Voxel-based mapping of irreversible ischaemic damage with PET in acute stroke

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Summary
Objective mapping of irreversible tissue damage in the acute stage of ischaemic stroke would be useful for prognosis and in assessing the efficacy of therapeutic manoeuvres in impeding extension of infarction. From our database of 30 patients studied with 15O-PET within 5–18 h after onset of first-ever middle cerebral artery territory stroke, we extracted a subgroup of 19 survivors (age 74.6 ± 8.5 years) in whom late CT coregistered with PET was available to determine final infarct topography. By means of a voxel-based analysis of the PET data, we determined putative thresholds for irreversible tissue damage as the lower limit of the 95% confidence interval calculated from all voxels within the ultimately non-infarcted brain parenchyma ipsilateral to the insult. The following values were found: 8.43 ml/100 ml/min, 0.87 ml/100 ml/min, 1.64 ml/100 ml, 0.27 and 2.21/min, for cerebral blood flow (CBF), oxygen consumption (CMRO2), blood volume (CBV), oxygen extraction fraction and the ratio CBF : CBV, respectively. Voxels below these thresholds occurred significantly more frequently in the final infarct region than in the non-infarcted parenchyma for CBF and CMRO2 (P = 0.016 and P = 0.0045, respectively, Wilcoxon test), but not for the other PET variables. Furthermore, with both CBF and CMRO2, the percentage of irreversible tissue damage voxels in the affected hemisphere relative to the opposite hemisphere was significantly positively correlated to both the volume of final infarct and the neurological outcome at 2 months (all P < 0.005, Spearman ranked test). These findings validate our voxel-based CBF and CMRO2 thresholds for probabilistic mapping of irreversible tissue damage within the 5–18 h interval after stroke onset; however, whether they would be applicable to earlier intervals remains to be determined. Transfer of our procedure for determination of irreversible tissue damage thresholds to other imaging modalities such as single proton emission computed tomography and diffusion-weighted MRI should be straightforward.

Keywords: stroke; cerebral blood flow; oxygen metabolism; PET

Abbreviations: GI = glabella–inion; MCA = middle cerebral artery; NIBP = non-infarcted brain parenchyma; CBF = regional cerebral blood flow; CMRO2 = regional cerebral oxygen consumption; OEF = regional oxygen extraction fraction; ROI = region of interest

Introduction
In several species, the core of irreversibly damaged brain tissue slowly increases in volume over several hours or even days following experimental occlusion of the middle cerebral artery (MCA) (Weinstein et al., 1986; Kaplan et al., 1991; Meier-Ruge et al., 1992; Touzani et al., 1995; Young et al., 1997). The widely assumed explanation for this intriguing phenomenon has been the progressive demise of the ‘penumbra’, a severely ischaemic but still potentially viable tissue (Hossmann, 1994). It would be of obvious value in clinical trials to obtain rapidly objective maps depicting the extent and topography of the irreversible tissue damage at start of therapy, so as to evaluate whether the agent under study impedes, or even interrupts, infarct growth. Such maps would also have considerable diagnostic and prognostic applications. However, currently such maps cannot be obtained with either CT or MRI scanning. Thus, early changes at CT or standard T2-weighted MRI are found in a fraction of the patients only and are not consistently predictive of irreversible tissue damage (Mohrt et al., 1995; Tarr et al., 1996; Grond et al., 1997; Ghokhar et al., 1998; Fieschi et al., 1999; Jaillard Serradj et al., 1999), while the predictive value of diffusion-weighted MRI for irreversible tissue damage is
still undecided (Baird and Warach, 1998; Powers and Zivin, 1998; Kidwell et al., 1999a, b; Lecouvet et al., 1999). Using PET, three earlier studies attempted to determine regional cerebral blood flow (CBF) and oxygen consumption (CMRO₂) threshold values below which tissue would consistently be infarcted on late CT, and all three concurred in the finding of a roughly similar CMRO₂ threshold ranging from 1.2 to 1.7 ml/100 ml/min, while the CBF threshold around 12 ml/100 ml/min was clearly less consistent (Baron et al., 1983; Powers et al., 1985; Ackerman et al., 1989). In retrospect, however, these classic PET studies all had serious limitations. First, the investigators all studied few cases and used first-generation PET devices with poor spatial resolution. Secondly, in all these studies, the approach used to determine an irreversible tissue damage threshold was to place a few geometrical regions of interest (ROIs) in topographically predetermined, mainly grey matter structures, so that the calculated thresholds would be applicable only to similarly shaped, sized and placed ROIs; also, sampling confined to grey matter led to conservatively high threshold values (Powers et al., 1985). Thus, these ‘classical’ thresholds would not be applicable for the mapping of irreversible tissue damage, i.e. a voxel-based, computer-driven search of the entire brain parenchyma. Thirdly, none of these early studies used PET-to-CT coregistration, and therefore the accuracy of the outcome of the tissue sampled by the ROIs should be questioned. Fourthly, the clinical material was either suboptimal or poorly described, as (i) the study carried out by Baron and colleagues (Baron et al., 1983) was not performed within the first 24 h; (ii) the only two acute stroke patients of Powers and colleagues (Powers et al., 1985) had suffered from subarachnoid haemorrhage with vasospasm; and (iii) the study carried out by Ackerman and colleagues (Ackerman et al., 1989) was reported in short abstract only. Fifthly, and finally, potential irreversible tissue damage thresholds for regional oxygen extraction fraction (OEF), regional cerebral blood volume (CBV) and the ratio CBF : CBV, an index of cerebral perfusion pressure (Gibbs et al., 1984; Sette et al., 1989; Schumann et al., 1998) were not systematically searched.

