The locations of CMBs were classified as follows: (1) lobar if present in the cortex, subcortex and white matter of the frontal, parietal, temporal, occipital and insular lobes; (2) deep if present in the head of the caudate, putamen, globus pallidus, internal capsule and thalamus and (3) infratentorial if present in the midbrain, pons, medulla and cerebellum.[16]

### Evaluation of HT and clinical outcome

HT was classified by using clinical and radiological criteria as follows: haemorrhagic infarction (HI, including HI-1 and HI-2), parenchymal haemorrhage (PH, including PH-1 and PH-2), extraischaemic haemorrhage and symptomatic ICH (sICH). An ICH was defined as sICH if the patient had clinical deterioration causing an increase of NIHSS ≥4 points and if the haemorrhage was likely to be the cause of the clinical deterioration.[17] Outcome was dichotomised into good clinical outcome (mRS 0–2) or poor clinical outcome (mRS 3–6) at 3 months.

### Reliability and validity of the radiological data

Two investigators blinded to the patients' clinical data jointly evaluated the MRI findings. A single trained observer (SY) evaluated the images of all patients twice, at an interval of 3 months apart. Another observer (XJ) independently made the same evaluation. Intraclass correlation coefficients (ICCs) and the κ test were used for this step. Discrepancies were resolved by consensus.

### Statistical analysis

The patients were trichotomised according to the HT subtypes or dichotomised according to the clinical outcome. Fisher's exact test was used to compare the dichotomous variables between groups, while an independent sample two-tailed t test or a Mann-Whitney U test was used for the continuous variables, depending on the normality of the distribution. A one-way analysis of variance or Kruskal-Wallis test was used between multiple groups. Variables with a $p<0.1$ in univariate regression analyses were included in the multinomial logistic regression or binary logistic regression model (backward stepwise conditional model). All analyses were performed blinded to the participant identifying information. Statistical significance was set at a probability value of $<0.05$. All statistical analysis was performed with an SPSS package (V.14.0 for Windows).

## RESULTS

The interobserver and intraobserver ICCs were 0.95 and 0.91 for the number of baseline CMBs, respectively. The interobserver and intraobserver reliabilities about the presence of CMBs (≥1) were also acceptable (κ value=0.87 and 0.81, respectively).

### Subject characteristics

A total of 350 consecutive patients with acute ischaemic stroke received MRI-guided intravenous thrombolysis during the study period. Seventeen patients were excluded due to the following reasons: without imaging 24 h after treatment (n=5), poor MRI quality for analysis (n=3) and combined endovascular and intravenous rtPA therapy (n=9). Thus, the remaining 333 patients were included for the final analysis. Demographic, clinical and laboratory data were not different between included and excluded patients, except that the baseline NIHSS score was higher in excluded patients, mainly because some patients with severe stroke were transferred to the intensive care unit or received surgical treatment, resulting in the failure of follow-up scans within 24 h. Of the included patients, 110 (33.0%) were women, with a median age of 67 years (mean 66.15 ±13.02 years, range 23–94 years). The mean time from onset to rtPA infusion was 234±92 min.

### Frequency and location of baseline CMBs

On the initial SWI, we observed 742 CMBs in 133 patients (39.9%). The median number of baseline CMBs was 2 (range 1–81). Eighty-four patients had 1–2 CMBs, 31 had 3–10 CMBs and 17 had more than 10 CMBs. There were 338 (45.5%) baseline CMBs in the lobar location, 285 (38.4%) in the deep location and 119 (16.0%) in the infratentorial location. Among 133 patients with baseline CMBs, 45 had lobar CMBs, 26 had deep CMBs, 6 had infratentorial CMBs, 20 had lobar and deep CMBs, 7 had lobar and infratentorial CMBs, 6 had deep and infratentorial CMBs, and 23 had lobar, deep and infratentorial CMBs. Among 95 patients with lobar CMBs, 26 had multiple baseline CMBs (≥3) in the lobar location.

