Infarction of ‘non-core–non-penumbral’ tissue after stroke: multivariate modelling of clinical impact

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There is considerable intersubject variability in early neurological course after anterior circulation stroke, yet the pathophysiology underlying this variability is not fully understood. Here, we hypothesize that, although not predicted by current pathophysiological models, infarction of ‘non-core–non-penumbral’ (i.e. clinically silent) brain tissue may nevertheless occur, and negatively influence clinical course over and above the established positive impact of penumbral salvage. In order to test this hypothesis, non-core–non-penumbral tissue was identified in two independent prospectively recruited cohorts, using computed tomography perfusion, and magnetic resonance perfusion- and diffusion-weighted imaging, respectively. Follow-up structural magnetic resonance imaging was obtained about 1 month later in all patients to map the final infarct. The volumes of both the acutely silent but eventually infarcted tissue, and the eventually non-infarcted penumbra, were determined by performing voxel-wise analysis of the acute and follow-up image sets, using previously validated perfusion thresholds. Early neurological course was expressed as change in National Institutes of Health Stroke Scale scores between the acute and 1-month assessments, relative to the acute score. The relationship between the acutely silent but eventually infarcted tissue volume and early neurological course was tested using a multivariate regression model that included the volume of non-infarcted penumbra. Thirty-four and 58 patients were recruited in the computed tomography perfusion and magnetic resonance perfusion cohorts, respectively (mean onset-to-imaging time: 136 and 156 min; 27 and 42 patients received intravenous thrombolysis,
Infarction of acutely silent tissue was identified in most patients in both cohorts. Although its volume (median 0.2 and 2 ml, respectively) was much smaller than that of salvaged penumbra (59.3 and 93 ml, respectively), it was substantial in ~10% of patients. As expected, salvaged penumbra strongly positively influenced early neurological course. Even after correcting for the latter effect in the multivariate model, infarction of acutely silent tissue independently negatively influenced early neurological course in both cohorts (P = 0.018 and 0.031, respectively). This is the first systematic study to document infarction of acutely silent tissue after anterior circulation stroke, and to show that it affects a sizeable fraction of patients and has the predicted negative impact on clinical course. These findings were replicated in two independent cohorts, regardless of the perfusion imaging modality used. Preventing infarction of the tissue not initially at risk should have direct clinical benefit.

Keywords: stroke; perfusion imaging; penumbra; clinical recovery; cerebral ischaemia
Abbreviations: A/U = affected-to-unaffected; FLAIR = fluid-attenuation inversion-recovery; NIHSS = National Institutes of Health Stroke Scale

Introduction

There is striking intersubject variability in neurological course following anterior circulation stroke (Lascelles and Burrows, 1965; Jones and Millikan, 1976; Bamford et al., 1991). Thus, while some patients experience partial or full recovery, others experience little or no improvement (Toni et al., 1998) or even deteriorate (Britton and Roden, 1985; Weimar et al., 2005; Kwan and Hand, 2006). The pathophysiology underlying these individual differences is not well understood, and a better understanding of it might lead to new therapeutic approaches.

Final infarct volume is a major, albeit not exclusive, determinant of clinical outcome in anterior circulation stroke (Beaulieu et al., 1999). Based on seminal non-human primate studies (Astrup et al., 1981; Jones et al., 1981; Hossmann, 1994), one key factor known to influence infarct volume is salvage of the ‘penumbra’, operationally defined as ischaemic tissue that (i) is electrophysiologically silent; (ii) whose cerebral blood flow stands below a critical threshold for neuronal function; and (iii) whose outcome is uncertain—i.e. it can be recruited into the irreversibly damaged ‘core’ or salvaged and resume function, depending on the subsequent events such as recanalization of the occluded artery (Muir et al., 2006a). Thus, both the core and the penumbra contribute to the clinical deficit and together constitute the ‘symptomatic tissue’. Accordingly, imaging studies in the clinical setting have shown that their combined volume correlates better with acute clinical scores than either considered separately (Furlan et al., 1996; Beaulieu et al., 1999; Baird et al., 2000), and that salvage of penumbral tissue is associated with commensurate improvements in clinical scores (Furlan et al., 1996; Heiss et al., 1998; Baird et al., 2000; Wintermark et al., 2002; Hillis et al., 2003; Markus et al., 2004; Muir et al., 2006b)—another key operational criterion defining the penumbra (Donnan and Davis, 2002; Muir et al., 2006a).

In the classic core/penumbra model, the surrounding mildly hypoperfused tissue (so-called ‘oligaemia’) is assumed to (i) cause no symptoms, i.e. be clinically ‘silent’; (ii) not be at risk of infarction; and, therefore, (iii) have no influence on clinical course (Muir et al., 2006a), and this applies even more to more peripheral, normally perfused tissue. However, a recent systematic search of the literature (Alawneh et al., 2009) found a handful of single-case reports where, based on the information provided, infarction of non-core–non-penumbral tissue apparently occurred, while this eventuality was mentioned but not commented in another article (Wu et al., 2006). Given the dynamic nature of ischaemic stroke, however, infarction of such acutely silent tissue is plausible under certain circumstances (Muir et al., 2006a); for instance, if the cerebral blood flow in oligaemia falls below the penumbral, and then the infarction threshold (Alawneh et al., 2009). Recently, Bang et al. (2010) reported the occurrence of ‘new diffusion-weighted imaging lesions’ at Day 7 post-stroke in >50% of anterior circulation stroke patients, predominantly involving areas initially affected by only mild hypoperfusion, but neither final infarction nor the precise clinical correlates of these new lesions were addressed. Thus, no study so far has systematically examined the issue of infarction of acutely silent tissue and, above all, its putative impact on clinical course after anterior circulation stroke. Indeed, if infarction of such tissue truly occurred, these patients’ neurological course would derive from two forces working in opposite directions: (i) salvage of the penumbra, causing clinical improvement and (ii) infarction of acutely silent tissue, causing absolute or relative neurological deterioration. This putative model is illustrated in Fig. 1.