Over a period of 4 years our group has prospectively accumulated a unique sample of 30 first-ever MCA territory ischaemic stroke patients, all selected according to strict and pre-defined entry criteria and in whom quantitative high-resolution seven-slice PET imaging of CBF, CMRO₂, OEF, CBV and the CBF : CBV ratio was performed within the first 18 h after stroke onset. This PET procedure was repeated around 3 weeks later in the survivors, together with an unenhanced CT scan coregistered with PET to map final infarction. To assess spontaneous outcome and recovery, each patient was prospectively followed-up from admission to either death or a 2-month end-point with quantitative stroke scales validated for MCA stroke and administered at pre-defined time-points. In a manner similar to the administration of a brain bank, this unique database, which contains around 2000 images representing different physiological and structural variables (i.e. ~1000 PET images for each of the two time-points plus ~150 CT images), was used to address a number of independent scientific questions, with the following major findings: (i) there exist distinct visually-defined patterns of acute-stage changes in CBF and CMRO₂, each associated with a distinct clinical outcome (Marchal et al., 1993, 1995); (ii) early spontaneous tissue hyperperfusion is a marker of good tissue outcome (Marchal et al., 1996a); (iii) clinical recovery from the acute to the subacute stage is not accompanied by parallel increases in CMRO₂ in the unaffected hemisphere, contradicting the classic trans-hemispheric diaschisis theory (Iglesias et al., 1996); (iv) using voxel-based analysis, a substantial part of the finally infarcted area still exhibited CMRO₂ values higher than the classical viability threshold of 1.4 ml/100 g/min in the acute stage, presumably representing still salvageable tissue (i.e. the penumbra) (Marchal, 1996b); and (v) also by means of voxel-based analysis, we were able to show that acutely ischaemic voxels with similar CBF, CMRO₂ and OEF values could either evolve to or escape from infarction, with the volume of the latter being proportional to the degree of subsequent clinical recovery, consistent with the concept of penumbra (Furlan et al., 1996). The aim of the present analysis was to exploit this database again to re-examine the issue of irreversibility thresholds by means of a voxel-by-voxel approach, and to determine whether mapping of the already irreversibly damaged tissue in acute stroke is feasible. To the best of our knowledge this has never been attempted either with PET or with any other imaging technology, presumably because of the complexities involved. For instance, Marchal and colleagues (Marchal et al., 1996b) assessed the ultimately infarcted penumbra by applying the above-mentioned CMRO₂ irreversibility threshold of 1.4 ml/100 ml/min; however, because many voxels below this threshold were observed in the white matter of both hemispheres, the authors argued that this cut-off probably overestimated the true voxel-level threshold, a problem which was judged to result in a conservative, rather than liberal estimate of the penumbra. Likewise, Heiss and colleagues (Heiss et al., 1998) recently applied the classic grey-matter ROI-based threshold of 12 ml/100 ml/min in a voxel-based analysis of semi-quantitative PET perfusion images obtained before and after thrombolysis in a sample of acute stroke cases. Thrombolysis was reported to salvage substantial volumes of voxels with CBF below this cut-off, indicating that the voxel-based threshold for irreversible tissue damage must be lower than this.