### Univariate and multivariate regression analysis of HT subtypes

Follow-up scans 24 h after treatment revealed HT in 102 (30.6%; 74 were HI and 28 were PH) patients, and sICH was observed in 8 (2.4%) patients. Ten (3.0%) patients had extraischaemic haemorrhage of whom 3 had sICH. In the patients without CMBs, 1% (2 in 201) developed sICH, while 4.2% (2 in 48) developed sICH in those with extensive (≥3) CMBs. Table 1 shows the characteristics of patients with different HT

**Table 1** Univariate comparison of characteristics between patients with different HT subtypes

| Variable | No HT (n=231) | HI (n=74) | PH (n=28) | p Value |
|---|---|---|---|---|
| Age (year) | 65.26±13.17 | 67.49±12.98 | 70.04±11.06 | 0.113 |
| Female | 80 (34.6%) | 21 (28.4%) | 9 (32.1%) | 0.614 |
| Comorbid conditions | | | | |
| Hypertension | 154 (66.7%) | 52 (70.3%) | 21 (75.0%) | 0.669 |
| Diabetes mellitus | 47 (20.3%) | 14 (18.9%) | 6 (21.4%) | 0.950 |
| Hyperlipidaemia | 115 (49.8%) | 32 (43.2%) | 12 (42.9%) | 0.531 |
| Atrial fibrillation | 74 (32.0%) | 33 (44.6%) | 17 (60.7%) | 0.004 |
| Clinical variables | | | | |
| NIHSS score | 9 (5–14) | 12 (8–17) | 17 (10–19) | <0.001* |
| Onset to treatment (min) | 229±97 | 240±80 | 256±73 | 0.247 |
| Previous use of aspirin or warfarin | 34 (14.7%) | 6 (8.1%) | 5 (17.9%) | 0.248 |
| Platelet ($10^9$/L) | 186±63 | 181±56 | 165±46 | 0.212 |
| Radiological data | | | | |
| Baseline infarct volume (mL) | 3 (1–12) | 14 (4–42) | 18 (5–76) | <0.001* |
| Leukoaraiosis volume (mL) | 5 (2–12) | 5 (2–14) | 7 (3–19) | 0.172* |
| Presence of CMBs | 85 (36.8%) | 29 (39.2%) | 19 (67.9%) | 0.007 |
| Number of CMBs | 0 (0–1) | 0 (0–1) | 1 (0–3) | 0.006* |
| Grade of CMBs | | | | 0.011 |
| Absent (0) | 146 (63.2%) | 46 (62.2%) | 9 (32.1%) | |
| Mild (1–2) | 58 (25.1%) | 16 (21.6%) | 10 (35.7%) | |
| Extensive (≥3) | 27 (11.7%) | 12 (16.2%) | 9 (32.1%) | |
| Poor clinical outcome, mRS ≥3 | 85 (36.8%) | 34 (45.9%) | 21 (75.0%) | <0.001 |

*Kruskal-Wallis test.
CMBs, cerebral microbleeds; HI, haemorrhagic infarction; HT, haemorrhagic transformation; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; PH, parenchymal haemorrhage.

subtypes. Patients with PH 24 h after rtPA infusion had a higher frequency of atrial fibrillation, a higher baseline NIHSS score and a larger baseline DWI volume than those with HI or without HT. Patients with baseline CMBs had a higher frequency of PH (14.3% vs 4.5%, p=0.007). In addition, patients with extensive (≥3) CMBs had a higher frequency of PH-2 than those with mild (1–2) CMBs (10.4% vs 7.1%, p=0.028).

The baseline NIHSS score, baseline infarct volume, history of atrial fibrillation and grade of CMBs (absent, mild and extensive) were included in the multinomial logistic regression model. Only the baseline NIHSS score was associated with HI independently, whereas the baseline NIHSS, history of atrial fibrillation and grade of CMBs were the independent factors associated with PH (table 2). The presence of extensive (≥3) CMBs had a higher OR than mild (1–2) CMBs (6.808 vs 3.105). When we replaced grade of CMBs with the number of CMBs, it still predicted PH (OR 1.041; 95% CI 1.004 to 1.079; p=0.031). The association of extensive CMB with PH remained significant after adjustment for the leukoaraiosis volume (OR 8.056; 95% CI 2.283 to 28.433; p=0.001).