The aim of the present study, carried out in two independent, prospectively recruited cohorts, was therefore to test the hypotheses that infarction of acutely silent tissue occurs after anterior circulation stroke, and that it negatively influences clinical course (expressed as the difference between chronic and acute neurological scores), over and above the expected positive impact of penumbral salvage.

To test these hypotheses, we obtained neurological scores and perfusion imaging (CT perfusion in Cohort 1 and magnetic resonance perfusion-weighted imaging in Cohort 2) on admission and, ~1 month later, repeat neurological scores and structural magnetic resonance to map the infarct. The ‘symptomatic’ tissue, i.e. (penumbra + core) was mapped voxelwise using validated perfusion thresholds, and then compared with the final infarct to determine the volume of salvaged symptomatic tissue; any finally infarcted tissue that was not part of the acutely symptomatic tissue was considered as acutely silent infarcted tissue. To test the effects of salvaged symptomatic tissue and acutely silent infarcted tissue on neurological course, their volumes were entered in a novel
multivariate model. To validate the findings, this analysis was carried out first on the CT perfusion cohort and then repeated on the perfusion-weighted imaging cohort.

**Patients and methods**

**Patients**

Patients of Cohort 1 (the ‘CT perfusion cohort’) were admitted to Addenbrooke’s Hospital, Cambridge and underwent CT perfusion on admission immediately after plain CT. Cohort 2 (the ‘perfusion-weighted imaging cohort’) includes patients who underwent diffusion-weighted imaging and perfusion-weighted imaging on their admission to centres participating in the I-KNOW European multicentre study, whose aim was to collect a large sample of anterior circulation stroke patients who underwent both admission and follow-up MRI to derive voxel-wise probabilistic maps of infarct prediction based on both clinical and magnetic resonance-based variables using multivariate models (http://www.i-know-stroke.eu).

All patients were recruited according to the same criteria: (i) anterior circulation stroke; (ii) CT perfusion or diffusion-/perfusion-weighted imaging performed as early as possible and within 12 h of onset; (iii) able and consenting to have follow-up structural MRI 1 month later; and (iv) no evidence of cerebral parenchymal haemorrhage type 1 or 2 (Berger et al., 2001) based on systematic 24 h plain CT. Lacunar stroke syndromes were excluded.

The neurological course was assessed using the National Institutes of Health Stroke Scale (NIHSS), which has been validated for anterior circulation stroke (Lyden et al., 1994). The NIHSS was recorded on admission and at the time of follow-up imaging. Clinical course was expressed as the change in NIHSS between admission and follow-up, as:

$$\Delta \text{NIHSS} = (\text{acute NIHSS} - \text{final NIHSS})$$

The following clinical data were also collected: age, gender, affected side, time from onset to perfusion imaging, past medical history, vascular risk factors, medications taken at time of stroke, treatments given such as thrombolysis, cause of stroke as per SITS MOST (Wahlgren et al., 2007), and outcome modified Rankin Scale (van Swieten et al., 1988).

Both protocols were approved by the relevant Ethics Committees and written informed consent was obtained from the patients or their legal representative.

**Imaging methodology**

**Computed tomography perfusion cohort**

In each patient plain CT and CT perfusion were acquired in succession using a Siemens Sensation 4. CT perfusion was acquired after injecting 50 ml of iodinated contrast (iopamidon 300) into the cubital vein with flow rate of 8 ml/s using an injection pump. The protocol consisted of a 40 s cine acquisition with a repetition time of 1 s on two contiguous 10 mm axial slices manually placed on plain CT at the level of the basal ganglia and the level above. Data was acquired with a field of view of 25 x 25 mm (512 x 512 matrix size).

Images were processed using an in-house software using Matlab version 7.3 (The MathWorks, Inc.). The methodology closely followed that used by Wintermark et al. (2008) in order to apply their validated thresholds. The arterial input function was derived from the anterior cerebral artery; if the signal from the anterior cerebral artery was poor a branch of the middle cerebral artery was selected (Wintermark et al., 2007). The cerebral blood volume was calculated from the area under the curve; the enhancement curve from the superior sagittal sinus was used as reference. The vascular mean transit time was derived for each voxel from the convolution of the arterial input function with a box-residue function. Cerebral blood flow was calculated following the central volume principle as cerebral blood flow = cerebral blood volume/mean transit time. To define symptomatic tissue, both the mean transit time and cerebral blood volume maps were used; mean transit time has been shown to best distinguish penumbra using the affected-to-unaffected (A/U) ratio $\geq 1.45$ (Wintermark et al., 2006).