To achieve our aim, we analysed the data for each of the above five PET parameters, and designed an entirely novel approach to image analysis. First, we defined an operational potential threshold for irreversible tissue damage for each PET parameter as the lower 95% confidence limit of voxel values in the ultimately non-infarcted parenchyma of the affected hemisphere. Secondly, to test the validity of the probabilistic thresholds thus obtained, we assessed how they performed in identifying irreversible tissue damage relative
to the CT-defined infarct in terms of both topography and volume. Finally, and to provide an independent additional validation, we also looked for correlations between the volumes of irreversible tissue damage so determined and neurological deficits measured at admission and 2 months later.

Subjects and methods

Subjects
Our database consists of 30 consecutive patients aged >18 years with first-time MCA territory stroke and persistent neurological deficits (14 men, 16 women; age 73.7 ± 10.4 years, mean ± standard deviation). They were prospectively studied by acute-stage PET (<18 h), clinical evaluation under standard medical management (i.e. spontaneous outcome) and a late CT scan coregistered with PET to determine final infarct topography (Marchal et al., 1995, 1996; Furlan et al., 1996). Exclusion criteria were previous stroke, marked somnolence, poor cooperation, lacunar syndrome, haemorrhagic infarct on admission CT, organ failure and recent myocardial infarction. Neurological deficits were scored with Orgogozo’s neurological MCA scale at admission and at 2 months follow-up (Orgogozo, 1998). Eligibility for the present study was availability of the late CT scan, for methodological reasons to be explained below.

General methodology

PET studies
PET studies were performed using the LETI TTV03 tomograph (Mazoyer et al., 1990) following the 15O equilibrium procedure that we have described in detail previously (Marchal et al., 1992). The patient’s head was restrained by a Laitinen stereotaxic headset and positioned according to the Fox method which consists of drawing on the skull X-ray the glabella–inion (GI) line, which is essentially parallel to the bicommissural line (Fox et al., 1985). Tissue attenuation was measured before each study with 68Ge. Trace amounts of C15O, 15O2 and C15O2 mixed with room air were sequentially delivered to the patient via a thermoplastic face mask, and three pairs of blood samples were taken during each PET data acquisition to measure arterial 15O concentrations. After image reconstruction and quantification with classic equations including correction for intravascular tracer, parametric images of CBV, OEF, CMRO₂ and CBF were obtained (Frackowiak et al., 1980; Pantano et al., 1985). This PET procedure has been approved by the Ethics Committee of Caen University.

CT scan
A CT scan was performed at admission to exclude haemorrhagic infarct, previous stroke or non-ischaemic insult. A late CT scan coregistered with PET (according to the same GI landmarks) was performed 2 weeks to 3 months after stroke onset to outline the final infarct (Furlan et al., 1996).

Matrix transformation and infarct ROI
After PET-to-CT realignment in the matrix (no reslicing was necessary as the CT cuts were directly obtained coregistered with PET), the 1 × 1 × 9 mm voxel matrix was transformed into a 8 × 8 × 9 mm matrix (Furlan et al., 1996; Marchal et al., 1996), the objective being to reduce both the volume of data and the statistical noise in each new voxel. In addition, this operational voxel size takes into account the true resolution of the PET device used.

Infarct ROI
The late CT was displayed on a Silicon Graphics Workstation and the contour of hypodensity [which depicts the infarcted brain tissue or ‘pan-necrosis’ (Garcia et al., 1996)], if any, was delineated plane-by-plane by means of the Miriam software developed in-house. The infarct ROI comprised all 8 × 8 × 9 mm voxels included in the cumulated areas of hypodensity delineated on the relevant CT cuts. Voxels which fell across contours were taken into account in the ROI only if >50% of the original 1 × 1 mm pixels fell within this ROI. In this case, only the resulting fraction of the 8 × 8 × 9 mm voxel was retained for further analysis (i.e. volume measurements, PET variables). This procedure also allowed calculation of a volume for infarction by summing all included 8 × 8 × 9 mm voxels (and fractions thereof).

PET data analysis
For the present work, we analysed six PET slices (all five parameters), from GI + 8 mm to GI + 68 mm, according to the methodology described below.

Determination of irreversible tissue damage thresholds
In this study, a probabilistic irreversible tissue damage threshold for each PET parameter was defined as the lower 95% confidence limit of the population of ultimately non-infarcted voxels in the brain parenchyma ipsilateral to the ischaemic insult. This procedure, which was applicable even if no infarct was detectable on the later CT, ran as follows.