For sICH, logistic regression analysis identified one significant independent predictor and one variable with a possible trend: the baseline NIHSS score (OR 1.117; 95% CI 1.005 to 1.243; p=0.041) was the independent predictor, and the presence of extensive (≥3) CMBs (OR 6.089; 95% CI 0.726 to 51.097; p=0.096) showed a possible trend towards significance. When we replaced the grade of CMBs with the number of CMBs, it no longer predicted sICH (OR 0.968; 95% CI 0.799 to 1.174; p=0.744). Patients with baseline any CMBs also revealed higher risk of extra ischaemic haemorrhage than those without (6.0% vs 1.0%; p=0.017), whereas the extraischaemic haemorrhage rate was not significantly different between the patients with or without multiple lobar CMBs (≥3) (7.7% vs 2.6%; p=0.179).

## Univariate and multivariate regression analysis of clinical outcome

Table 3 shows the characteristics of patients with good and poor clinical outcomes. Patients with a poor clinical outcome were older, and had a higher frequency of atrial fibrillation, a higher baseline NIHSS score, a lower serum platelet level and a larger baseline DWI volume than those with a good clinical outcome. A poor clinical outcome was more frequent in patients with extensive (≥3) CMBs than those with HI or without HT

**Table 2** Multinomial logistic regression of presence of CMBs for prediction of HT

| | OR | 95% CI | p Value |
|---|---|---|---|
| For HI (set no HT as reference) | | | |
| NIHSS score | 1.073 | 1.022 to 1.127 | 0.005 |
| Baseline infarct volume (mL) | 1.003 | 0.996 to 1.009 | 0.420 |
| Atrial fibrillation | 1.476 | 0.847 to 2.574 | 0.169 |
| Grade of CMBs (set 'absent' as reference) | | | |
| Mild (1–2) | 0.888 | 0.458 to 1.742 | 0.726 |
| Extensive (≥3) | 1.517 | 0.693 to 3.342 | 0.297 |
| For PH (set no HT as reference) | | | |
| NIHSS score | 1.160 | 1.077 to 1.249 | <0.001 |
| Baseline infarct volume (mL) | 1.002 | 0.994 to 1.011 | 0.596 |
| Atrial fibrillation | 2.686 | 1.129 to 6.391 | 0.025 |
| Grade of CMBs (set 'absent' as reference) | | | |
| Mild (1–2) | 3.105 | 1.136 to 8.488 | 0.027 |
| Extensive (≥3) | 6.808 | 2.227 to 20.817 | 0.001 |

CMBs, cerebral microbleeds; HI, haemorrhagic infarction; HT, haemorrhagic transformation; NIHSS, National Institutes of Health Stroke Scale; PH, parenchymal haemorrhage.

**Table 3** Univariate comparison of characteristics between patients with good and poor clinical outcomes

| Variable | Clinical outcome | | p Value |
|---|---|---|---|
| | Good (mRS <3, n=193) | Poor (mRS ≥3, n=140) | |
| Age (year) | 63.75±13.00 | 69.46±12.34 | <0.001 |
| Female | 67 (34.7%) | 43 (30.7%) | 0.480 |
| Comorbid conditions | | | |
| Hypertension | 124 (64.2%) | 103 (73.6%) | 0.075 |
| Diabetes mellitus | 36 (18.7%) | 31 (22.1%) | 0.489 |
| Hyperlipidaemia | 100 (51.8%) | 59 (42.1%) | 0.096 |
| Atrial fibrillation | 61 (31.6%) | 63 (45.0%) | 0.016 |
| Clinical variables | | | |
| NIHSS score | 7 (4–13) | 14 (10–17) | <0.001* |
| Onset to treatment (min) | 233±95 | 234±88 | 0.923 |
| Previous use of aspirin or warfarin | 27 (14.0%) | 18 (12.9%) | 0.871 |
| Platelet ($10^9$/L) | 189±61 | 174±59 | 0.029 |
| Radiological data | | | |
| Baseline infarct volume (mL) | 2 (1–8) | 13 (4–57) | <0.001* |
| Leukoaraiosis volume (mL) | 4 (2–10) | 7 (3–18) | 0.001* |
| Presence of CMBs | 67 (34.7%) | 66 (47.1%) | 0.024 |
| Number of CMBs | 0 (0–1) | 0 (0–2) | 0.009* |
| Grade of CMBs | | | 0.012 |
| Absent (0) | 127 (65.8%) | 74 (52.9%) | |
| Mild (1–2) | 47 (24.4%) | 37 (26.4%) | |
| Extensive (≥3) | 19 (9.8%) | 29 (20.7%) | |
| Haemorrhagic transformation | | | <0.001 |
| HI | 40 (20.7%) | 34 (24.3%) | |
| PH | 7 (3.6%) | 21 (15.0%) | |
| sICH | 1 (0.5%) | 7 (5.0%) | 0.011 |