In anterior circulation stroke, it is expected that both penumbra and core will exhibit high mean transit time. However, due to low contrast signal in severely hypoperfused tissue, particularly in white matter,
mean transit time estimation may become unreliable. It is, therefore, recommended to apply an additional criterion for core such as cerebral blood volume, which is reduced in severely ischaemic tissue. Cerebral blood volume maps were, therefore, used as a second criterion to identify symptomatic tissue, using $0.82 \, \text{ml/100 g}$ as the threshold previously reported to best characterize core in white matter (Murphy et al., 2008). Thus, symptomatic tissue was defined as any voxel with mean transit time $A/U \geq 1.45$ or cerebral blood volume $\leq 0.82 \, \text{ml/100 g}$, or both; this in turn allowed a binary map (i.e. symptomatic tissue or not) to be generated. The unaffected side mask used to calculate the mean transit time ratio included all voxels in the cerebral hemisphere contralateral to the stroke. Voxel representing CSF were automatically excluded from the analysis.

### Perfusion-weighted imaging cohort

The protocol included diffusion-weighted imaging (with 3 or 12 directions; repetition time $> 6000\,$s, field of view $24\,$cm, matrix $128 \times 128$, slice thickness $3$ or $5\,$mm), as well as gradient echo, $T_1$-weighted, $T_2$-weighted, Time of Flight magnetic resonance angiography and perfusion-weighted imaging. The perfusion-weighted imaging sequence (echo time $30$–$50\,$ms, repetition time $1500\,$ms, field of view $24\,$cm, matrix $128 \times 128$, slice thickness $5\,$mm) was obtained after Gadolinium contrast ($0.1\,$mmol/kg) intravenous injection at $5\,$ml/s followed by $30\,$ml physiological saline. Perfusion maps were calculated according to Ostergaard et al. (1996) using block-circulant singular value decomposition to fit an arterial input function from the contralateral middle cerebral artery (Wu et al., 2003). Cerebral blood flow corresponds to the peak of the deconvolved curve; cerebral blood volume is calculated from the area under the curve; and mean transit time from the equation:

$$\text{Mean transit time} = \frac{\text{cerebral blood volume}}{\text{cerebral blood flow}}$$

Regions of high diffusion-weighted imaging were drawn using seed thresholding; this produced the acute diffusion-weighted imaging lesion mask. The rest of the processing was identical to the CT perfusion above, except that the core was defined by the diffusion-weighted imaging lesion mask (as no clear or reliable perfusion-weighting imaging derived absolute cerebral blood volume threshold has been described) (Kumar et al., 2010) and a mean transit time $A/U$ ratio $\geq 1.63$ was used as penumbra threshold (Rohl et al., 2001). Thus, in the perfusion-weighted imaging cohort, symptomatic tissue was defined as the diffusion-weighted imaging lesion plus any voxel outside the latter with mean transit time $A/U$ ratio $\geq 1.63$.

### Follow-up magnetic resonance imaging

In both cohorts, follow-up structural MRI was acquired on $1.5$ or $3T$ magnets. The protocol included spoiled-gradient $T_1$-weighted volume and standard $T_2$-weighted, fluid attenuation inversion recovery (FLAIR) and diffusion-weighted imaging. All images were coregistered to the $T_1$-weighted data set using Statistical Parametric Mapping (SPM; http://www.fil.ion.ucl.ac.uk/spm). An infarct region of interest was defined as the diffusion-weighted imaging lesion plus any voxel not part of symptomatic tissue. The voxel-wise superimposition of the final infarct region of interest and the symptomatic tissue binary maps (from either CT perfusion or diffusion-/perfusion-weighted imaging) allowed determination of the volumes of salvaged symptomatic tissue (i.e. any symptomatic tissue voxel not included in the infarct region of interest) and acutely silent infarcted tissue (any infarcted voxel not part of symptomatic tissue; Fig. 1). The entire image analysis was carried out blinded to clinical data.

### Quantitative model

A quantitative multivariate model relating salvaged symptomatic tissue and acutely silent infarcted tissue to clinical course was designed. First, according to our hypothesis, infarction of acutely silent tissue would negatively influence clinical course, over and above the expected positive impact of penumbral salvage, which can be mathematically expressed as follows:

$$\Delta \text{NIHSS} = a(\Delta \text{ST} - \Delta \text{ASIT}) \quad (1)$$

where $a$ is the constant describing the relationship between $1\,$ml of infarcted tissue and $1$ point change in NIHSS score, $\Delta \text{ST} = \text{salvaged symptomatic tissue}$ and $\Delta \text{ASIT} = \text{acutely silent infarcted tissue}$. To account for the heterogeneous contribution to the NIHSS of different brain regions on the right and left hemispheres (Hillis et al., 2003; Lyden et al., 2004), we normalize $\Delta \text{NIHSS}$ by acute NIHSS, and express it as a percentage, i.e.

$$\Delta \text{NIHSS}\% = \frac{\Delta \text{NIHSS}}{\text{Acute NIHSS}} \times 100 \quad (2)$$

To incorporate $\Delta \text{NIHSS}\%$ in the left side of Equation (1), the right side must also be divided by acute NIHSS, as follows:

$$\Delta \text{NIHSS}\% = \frac{a(\Delta \text{ST} - \Delta \text{ASIT})}{\text{Acute NIHSS}} \quad (3)$$

However, as explained in the ‘Introduction’ section, acute NIHSS is proportional to the volume of symptomatic tissue (ST), as follows:

Acute NIHSS = $bST$ (4)

where $b$ is a constant that describes the relationship between $1\,$ml of symptomatic tissue and $1$ point in acute NIHSS. Replacing acute NIHSS by $bST$ in Equation (3) yields:

$$\Delta \text{NIHSS}\% = \frac{a}{b} \left[ \frac{\Delta \text{ST}}{\text{ST}} - \left( \frac{\Delta \text{ASIT}}{\text{ST}} \right) \right] \quad (5)$$

where $\Delta \text{ST}/\text{ST}$ expresses the fraction of symptomatic tissue that escapes infarction (to be referred to as ST% below); and $\Delta \text{ASIT}/\text{ST}$ is the ratio of the acutely silent infarcted tissue volume relative to symptomatic tissue (to be referred to as ASIT% below). Thus, replacing these terms in Equation (5) yields:

$$\Delta \text{NIHSS}\% = c(\Delta \text{ST}/\text{ST} - \Delta \text{ASIT}/\text{ST}) \quad (6)$$

where $c = a/b$ (i.e. a ratio of constants).

In-keeping with our hypothesis, Equation (6) predicts that percentage changes in clinical scores will be influenced in a positive direction by the (relative) volume of salvaged penumbra, and in a negative direction by the (relative) volume of acutely silent tissue evolving to infarction. This is the model that was tested using multiple linear
regression in both the CT perfusion and the perfusion-weighted imaging cohorts.

Statistical analyses

Descriptive statistics are presented for the demographic data, clinical scores and map-derived volumes. Pearson linear regression was used to test the expected relationships between clinical scores and tissue volumes. In order to test our central hypothesis, we first ran the univariate correlation between ΔNIHSS% and ASIT%; and then the same correlation this time in a general linear model after including SST% as a second independent variable, according to Equation (6) above. Two-tailed \( P < 0.05 \) was regarded as significant.

Results

Clinical data

Computed tomography perfusion cohort

Thirty-four patients fulfilled the inclusion criteria. The demographics and pertinent clinical data are summarized in Table 1 and the individual data are presented in the online Supplementary Table 1A. The cause of stroke was large vessel disease in 12%, cardioembolic in 56% and other in 32%. Mean onset-to-imaging time was 136 min; one patient (Patient 22) had awakening stroke but based on plain CT and CT perfusion was judged to have had it close to or on awakening (Hellier et al., 2006). Thrombolysis was performed in 27 patients (79%). At follow-up, median \( \Delta \text{NIHSS}\% \) was 85% (Q1, Q3: 50, 100); all patients improved apart from one (Patient 28) who deteriorated. Good outcome (modified Rankin Scale 0–2) was observed in 76%.

Perfusion-weighted imaging cohort

Fifty-eight patients fulfilled the inclusion criteria (see Table 1 for a summary and Supplementary Table 1B for individual data). Cause of stroke was large vessel disease in 32.7%, cardioembolic in 46.6% and other in 20.7%. Mean onset-to-imaging time was 156 min. Thrombolysis was performed in 42 patients (72.4%). At follow-up median \( \Delta \text{NIHSS}\% \) was 78% (Q1, Q3: 63, 100); all patients improved apart from one who deteriorated (Patient 32) and one whose scores remained level (Patient 7). Good outcome (modified Rankin Scale 0–2) was observed in 69%.

Infarct volume

In the CT perfusion cohort, both Infarct\(_{\text{whole}}\) (range: 0.07–210 ml) and Infarct\(_{\text{2slice}}\) (range: 0–65 ml) positively correlated with final NIHSS \( (r = 0.727 \text{ and } r = 0.679, \text{ respectively}; P < 0.001 \text{ for both}) \) as well as with each other \( (r = 0.93, P < 0.001) \). In the perfusion-weighted imaging cohort, Infarct\(_{\text{whole}}\) (range 0–213 ml) also positively correlated with final NIHSS \( (r = 0.75, P < 0.001) \) (Table 2).

Symptomatic tissue

In both the CT perfusion and the perfusion-weighted imaging cohorts, the volume of symptomatic tissue (range: 19–121 ml and 23–271 ml, respectively) positively correlated with acute NIHSS \( (r = 0.426, P = 0.012; \text{ and } r = 0.438, P < 0.001, \text{ respectively}) \) (Table 2).

Salvaged symptomatic tissue

Median (and quartiles) salvaged symptomatic tissue and SST% values are shown in Table 2 for the CT perfusion and perfusion-weighted imaging cohorts. The range of SST% was 39–100% and 30–100% in the CT perfusion and perfusion-weighted imaging cohorts, respectively (Table 2).