Step 1. The contours of the non-infarcted brain parenchyma, excluding the final infarct area (if any) and the ventricles, were delineated on each of the six CT cuts, as illustrated in Fig. 1. The 8 × 8 × 9 mm voxels selected within these contours defined what we refer to below as the ‘non-infarcted brain parenchyma’ (NIBP) ROI. Regarding the voxels that
overlapped the parenchyma contours, the same rule as that described above for the infarct ROI was applied.

**Step 2.** Following selection of the voxels within the NIBP, we calculated, for each of the five PET parameters and across all the eligible patients, the lower 95% confidence limit (one-tailed, i.e. $t = 1.64$) for this entire population of voxel values.

**Validation of the putative irreversible tissue damage thresholds**

To assess the validity of the probabilistic thresholds obtained from the above procedure to identify irreversible tissue damage, we tested whether, across the patients sample, and as would be expected for a useful threshold, those voxels whose values fall below each of the five thresholds (i.e. one per PET parameter) preferentially projected within the final CT-defined infarcts. In other words, voxels with values lower than a valid irreversible tissue damage threshold should preferentially distribute within the ultimate infarct than in the non-infarcted parenchyma, i.e. significantly more likely than chance. This procedure ran as follows.

**Step 1.** Screening for below-threshold voxels within the infarct for each PET parameter. For each of the five PET parameters, all the voxels characterized by a PET value below the given threshold and located within the infarct ROI were computer-selected and their integrated volume calculated. To account for the different infarct sizes among patients, this volume was expressed as percentage of infarct volume. They will be referred to as "infarct %".

**Step 2.** Selection of below-threshold voxels within the non-infarcted brain parenchyma. As for the infarct, the below-threshold voxels for each PET parameter were computer-selected within the NIBP region, and their volume was expressed as percentage of the whole region volume, as above. This will be referred to as "NIBP %".

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**Fig. 1** Illustration of the method used to determine, on the late CT scan, the contours of the ultimately non-infarcted parenchyma ipsilateral to the infarct, and the resulting $8 \times 8 \times 9$ mm voxels selected. In the patient illustrated, the voxels are displayed according to a pseudo-colour scale showing the CMRO$_2$ values from zero (white) to 5 ml/100 ml/min (red), across five relevant PET slices (from +20 to +68 mm relative to the GI line, see Subjects and methods).
Step 3. We compared for each PET parameter the infarct % values with the NIBP % values across all eligible patients. A validated irreversible tissue damage threshold would result in a statistically larger value for the former.

Correlations with neurological deficit and infarct volume

To assess further the validity of those thresholds that would pass the previous test, if any, we evaluated the correlations between the volume of putative irreversible tissue damage voxels in the affected hemisphere and both the final infarct volume and neurological scores (at day 0 and day 60). However, to obtain a clinically meaningful value for the volume of irreversible tissue damage voxels in the affected hemisphere, i.e. at a stage where the final infarct contours are unpredictable, it was necessary to take into account the chance occurrence of such voxels in both hemispheres inherent to the use of probabilistic thresholds. To this end, for each subject, the total number of irreversible tissue damage voxels (across all selected planes) occurring in the non-affected hemisphere was subtracted from that occurring in the affected hemisphere, so that this difference would more closely represent true irreversible tissue damage. This required that a ROI for the non-affected hemisphere was first delineated. This was done in the same way as for the affected hemisphere (see above), except that obviously no infarct needed to be excluded. The irreversible tissue damage volumes obtained were correlated with both infarct volumes and MCA scores at days 0 and 60.

Statistical analysis

The statistical analysis was performed using the BMDP package software (BMDP, Calif., USA) on a Sun/Unix Workstation. Results are expressed as means (± standard deviation). Because the CMRO2, CBF, CBV, OEF and CBF : CBV samples of non-infarcted voxels all had skewed distributions (P < 0.001), they were normalized by squared-root transformation. The 95% lower confidence limit was then calculated for each PET variable by taking the reciprocal function of the transformed variable as follows:

\[ V_i = \frac{\mu - Z_{0.025} \times SD}{SD} \]

where \( V_i \) represents the threshold value of PET variable \( i \), \( \mu \) the mean value of the squared-root data of the PET variable \( i \) within the NIBP; \( Z_{0.025} = 1.645 \) represents the one-tailed Z-score value for 95% CI, and SD the standard deviation of the squared-root data of the PET variable \( i \).

We used the non-parametric Wilcoxon test to assess whether voxels with values below the threshold were distributed electively within the final infarct areas or within the ultimately non-infarcted brain parenchyma. Correlations between irreversible tissue damage volumes and both infarct volumes and MCA scores were assessed with non-parametric Spearman ranked tests. A P-value of 0.05 was considered to be significant.