*Mann-Whitney U test.
CMBs, cerebral microbleeds; HI, haemorrhagic infarction; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; PH, parenchymal haemorrhage; sICH, symptomatic intracranial haemorrhage.

(75.0% vs 45.9% vs 36.8%; p<0.001). The presence of extensive (≥3) CMBs was a significant independent predictor for a poor clinical outcome after adjusting for the baseline NIHSS score, age and baseline infarct volume in the binary logistic regression model (table 4). Interestingly, it remained to be an independent predictor after adjusting for HT subtypes (OR 2.184; 95% CI 1.002 to 4.760; p=0.049). The association of extensive CMB with poor clinical outcome remained significant after adjustment for the leukoaraiosis volume (OR 2.455; 95% CI 1.086 to 5.549; p=0.031).

## DISCUSSION

This study is a systematic investigation of the influence of CMBs severity on different HT subtypes and clinical outcome. We found that the presence of extensive (≥3) CMBs independently predicted PH and poor clinical outcome after intravenous thrombolysis. In addition, there was a trend for a higher sICH risk in those patients with baseline CMBs.

Whether pre-existing CMBs increase the risk of post-thrombolysis ICH and whether their presence is a marker of poor functional outcome are controversial. A recent study indicated that CMBs detected on pretreatment SWI did not increase the risk for ICH or worsen outcome.[18] However, they also enrolled the patients treated with endovascular therapy, or intravenous thrombolysis followed by endovascular therapy. Actually, the presence of CMBs was correlated with sICH in the subgroup of patients treated with intravenous thrombolysis in their study.[18] Both sICH and asymptomatic ICH rate were revealed higher in endovascular therapy and bridging groups than intravenous thrombolysis group (6.0% vs 8.8% vs 3.4%, for sICH; 20.7% vs 27.9% vs 14.4%, for asymptomatic ICH) in their study.[18] Thus, the possible increased risk of ICH with endovascular therapy might, to some extent, dampen the practical significance of the results. A recent study indicates a higher risk of sICH and PH after intravenous thrombolysis when multiple CMBs are present,[19] which is similar to our finding. However, in their study, multiple CMBs were related with clinical outcome in univariate but not multivariate analysis, mainly because the low number of patients with more than 5 CMBs (10 in 326) limited their statistical power.

The BRASIL study is the largest prospective study to date that investigated the relation between the presence of CMBs and the risk of post-thrombolysis ICH from 13 centres.[11] However, their reported prevalence of CMBs varied largely across the participating centres, ranging from 0.0% to 36.8%. While it is

**Table 4** Binary logistic regression analysis of risk factors for poor clinical outcome

| | OR | 95% CI | p Value |
|---|---|---|---|
| Age (year) | 1.033 | 1.011 to 1.056 | 0.003 |
| NIHSS score | 1.111 | 1.060 to 1.165 | <0.001 |
| Baseline infarct volume (per 10 mL) | 1.260 | 1.127 to 1.409 | <0.001 |
| Grade of CMBs (set 'absent' as reference) | | | |
| Mild (1–2) | 1.224 | 0.665 to 2.253 | 0.516 |
| Extensive (≥3) | 2.340 | 1.086 to 5.043 | 0.030 |

CMBs, cerebral microbleeds; NIHSS, National Institutes of Health Stroke Scale.

increasingly known that longer echo time, higher spatial resolution and increased magnetic field strength can increase the sensitivity of CMBs detection,[2] we consider it possible that the presence, number and size of CMBs may vary substantially with the parameters of GRE in different centres and some individuals might be misclassified as having no CMBs. Moreover, owing to the small size of CMBs, slice thickness and interslice gap are particularly important in determining whether a CMB is present or not. Therefore, it is important to use a contiguous, thin-slice, 3D sequence for CMBs detection. Our results are thus credible, based on patients with large sample size of acute ischaemic stroke who received only intravenous thrombolysis with a sensitive sequence for CMBs detection (finished within 2 min) on a 3 T MRI unit.