Acutely silent infarcted tissue

Infarction of acutely silent tissue was detected in the majority of patients in both cohorts, but the volume involved was small in

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Table 1 Summary of demographics and main clinical data for the two patient cohorts

<table>
<thead>
<tr>
<th></th>
<th>CT perfusion cohort (n = 34)</th>
<th>Perfusion-weighted imaging cohort (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), Mean (SD)</td>
<td>62.7 (12.9)</td>
<td>68.1 (11.2)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (59)</td>
<td>33 (57)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (41)</td>
<td>25 (43)</td>
</tr>
<tr>
<td>Stroke side, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>15 (44)</td>
<td>26 (45)</td>
</tr>
<tr>
<td>Left</td>
<td>19 (56)</td>
<td>32 (55)</td>
</tr>
<tr>
<td>Stroke onset to perfusion imaging in minutes, mean (SD)</td>
<td>136 (52)(^a)</td>
<td>156 (83)</td>
</tr>
<tr>
<td>Onset to follow-up in days, median (Q1,Q3)</td>
<td>43 (35, 59)</td>
<td>31 (20, 90)</td>
</tr>
<tr>
<td>Thrombolysis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 (79)</td>
<td>42 (72)</td>
</tr>
<tr>
<td>No</td>
<td>7 (21)</td>
<td>16 (28)</td>
</tr>
<tr>
<td>NIHSS, median (Q1,Q3)</td>
<td>15 (6.75, 19.25)</td>
<td>10 (6, 16.25)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>2 (0, 6.75)</td>
<td>2 (0, 4.25)</td>
</tr>
<tr>
<td>ΔNIHSS%</td>
<td>85 (50, 100)</td>
<td>78 (63, 100)</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (32)</td>
<td>40 (69)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>4 (12)</td>
<td>21 (36)</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>4 (12)</td>
<td>27 (47)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2 (6)</td>
<td>12 (21)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1 (3)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Smoking</td>
<td>8 (24)</td>
<td>10 (17)</td>
</tr>
<tr>
<td>Previous transient ischaemic attack</td>
<td>4 (12)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>1 (3)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Cause of stroke, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery with severe carotid stenosis</td>
<td>1 (3)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Other large artery</td>
<td>3 (9)</td>
<td>10 (17)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>19 (56)</td>
<td>27 (47)</td>
</tr>
<tr>
<td>Other determined</td>
<td>1 (3)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>10 (29)</td>
<td>8 (14)</td>
</tr>
</tbody>
</table>

\(^{a}\) Excluding one patient with awakening stroke.
most. However, it was considered substantial (>1 ml and >10 ml in the CT perfusion and perfusion-weighted imaging cohorts, taking into account the limited versus full brain coverage, respectively) in 7/34 (21%; up to 8 ml) and 11/58 (19%; up to 86 ml) patients, respectively, representing on average 11% (up to 83%) of Infarct_{2Slices} and 27% (up to 53%) of Infarct_{whole} (see below and Table 4 for further details). In these patients, the topography of acutely silent infarcted tissue varied, but in both cohorts was always in direct contact with the main infarct. Illustrative examples are shown in Fig. 2.

In neither cohort was there a significant relationship between acutely silent infarcted tissue volume or ASIT% and cause of stroke.

### Table 2  Median (and interquartile) volumes in millilitre (or per cent)

<table>
<thead>
<tr>
<th>CT perfusiona</th>
<th>ST</th>
<th>Infarct_{2Slices}</th>
<th>Salvaged symptomatic tissue</th>
<th>SST%</th>
<th>Acutely silent infarcted tissue</th>
<th>Acutely silent infarcted tissue/Infarct_{2Slices} (%)</th>
<th>Infarct_{whole}</th>
<th>ASIT%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>78.4</td>
<td>8</td>
<td>59.3</td>
<td>86</td>
<td>0.2</td>
<td>1.3</td>
<td>23</td>
<td>0.2</td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>42, 93</td>
<td>2.7, 25</td>
<td>37, 70</td>
<td>74, 96</td>
<td>0, 0.7</td>
<td>0.5, 8</td>
<td>6, 79</td>
<td>0.1, 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Perfusion-weighted imagingb</th>
<th>Acutely silent infarcted tissue/Infarct_{whole} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>103</td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>68, 166</td>
</tr>
</tbody>
</table>

Infarct_{2Slices} = infarct volume in the two CT perfusion slices only; Infarct_{whole} = whole-infarct volume; MD = median; N/A = Not applicable; ST = symptomatic tissue; SST% and ASIT% = percentages relative to symptomatic tissue.

a CT perfusion cohort (n = 34; volumes shown are for the two CT perfusion slices only, except for Infarct_{whole}).
b perfusion-weighted imaging cohort (n = 58).

**Figure 2** (A) CT perfusion (CTP) cohort. Illustrative acute mean transit time (MTT) and cerebral blood volume (CBV) maps, follow-up FLAIR (F/U FLAIR) and colour map to show infarction of acutely silent tissue in Patient 18. (B) Perfusion-weighted imaging (PWI) cohort. Illustrative acute mean transit time map, acute diffusion-weighted imaging (DWI) image, follow-up FLAIR and colour map to show infarction of acutely silent tissue in Patient 7. Yellow = symptomatic tissue; red = infarct within symptomatic tissue; blue = infarct outside symptomatic tissue.
Influence of salvaged symptomatic tissue and acutely silent infarcted tissue on neurological course: univariate and multivariate analyses

Salvaged symptomatic tissue
In both the CT perfusion and the perfusion-weighted imaging cohorts, SST% strongly positively correlated with ΔNIHSS% ($r = 0.428$, $P = 0.012$ and $r = 0.415$, $P = 0.001$, respectively). The scatterplots are shown in Fig. 3. Note that in the perfusion-weighted imaging cohort the correlation was strongly significant despite one extreme outlier (Patient 32). As this outlier’s Z-score relative to the rest of the sample was >5, this patient was excluded from all subsequent analyses.

Acutely silent infarcted tissue
ASIT% negatively correlated with ΔNIHSS% in both cohorts ($r = -0.403$, $P = 0.018$; and $r = -0.62$, $P < 0.001$, respectively; Fig. 3).