Results

Patient population

Out of the 30 patients in our database, 11 were non-eligible for this analysis because of lack of an adequate late CT scan, due to early death (n = 6), patient refusal (n = 3) or technical problems with the CT-to-PET registration (n = 2). The relevant clinical and CT data of the remaining 19 patients (mean age 74.6 ± 8.5 years) are shown in Table 1. All of them but one were part of previous analyses from the same data bank (see Table 1). The time of the late CT ranged from 14 to 85 days after symptom onset (mean 53 days). No definite infarct was detected in two patients (7 and 15).

Irreversible tissue damage thresholds

Across the 19 eligible patients, 8852 voxels (or fractions thereof, see Subjects and methods) were selected within the NIBP region. The mean (±SD) CBF, CMRO2, CBV, OEF and CBF : CBV values for this sample of 8852 voxels were 23.25 ± 11.55 ml/100 ml/min, 2.04 ± 0.78 ml/100 ml/min, 4.02 ± 2.13 ml/100 ml, 0.515 ± 0.173/min and 7.20 ± 4.17/min, respectively.

The lower 95% confidence limits calculated from these samples according to the method described above were 8.43 ml/100 ml/min, 0.87 ml/100 ml/min, 1.47 ml/100 ml, 0.27 and 2.21/min, respectively.

Validation of the putative irreversible tissue damage thresholds

As this procedure could be applied only to the patients in whom infarcted voxels existed, the data from patients 2, 3, 7 and 15 were not used (see Table 1). In the remaining 15 patients, a clear-cut distribution of CBF and CMRO2 irreversible tissue damage voxels within the final infarct area was evident by simple visual inspection, especially in the eight cases with the largest infarcts (see Fig. 2 for illustration in two cases). Table 2 shows, for each PET parameter, the mean infarct % and NIBP % values across the 15 subjects. The former was significantly larger than the latter for CBF and CMRO2 (P = 0.0159 and 0.0045, respectively), but not for CBV, OEF and CBF : CBV. For comparison, Table 2 also shows the percentages of below threshold voxels present in the cerebral hemisphere contralateral to the insult.

Correlation of irreversible tissue damage volume with infarct volume and neurological outcome

Because the findings above indicate that irreversible tissue damage thresholds are statistically valid solely for CBF and
<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Past medical history</th>
<th>Side of infarct</th>
<th>Neurological exam</th>
<th>MCA score</th>
<th>Stroke-to-PET delay (h)</th>
<th>Late CT (days)</th>
<th>Infarct volume (ml)*</th>
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<td>86</td>
<td>M</td>
<td>Coronary artery ischaemic heart disease</td>
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<td>55</td>
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<tr>
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<td>F</td>
<td>HBP</td>
<td>L</td>
<td>Hemiparesis, aphasia</td>
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<td>100</td>
<td>15</td>
<td>32</td>
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<td>F</td>
<td>–</td>
<td>R</td>
<td>Hemiparesis, visual neglect</td>
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MCA score = Orgogozo’s neurological middle cerebral artery score (Orgogozo, 1998); R = right; L = left; HBP = high blood pressure; α, β, γ = patients previously reported in Furlan et al. (1996) and Marchal et al. (1996a, b), respectively. *Calculated as described in Subjects and methods; †infarct present but too small to include >50% of even one $8 \times 8 \times 9$ mm voxel (see Subjects and methods); ‡no definite infarct detected on the late CT.
CMRO₂, correlations with infarct volume and neurological data were only evaluated for irreversible tissue damage volumes obtained with these two variables. All 19 patients (i.e. including the patients without detectable or measurable infarct) were used in these correlations.

There was a significant positive correlation between irreversible tissue damage volumes and final infarct volumes for both CBF and CMRO₂ (rho = 0.86, P = 0.0003 and rho = 0.68, P = 0.0041, respectively) (Fig. 3). Likewise, there were significant positive correlations between irreversible tissue damage volumes and both day 0 and day 60 MCA scores (for CBF rho = −0.71, P = 0.0027 and rho = −0.77, P = 0.0011, respectively; for CMRO₂ rho = −0.55, P = 0.0198 and rho = −0.67, P = 0.0045, respectively) (Fig. 4). These correlations with infarct volume and neurological scores were not altered if the raw irreversible tissue damage volumes for the affected hemisphere, rather than the values adjusted to the non-affected hemisphere, were used instead (data not shown). Finally, the correlation between irreversible tissue damage volumes and time elapsed between stroke onset and PET was not statistically significant.