Two main pathogenic mechanisms for CMBs production were suggested: leakage from damaged vessels due to hypertension; or due to vascular amyloid deposition.[7 20] Histopathological analysis confirmed that CMBs consist of hemosiderin accumulations from red blood cells, which presumably have leaked out of small vessels.[7] Thus, the possibility that CMBs are simply a marker of impaired blood–brain barrier function may explain the high risk of PH. Interestingly, we found that both the grade and number of CMBs predicted the occurrence of PH. Moreover, patients with baseline CMBs revealed a higher risk of extraischaemic haemorrhage. These observations, similar to the finding of bimodal microbleed and macrobleed distribution, raise the possibility of a threshold mechanism, whereby a CMB can either remain stable or reach a crucial size that causes it to enlarge into PH in avalanche after intravenous thrombolysis, since rtPA itself increases blood–brain barrier disruption in acute ischaemic stroke. Our finding that there is no association between baseline CMBs and HI after thrombolysis also lends support to this assumption, since the occurrence of HI may represent early successful reperfusion after intravenous thrombolysis.[15] It thus may be of interest to explore whether post-thrombolysis bleeds co-localised with pre-existing CMBs in the future studies.

Another interesting finding is that only the presence of extensive (≥3) CMBs increased the frequency of poor clinical outcome, but not mild (1–2) CMBs, although they both predicted PH after intravenous thrombolysis. One explanation is that patients with extensive CMBs had a higher frequency of PH-2 than those with mild CMBs (10.4% vs 7.1%), since only the presence of PH-2 independently modifies the risk of a worse outcome and that PH-1 increases the risk of early deterioration but not of a worse long-term outcome.[21 22] Neuropathological analyses of CMBs generally indicate that these lesions are associated with surrounding tissue damage. Moreover, the number of CMBs was found to be independently associated with functional dependence in one study of patients with CADASIL.[23] And extensive CMBs remained to be an independent predictor after adjusting for HT subtypes. Therefore, our finding may suggest an additional effect of extensive (≥3) CMBs on neurological outcome, besides the increased HT risk, in patients with ischaemic stroke.

Limitations include a retrospective design, though we prospectively collected data using a stroke registry and MRI protocol, and might have a potential risk of selection bias. Some patients with severe stroke might be transferred to intensive care unit or receive surgical treatment next day so that they were unable to undergo follow-up scans within 24 h. Second, there was no control group without thrombolytic therapy. It was still questionable whether the CMBs-related ICH was likely to exceed the benefits of thrombolytic therapy. The decisions regarding the use of thrombolysis in patients with different grades of CMBs still need further exploration. Third, the requirement for pre-thrombolysis SWI to detect CMBs may take a longer time than CT-based treatment, which can be hard to justify, given the strong effect of time on outcomes. However, with the advanced MRI technique, it is possible to obtain the goal of shortening the examination time while preserving adequate image quality in the future. Fourth, the onset to treatment was long in our study since some patients received thrombolysis beyond the time window based on multimodal MRI, which may increase the risk of HT. Although we did not find the difference of onset to treatment among patients with different grades of CMBs, this restriction could potentially limit the general application of our results to the patients with stroke within the time window.

In summary, we concluded that the presence of extensive (≥3) CMBs increased the risk of PH 24 h after intravenous thrombolysis, and predicted poor clinical outcome independently. Future prospective studies with much larger sample sizes are required to clarify our results, and standardisation of imaging parameters for CMBs detection across centres is encouraged.

**Contributors** ML and SY conceived and designed the experiments. SY, XJ, XZ and SZ performed the experiments. SY, XJ, XZ, SZ and ML analysed the data. XJ, XZ, SZ and ML contributed reagents/materials/analysis tools. SY, DSL and ML wrote the paper.

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**Patient consent** Obtained.

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Shenqiang Yan, Xinchun Jin, Xuting Zhang, Sheng Zhang, David S Liebeskind and Min Lou

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