Multivariate analysis
In the general linear model with ΔNIHSS% as the dependent variable, both SST% and ASIT% remained significant as independent variables in the expected direction (i.e. positive and negative, respectively) in both cohorts. The general linear model was then repeated after adding age and acute NIHSS, which did not emerge as independent predictors in either cohort and did not change the significance of the model. The adjusted $P$-values for SST%, ASIT%, age and acute NIHSS were 0.002, 0.018, 0.495 and 0.073, respectively, in the CT perfusion cohort; and 0.015, 0.038, 0.626 and 0.263, respectively, in the perfusion-weighted imaging cohort. Note that the non-significant influence of age and admission NIHSS on neurological recovery here does not negate their well-known influence on clinical and functional outcome (Table 3).

Accuracy of delineation of acutely silent tissue
In order to define symptomatic tissue as accurately as possible, we used thresholds derived from the literature, as detailed above.

![Figure 3](https://academic.oup.com/brain/article-abstract/134/6/1765/375112)
However, to further establish this classification was sufficiently accurate, three additional analyses were performed, as follows.

**Perfusion parameters within the acutely silent infarcted tissue**

The population of voxels classified as acutely silent infarcted tissue was divided arbitrarily into two compartments comprising all voxels with mean transit time ratio $\geq 1.2$ and $<1.2$, thought to represent oligaemia and normoperfused tissue, respectively, and the perfusion parameters within these two subsets of voxels was scrutinized in the 7 and 11 patients of the CT perfusion and perfusion-weighted imaging cohorts, respectively, with significant ASIT volume as defined above. The results are shown in Table 4. On average, 43% and 36% of the volume of acutely silent infarcted tissue had a mean transit time ratio $\geq 1.2$, respectively. In the vast majority of cases, the tissue classified as clinically silent did have haemodynamic characteristics consistent with non-core–non-penumbral tissue. However, in three patients of the CT perfusion cohort (Patients 10, 19 and 28) and three of the perfusion-weighted imaging cohort (Patients 31, 33 and 36), the cerebral blood flow was considered inordinately high ($>150\%$ of contralateral) or low ($<50\%$) and/or the cerebral blood volume inordinately low ($<50\%$), which could represent partially or fully reperfused core. We, therefore, reanalysed our data excluding any voxels with extreme cerebral blood volume or cerebral blood flow (i.e. $<0.5$ or $>1.5$) within the acutely silent infarcted tissue whenever the latter was $>1\text{ml}$. Both SST\% and ASIT\% remained significantly related to $\Delta$NIHSS\% on the general linear model.

### Table 4

Volumes (in millilitre and in per cent of Infarct$_{2\text{slice}}$ and Infarct$_{\text{whole}}$) of, and mean perfusion parameters within, the acutely silent infarcted tissue in the subset of cases with substantial acutely silent infarcted tissue volumes

<table>
<thead>
<tr>
<th>CT perfusion</th>
<th>Infarct$_{2\text{slice}}$</th>
<th>Acutely silent infarcted tissue (%)*</th>
<th>Salvaged symptomatic tissue contribution to $\Delta$NIHSS (%)</th>
<th>Acutely silent infarcted tissue contribution to $\Delta$NIHSS (%)</th>
<th>Acutely silent infarcted tissue with mean transit time $&lt;1.2$</th>
<th>Acutely silent infarcted tissue with mean transit time $\geq 1.2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.3</td>
<td>8.0 (46)</td>
<td>82</td>
<td>18</td>
<td>2.54</td>
<td>1.03</td>
</tr>
<tr>
<td>4</td>
<td>65.2</td>
<td>1.4 (2)</td>
<td>97</td>
<td>3</td>
<td>1.06</td>
<td>0.98</td>
</tr>
<tr>
<td>10</td>
<td>16.1</td>
<td>1.0 (6)</td>
<td>98.5</td>
<td>1.5</td>
<td>0.84</td>
<td>0.67</td>
</tr>
<tr>
<td>18</td>
<td>14.0</td>
<td>2.6 (19)</td>
<td>96.5</td>
<td>3.5</td>
<td>0.81</td>
<td>1.18</td>
</tr>
<tr>
<td>19</td>
<td>5.8</td>
<td>1.7 (29)</td>
<td>97</td>
<td>3</td>
<td>1.53</td>
<td>1.36</td>
</tr>
<tr>
<td>22</td>
<td>1.8</td>
<td>1.5 (83)</td>
<td>93</td>
<td>7</td>
<td>1.28</td>
<td>0.99</td>
</tr>
<tr>
<td>28</td>
<td>31.8</td>
<td>5.3 (17)</td>
<td>91</td>
<td>9</td>
<td>3.98</td>
<td>0.78</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Perfusion-weighted imaging</th>
<th>Infarct$_{\text{whole}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29</td>
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<tr>
<td>7</td>
<td>182</td>
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<td>98</td>
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<td>142</td>
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<td>36</td>
<td>99</td>
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<td>77</td>
</tr>
<tr>
<td>45</td>
<td>71</td>
</tr>
<tr>
<td>48</td>
<td>69</td>
</tr>
</tbody>
</table>

Also indicated are the percentage contributions of salvaged symptomatic tissue and acutely silent infarcted tissue to $\Delta$NIHSS in these patients. $r$ cerebral blood volume, $r$ cerebral blood flow and mean transit time are relative to healthy side.