**Discussion**

The probabilistic thresholds calculated from this sample of 19 patients studied in the 5–18 h interval after stroke onset were validated for CBF and CMRO₂ (with values of 8.43 and 0.87 ml/100 ml/min, respectively), but not for CBV, OEF or CBF : CBV. The validity of the CBF and CMRO₂ thresholds was further supported by the significant positive correlation found between the number of irreversible tissue damage voxels selected in the affected relative to the non-affected hemisphere, and both final infarct volume and same-day or 2-month neurological scores.

Our negative findings concerning CBV and OEF are consistent with Powers and colleagues (Powers et al., 1985) who found a large overlap of ROI values for these two variables between areas of infarction and regions with viable cerebral tissue. The situation is less definite regarding the CBF : CBV ratio, which has never been assessed previously, as there was a trend for these voxels to be distributed in the final infarct area (P = 0.087, Wilcoxon test; P = 0.015, paired *t*-test).

The present study analysed a sample of patients from our database that overlapped with earlier articles (see Table 1), but it differs from these articles as it is the first to determine thresholds for irreversibility (see Introduction). Only the article by Furlan and colleagues (Furlan et al., 1996) bears some relationship with the present study in that it reported the lower 95% confidence limit of CBF for the family of voxels identified as penumbral, but this value was obtained with an entirely different analysis from the one that was employed here (see below for a comparison between values).

Three methodological issues need to be raised regarding the irreversible tissue damage thresholds for CBF and CMRO₂ reported here. First, they were calculated with reference to tissue infarction (i.e. pan-necrosis), as identified by sharply demarcated hypodensity on the late CT, and thus may not apply to partial neuronal loss and incomplete infarction, which have been reported at post-mortem in a few stroke patients as a CT-negative finding (Lassen et al., 1982), but which still have unclear clinical expression (Garcia et al., 1996). Secondly, we included all parenchymal voxels in our automatic irreversible tissue damage threshold determination, whereas grey and white matter, which have different physiological CBF and CMRO₂ (Marchal et al., 1992), might have different thresholds for irreversibility (Marcoux et al., 1982; Pantoni et al., 1996). While this has not been fully documented thus far, it would be theoretically possible to apply our methodology to a set of high-resolution PET data coregistered with T₁-weighted MRI to classify each voxel as grey or white matter and then search for separate irreversible tissue damage thresholds for each of these two subsets. However, such data were not available to us in this study. Furthermore, even if distinct irreversible tissue damage thresholds for grey and white matter existed, there would be no straightforward method for applying them to PET images acquired in the acute-stage setting, unless T₁-MRI together with fast computing algorithms were also available. This admixture of grey and white matter voxels in our analysis might also, in part, explain the fact that our thresholds for both CBF and CMRO₂ are lower than the classic ones, which were determined with ROIs placed on to mainly cortical regions; in other words, it is possible that our irreversible tissue damage thresholds do not detect all the already irreversibly damaged grey matter. For the present purpose of mapping irreversible tissue damage, however, we believe it
is better in acute stroke to be conservative rather than liberal in assigning brain tissue as irreversibly damaged. Thirdly, our analysis might be affected by partial volume effects. Thus, voxels with very low PET values at the periphery of brain tissue (i.e., at the borders with skull and ventricles) were included in our parenchyma ROI and might have affected the calculation of thresholds. To reduce such effects, we employed a method that excluded those voxels which belonged to non-brain spaces for >50% of their volume, and only counted in the final calculation the original 1 x 1 mm voxels that fell within brain tissue (see Subjects and methods). Nevertheless, and quite expectedly, this did not entirely solve the problem, as illustrated in Fig. 2, where irreversible tissue damage voxels occasionally fell at or near these borders. Again, the use of high-resolution PET with co-registered T1-weighted MRI would clearly have limited such effects, although voxels smaller than the ones we used here might have led to spurious findings, due to a low signal-to-noise ratio. Regardless, the validation approach we designed for the irreversible tissue damage thresholds controlled for this effect since the infarct %, NIBP % and irreversible tissue damage volume values were calculated by reference to the contralateral hemisphere (see Subjects and methods). Another potential side-effect of partial voluming is that larger infarcts should express lower voxel values, and therefore the chance that irreversible tissue damage voxels would distribute within a particular infarct would depend on its size. To account for this problem, we purposely expressed the number of irreversible tissue damage voxels relatively to infarcted or non-infarcted brain volumes. This procedure was effective for CMRO2, but less so for CBF, as shown by the significant positive Spearman correlation between infarct % values and infarct volumes (P = 0.01 for CBF, but not significant for CMRO2). Thus, for CBF but not for CMRO2, the sensitivity of our mapping method to identify irreversible tissue damage presumably remains slightly but not substantially affected by the extent of the damage already present. Again, this effect might be better controlled with irreversible tissue damage