$a$ Acutely silent infarcted tissue/Infarct$_{2\text{slice}}$ in CT perfusion and whole in perfusion-weighted imaging).

NA = not applicable as no change in NIHSS was observed.
model in both the CT perfusion cohort ($P = 0.01$ and 0.043, respectively; whole model $P < 0.005$) and the perfusion-weighted imaging cohort ($P = 0.001$ and 0.01, respectively; whole model $P < 0.001$). For completeness, Table 4 also lists for this subset of patients, the total acutely silent infarcted tissue volume as well as the percentage volume of acutely silent infarcted tissue relative to Infarc$^{2}_{\text{slice}}$ and Infarc$^{\text{whole}}$ (for the CT perfusion and perfusion-weighted imaging cohorts, respectively), and the respective contribution of salvaged symptomatic tissue and acutely silent infarcted tissue to $\Delta$NIHSS as calculated from the standardized coefficients of the general linear model (Table 3).

**Contribution of acutely silent infarcted tissue to acute neurological deficit**

According to our model, the contribution of acutely silent infarcted tissue to acute NIHSS is expected to be negligible, or at least much smaller than its influence on final NIHSS. This assumption was tested using regression analysis. In the CT perfusion cohort, acutely silent infarcted tissue volume explained only 0.5% of the variance in acute NIHSS, as compared with 20% of that of final NIHSS; corresponding values in the perfusion-weighted imaging cohort were 18% and 48%, respectively.

**Using less conservative penumbral thresholds**

Our aim here was to purposely increase symptomatic tissue volumes and decrease acutely silent infarcted tissue volumes, and see if the main findings still held. The mean transit time A/U threshold for symptomatic tissue was decreased from 1.45 to 1.30, and from 1.63 to 1.45, for the CT perfusion and perfusion-weighted imaging cohorts, respectively. Despite these changes, for both cohorts both SST% and ASIT% remained independently associated with $\Delta$NIHSS% on general linear model ($P = 0.009$ and 0.0021, respectively; whole model $P < 0.001$, for the CT perfusion cohort; and $P = 0.003$ and 0.044, respectively; whole model $P < 0.001$, for the perfusion-weighted imaging cohort).

**Discussion**

Consistent with our hypotheses, this prospective study documented first that tissue assumed not to be at risk of infarction acutely can eventually be part of the final infarct; and second, that based on a multivariate model controlling for salvaged penumbra, age and acute NIHSS, infarction of this tissue negatively influences clinical course. Both findings were replicated in two independent, reasonably large cohorts using distinct imaging modalities, both widely used in the clinical setting.

The negative clinical influence of infarction of acutely silent tissue observed here was markedly smaller than the positive impact of salvaged penumbra—itsself enhanced by the fact that the majority of the patients underwent early thrombolysis in both cohorts. This prominent impact of penumbral salvage replicates reports from a wide range of imaging modalities (Furlan et al., 1996; Heiss et al., 1998; Baird et al., 2000; Wintermark et al., 2002; Markus et al., 2004; Muir et al., 2006b). Also expected, (core + penumbra) volumes highly correlated with acute NIHSS, confirming that this tissue is indeed ‘symptomatic’. The consistency of these findings in both cohorts not only provides further validation of the core/penumbra model but also supports the validity of the methodology implemented here.

Even though, consistent with the core/penumbra model, the volume of acutely silent but eventually infarcted tissue was small in most patients, it was substantial in a sizeable subset of the patients (Table 4). Although this was particularly clear in the perfusion-weighted imaging cohort where the whole brain was sampled as compared with two 10 mm slices in the CT perfusion cohort, the results were similar when expressed relative to infarct volume in the sampled tissue, ranging from 2 to 83% and 13 to 53%, respectively. In absolute terms, and considering only the perfusion-weighted imaging cohort, 6/58 patients (10%) had acutely silent infarcted tissue volumes >20 ml, which is clinically relevant. To address directly the clinical impact of acutely silent infarcted tissue, we calculated its contribution to $\Delta$NIHSS in the same subset of patients (Table 4). Although, as expected, this impact was small in the CT perfusion cohort due to limited brain coverage, it was substantial in the perfusion-weighted imaging cohort (>10% in six patients and as high as 41% in one). Thus, this under-appreciated phenomenon may be quite significant in at least a fraction of anterior circulation stroke patients, which has clinical implications. Of note, as predicted by our quantitative model, both salvaged penumbra and infarction of acutely silent tissue exerted the same impact per ml of tissue on neurological course, as shown by the similar standardized coefficients from the general linear model (Table 3).