Fig. 3 Relationships across 19 patients between irreversible tissue damage (ITD) volumes calculated with CBF (A) and CMRO2 (B) acute-stage PET on the one hand and final infarct volume (as assessed by the late CT) on the other hand, showing significant positive correlations by non-parametric Spearman ranked test (P = 0.0003 and 0.0041, respectively). With Pearson’s correlations, the line equations are y = 0.49, x -0.12 and y = 0.48, x -0.06 for A and B, respectively (r^2 = 0.69, P < 0.0001 for each), both significantly different from the identity line (P < 0.05, t-test), consistent with the idea that across the sample a fraction of final infarct volume was not irreversibly damaged at the time of PET.

Fig. 2 Two illustrative examples of topographic relationships between irreversible tissue damage voxels and final infarcts. For each of the five PET parameters the irreversible tissue damage voxels from acute-stage PET (in red) are shown projected on to the relevant late CT cuts (coregistered with PET). In patient 13 (top), both the CMRO2 and CBF irreversible tissue damage voxels project preferentially on to the final infarct (representing 40 and 36% of infarct volume for CMRO2 and CBF, respectively, see Subjects and methods), while in patient 16 (bottom), this is less evident (18 and 20% of infarct volume for CMRO2 and CBF, respectively), indicating that in this patient a large fraction of the final infarct was probably still viable at the time of the PET study. In patient 13, the OEF and CBF : CBV irreversible tissue damage voxels also tended to distribute preferentially within the final infarct, but this was not statistically significant across the whole sample (see Results and Table 2). Note also the occasional distribution of selected voxels near boundaries of brain parenchyma over both hemispheres.
Fig. 4 Relationships across 19 patients between irreversible tissue damage (ITD) volumes calculated with CBF (A) and CMRO$_2$ (B), acute-stage PET, and Orgogozo's MCA neurological score at day 0 (top) and day 60 (bottom), showing significant positive correlations by non-parametric Spearman ranked test (CBF, $P = 0.0027$ and 0.0011, respectively; CMRO$_2$, $P = 0.0019$ and 0.0045, respectively).
thresholds determined with smaller-sized voxels and with the use of a higher resolution PET device.

As detailed in the Introduction, previous attempts to determine viability thresholds from PET data (Baron et al., 1983; Powers et al., 1985; Ackerman et al., 1989) were fraught with uncertainties due principally to the poor spatial resolution of the imaging devices used, the few acute ischaemic stroke patients studied, the use of ROIs placed in grey matter and the lack of CT-to-PET registration. These differences with the present study explain why the threshold of around 1.4 ml/100 ml/min for CMRO₂ reported in earlier studies is larger than that found here based on a voxel-by-voxel analysis, which considered the entire brain parenchyma. Likewise, we found here a robust threshold for CBF of around 8.5 ml/100 ml/min, while the inconsistent findings in previous studies likely reflected major methodological differences. For instance, while Baron and colleagues (Baron et al., 1983) determined a CBF threshold separately for areas with high OEF (i.e. misery-perfusion), Powers and colleagues (Powers et al., 1985) included some subacute-stage PET studies with late reperfusion in established infarcts. Again, and at variance with previous attempts, the thresholds found here were designed for the mapping of irreversible tissue damage, meaning a voxel-based, automatic and objective display method.

How do our CBF and CMRO₂ irreversible tissue damage threshold values compare with earlier literature, apart from the three landmark PET studies discussed above? Our irreversible tissue damage threshold for CBF of 8.43 ml/100 ml/min would fit well the classic infarction threshold for permanent MCA occlusion of about 7–10 ml/100 ml/min found in various non-human primate species (Astrup et al., 1981; Jones et al., 1981; Garcia et al., 1983). Regarding CMRO₂, few studies have considered the critical value of oxygen consumption necessary to maintain cerebral viability in man. In brain death, whole brain CMRO₂ was found to lay below 0.6 ml/100 ml/min (Shalit et al., 1972), a value which is close to our value of 0.87 ml/100 ml/min. With PET, CMRO₂ values as high as 2.6 ml/100 ml/min within subacute or chronic infarcts have occasionally been reported (Pantano et al., 1985; Powers, 1985), but these were small infarcts relative to the poor resolution of the cameras used, and ROIs were employed; in addition, the massive macrophage infiltration of the necrotic area in the subacute stage may result in transiently increasing oxygen consumption (Wise et al., 1983). As discussed above, we previously determined the lower 95% CBF limit for penumbral voxels, including the eventually infarcted penumbra; the reported value of ~7.0 ml/100 ml/min (Furlan et al., 1996) stands remarkably near the present threshold of 8.43 ml/100 ml/min.

Independently validating the CBF and CMRO₂ thresholds, there was a positive correlation between the calculated affected-hemisphere irreversible tissue damage volumes and infarct volume. In other words, the volume of irreversible damage determined at a single time-point in the acute phase of stroke was statistically predictive of final infarct volume, a finding with obvious clinical implications. That the slope of this relationship was significantly less than 1 (see Fig. 3), i.e the irreversible tissue damage volume underestimated final infarct size is consistent with the notion that in some patients the infarct may grow for hours at the expense of the penumbra (Heiss et al., 1992; Baird et al., 1997; Barber et al., 1999). Accordingly, in eight patients of the present series, we previously documented that a sometimes substantial part of the final infarct volume was still penumbral in the acute-stage PET (Marchal et al., 1996b). The correlation found between irreversible tissue damage volumes and concomitant neurological scores as well as neurological outcome further validates the CBF and CMRO₂ thresholds and indicates our method also has clinical predictive value.

Several questions arise regarding the clinical applicability of this method. First, which of CBF or CMRO₂ better identifies irreversible tissue damage? To address this point, we assessed in a complementary analysis the population of finally infarcted voxels separately identified by CBF and CMRO₂ irreversible tissue damage thresholds. This showed that CMRO₂ was not significantly more sensitive than CBF alone, but both the use of CBF after CMRO₂, and of CMRO₂ after CBF, allowed additional irreversible tissue damage voxels to be identified (22 and 30% on average, respectively, both P < 0.05, paired t-test). Thus, although the CBF threshold and the CMRO₂ threshold are separately valid, using both rather than either alone may allow a more comprehensive identification of the irreversibly damaged tissue. Secondly, as the irreversible tissue damage thresholds determined here were obtained from studies performed 5–18 h after clinical onset, they should be applicable to this interval only, at least as a first approximation. Thus, although this constraint likely applies to CBF whose infarction threshold in the non-human primate is known to be time-dependent (Jones et al., 1981; Garcia et al., 1983), it may not necessarily be the case for CMRO₂, whose infarction threshold may be time-independent, as discussed extensively elsewhere (Baron and Marchal, 1996). Unfortunately, no data on this important issue is available at the moment and only future studies at earlier time-points may clarify it. It may also be argued that the time-frame of our study may be irrelevant to the issue of salvaging at-risk brain tissue. However, although the advocated therapeutic window for intravenous thrombolysis in acute stroke is presently 3 h (NINDS, 1995), other trials suggest that this benefit may extend to 6 h (Hacke et al., 1998), while one trial of intra-arterial thrombolysis of up to 6 h in patients selected on the basis of proven MCA stem occlusion revealed striking benefits (Furlan et al., 1999). Furthermore, although none of the neuroprotective agents has shown reproducible benefit so far, they might do so for longer time-windows than thrombolysis, because, in contrast to the latter, their
potential benefit should not be hampered by an increased risk of symptomatic haemorrhagic transformation. Finally, PET and diffusion-weighted MRI in both man and non-human primates suggest salvageable tissue may be present until nearly 24 h after stroke onset in at least a fraction of the cases (Heiss et al., 1992; Touzani et al., 1995; Marchal et al., 1996b; Baird et al., 1997; Read et al., 1998; Barber et al., 1999). Thus, although in all rigour our thresholds should now be prospectively validated in another group of patients, they may already have clinical utility in trials with extended therapeutic windows and whenever treatment options are based on physiological neuroimaging. Because PET is of limited access, however, our approach might find wider clinical applications with more accessible imaging tools such as SPECT or diffusion-weighted MRI (Baird and Warach, 1998; Barber et al., 1998, 1999), with which the transfer of our procedure for determination and validation of irreversible tissue damage thresholds would be straightforward. The capacity to map irreversible tissue damage in acute stroke opens new avenues for both predicting spontaneous outcome and monitoring the effects of therapy.

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References


Pantoni L, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischemia. Stroke 1996; 27: 1641–7.


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