A significant challenge in this investigation was to define symptomatic tissue (and from there silent tissue) as accurately as possible. To this end, we used previously published thresholds. However, these thresholds have only partially been validated. Although the above clinical correlations with acute NIHSS and $\Delta$NIHSS indirectly support their validity, three additional post hoc analyses were performed to further establish the robustness of our findings: (i) the perfusion parameters of the acutely silent infarcted tissue were scrutinized in order to rule out any misclassified core; (ii) we verified that acutely silent infarcted tissue does not substantially contribute to acute NIHSS, i.e. was effectively ‘silent’; and (iii) the entire analysis was repeated using deliberately less conservative thresholds. The first analysis showed that in the vast majority of cases, the perfusion values within acutely silent infarcted tissue were indeed consistent with normoperfused or oligemic tissue. In a few cases, however, part of acutely silent infarcted tissue could have represented partially or fully reperfused core, but excluding these suspicious voxels from the analysis did not alter the main findings from our study. The results from the second analysis supported our assumption that acutely silent tissue, as defined here, had negligible impact on acute NIHSS as compared with its expected strong influence on final NIHSS when it progressed to infarction. Finally, the third analysis showed that using markedly less conservative mean transit time threshold for penumbra, i.e. deliberately decreasing the volume of acutely silent tissue, did not alter the significance of our findings, i.e. the latter are not exquisitely dependent on errors in the estimated tissue volumes.

An additional possible point of concern regards potential non-linearity and non-normal distributions in our data set. To address this, we reanalysed our data using non-parametric tests,
specifically partial Kendall τ correlations (Maghsoodloo, 1975). The results were not different, i.e. ASIT% again significantly correlated with ΔNIHSS% independently of SST% (partial Kendall τ = 0.308 and 0.319; P < 0.01 and < 0.001 for the CT perfusion and perfusion-weighted imaging cohorts, respectively).

Based on a very large individual Z-score, one extreme outlier was excluded from the general linear model in the perfusion-weighted imaging cohort. This patient had significant salvage of symptomatic tissue (>80%) and relatively small acutely silent infarcted tissue volume. Despite this, however, he sustained marked clinical deterioration (Supplementary Table 1B and Fig. 3B). His perfusion maps revealed that acutely, a large volume of the middle cerebral artery territory was severely affected. Detailed review of his follow-up imaging showed that most of the cortical areas had escaped infarction, yet the observed small volumes of acutely silent infarcted tissue was located in the deep structures (perhaps reflecting retrograde extension of the thrombus), which may have caused the disproportionate clinical worsening. This is a known limitation of all currently available stroke scales including the NIHSS, which is heavily weighted by motor function but much less so by dysphasia, neglect and impaired higher cognitive functions particularly those sustained by the frontal lobes and the non-dominant hemisphere (Hillis et al., 2003; Lyden et al., 2004). Thus, stroke scales such as the NIHSS do not allow a one-to-one relationship between patient’s symptoms, signs on examination, affected hemisphere and volume of impaired brain tissue, which may have influenced some of our results. Note that there was a similar, albeit less extreme, case in the CT perfusion cohort (Patient 28; Fig. 3A).

Aside from further validating the penumbra/core model and documenting again the major clinical impact of preventing infarction of penumbral tissue, we prospectively document here that surrounding non-core–non-penumbral regions may become at-risk. Other early events may involve backward propagation of the middle cerebral artery thrombus (as alluded above) or embolization of a fragmented clot into a distal branch of the artery that was originally occluded, which could account for the contiguous pattern and the large volume of acutely silent infarcted tissue in a proportion of the patients. Also, small and otherwise asymptomatic emboli may lead to infarction if they involve the oligoemia, through synergistic mechanisms (Bang et al., 2010). Repeated large emboli from e.g. cardiac thrombi were in principle excluded since clinically definite recurrent stroke was a cause of secondary exclusion; note also that there was no relationship between stroke subtypes and volume of acutely silent infarcted tissue. Further work using systematic sequential perfusion imaging and angiography as well as physiological monitoring is needed to clarify these possibilities. Finally, delayed tissue damage from secondary events such as peri-infarct inflammation may also contribute (Hughes et al., 2010). In view of its expected direct clinical benefit, preventing infarction of the initially not-at-risk tissue by avoiding these complications is an important future goal.

Our study has limitations. First, regarding the protocol, only patients who survived to the follow-up structural MRI were eligible because this study required final infarct mapping. However, early deaths would be expected if anything to entail larger, rather than smaller, infarction of silent tissue. Indeed, ‘malignant’ infarcts involve extensive vasogenic oedema and in turn increased local pressure, leading to hypoperfusion and expanding infarction of surrounding tissue (Alawneh et al., 2009). Second, the use of only two 10 mm perfusion slices in the CT perfusion cohort constrained the available tissue compartments for the general linear model. However, there was a strong correlation between whole-infarct volume and infarct volume in those two slices, suggesting the latter was an acceptable surrogate for the former. Moreover, findings in the CT perfusion cohort were entirely mirrored in the perfusion-weighted imaging cohort where brain coverage was full. Third, and final, uncertainties in the perfusion thresholds used and errors due to the algorithms implemented to generate perfusion maps may account for the small acutely silent infarcted tissue volumes derived in most patients, although as discussed above, are unlikely to explain the large volumes observed in a subset. Future work implementing systematic follow-up perfusion imaging within the first few hours after the initial session to identify new areas of penumbral hypoperfusion in correlation with clinical changes would be of value in establishing the validity and significance of the phenomenon reported here.

Conclusion

This is the first study to systematically examine infarction of non-core–non-penumbral tissue in acute ischaemic stroke. In two independent cohorts, regardless of the imaging modality and exact perfusion thresholds used, infarction of acutely silent tissue was demonstrated to be substantial in at least a subset of subjects, and had the hypothesized negative impact on clinical course over and above the positive impact of penumbral salvage. Researching the mechanism(s) leading to infarction of acutely silent tissue is an important goal.

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Supplementary material

Supplementary material is available at Brain online.

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