Time Is Penumbra: Imaging, Selection and Outcome

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Abstract
The foundation of modern therapy for ischaemic stroke involves reperfusion of the ischaemic penumbra and salvage of threatened but potentially viable brain tissue. Work on imaging of the penumbra and clinical trials using penumbral evaluation or selection have been a major focus of our collaborative work over several decades. We review the original description of the ischaemic penumbra, its measurement using a variety of imaging techniques, the duration of the penumbra and its potential salvage up to 48 h after stroke onset. The penumbra can now be accurately measured using automated thresholded techniques in real time with MRI or CT perfusion (CTP). Particular advances include more precise definitions of mismatch with validation of the measures for the ischaemic core and exclusion of benign oligaemia. While there has been greater trial experience with MRI perfusion/diffusion mismatch, CTP mismatch using a similar thresholded perfusion metric (T max 6 s) and relative blood flow (around 31%) to estimate the ischaemic core is generally more available and practicable in our experience. We review the completed clinical trials, which generally demonstrate the clinical benefits of acute reperfusion in penumbral patients, provided that large ischaemic cores are excluded. Our EPITHET trial was the first randomized controlled trial of tissue plasminogen activator (tPA) versus placebo at delayed times to test the concept of penumbral selection. We showed that in patients with a penumbra receiving thrombolysis, there was substantially increased reperfusion. Major reperfusion times were associated with reduced growth of the ischaemic core and improved clinical outcomes. Our current trial programme involves the application of penumbral imaging to attempt to extend the time window for intravenous tPA and treat wake-up strokes, to test the benefits of endovascular therapy in patients who have already received tPA but still have both substantial penumbra and an occluded vessel, and, finally, to use penumbral imaging to define a responder population in a phase III trial testing intravenous tenecteplase versus tPA within the current 4.5-hour time window. We believe that confirmation of these hypotheses will substantiate the role of multimodal imaging of the penumbra as a routine part of acute stroke management.

Part 1: The Penumbral Story

The term ‘ischaemic penumbra’ was almost certainly coined by Lindsay Symon and Anthony Strong in the early 1970s [1]. Lindsay Symon [1] describes, ‘at that time Anthony Strong was one of our research fellows and I recall going across Guildford Street from the lab to the dining room discussing this curious zone which we had documented. It seemed to me it was rather like the area around the centre of a candle flame, where there is a small bright zone known as the penumbra. We considered the correct name for this area was the ischaemic penumbra and so it proved. Astrup and Lassen pointed out that in a compete eclipse of the sun there was a bright zone around, which was also known as the penumbra. Whichever one likes to take as a scientific basis of the sobriquet is a matter of choice’. The need to describe the zone of electrical silence and the preservation of ultimate function around the infarct was based on their elegant work on baboons. It is now part of history that they demonstrated the thresholds of ischaemia as cerebral blood flow (CBF) was steadily reduced to around 40% of the original level to produce electrical dysfunction as measured by the then recently described somatosensory potentials. As CBF was reduced to around 30%, electrical failure became complete and at around 10%, release of potassium occurred as did subsequent cell death. Importantly, they were able to show restoration of electrical function with reperfusion, thus demonstrating the potentially salvageable nature of the electrically silent tissue [2].

The prediction that the demonstration of viable tissue in humans may eventually lead to therapies was made by the Chair of this Session, Jean-Claude Baron [3]. In 1980 and 1981, he described ‘misery perfusion’ using positron emission tomography (PET). Here, there was a mismatch between reduced CBF and the presence of a relatively preserved or even normal cerebral metabolic rate of oxygen consumption. This mismatch resulted in an increased oxygen extraction fraction, usually above the 30–40% seen in normal human brains up to a theoretical maximum of 100%. Others also confirmed these findings, showing that there was a transition from ischaemia to infarction and that the distribution of penumbral tissue was mainly in the cortex rather than the sub-cortex [4]. By 1993, Baron’s group had again published a landmark series of papers in patients with acute ischaemic stroke showing how various blood flow and metabolic patterns were associated with vastly differing outcomes [5, 6].
Definitions and Criteria

Over the years, there have been a number of definitions used to describe the ischaemic penumbra [1]. Most of the concepts are similar, although the techniques used to measure the various components vary. A summary of these definitions represented in brief is outlined in table 1. Mention should also be made here about the molecular penumbra proposed by Sharp et al. [7]. This is an important concept now well studied in animal models and may even have the potential to be imaged clinically. The molecular penumbras are outlined in table 2 together with their appropriate markers. They will not be discussed further in this paper because of space limitations.

The criteria for the ischaemic penumbra are important to establish so that the burgeoning number of imaging techniques used can be validated [8]. A useful set of criteria are shown in table 3, which were modified from those established earlier by Baron.

In vivo Imaging of the Ischaemic Penumbra

There have been remarkable developments in the in vivo imaging of the ischaemic penumbra since the original description of 'misery perfusion' mentioned earlier. From the time of the first images using $^{15}$O$_2$/$^{15}$O PET, more convenient methods of imaging have been introduced, a summary of which is shown in table 4. As can be seen, while the $^{15}$O$_2$/$^{15}$O PET remains the gold standard, it is expensive and impractical for standard clinical practice. Similarly, other PET techniques including CBF/$^{11}$C-flumazinal mismatch and $^{18}$F-fluoromisonidazole ($^{18}$FMISO) PET are not really practical for anything beyond relatively modest sample-sized clinical studies. However, the former provides critical information about neuronal integrity since flumazinal binding is almost ex-
clusively restricted to these cells and, therefore, provides unique data about, for example, selective neuronal loss [9]. \(^{18}\)FMISO PET has also proven to be a useful research tool given that it identifies tissue under hypoxic stress which is potentially salvageable [10, 11]. While it is a simpler one-agent PET technique, there is some evidence from animal studies that at least some of the FMISO may bind to the infarct core [12]. This would limit its everyday practical use.

Other methods are simpler, but each has its drawbacks. For example, MR using diffusion-weighted imaging (DWI)/perfusion imaging (PI) still has issues related to thresholding and the inability to use in patients with contraindications to MR, usually about 10% in most series [8]. MR may take up to 20 min or so, but this may be minimized by deploying a more limited MR approach, such as DWI/clinical mismatch [13]. This has some merit in that the clinical score replaces the perfusion measure based on the assumption that the latter volume represents functionally inert tissue and is responsible for the concurrent clinical deficit. Its main drawback is its relatively modest sensitivity and specificity for the presence of penumbral tissue.

We have become more comfortable with the use of CT perfusion (CTP) in everyday clinical trial use [14, 15]. One of the main reasons for this is that at our centres 24-hour access to this technique is quite common. Conversely, 24-hour access to research-based MRI is more difficult to come by. While this may vary from country to country, there is no doubt that CT is more readily available worldwide than MRI. Other advantages of CTP include its rapidity of use, only 5 min or so, in experienced hands. Also, if additional information is required about the collateral circulation for research purposes, the quality of these data is much greater than with MRI, although slightly longer acquisition times are required. One of the main concerns about the routine use of CTP in the emergency room setting of acute stroke assessment is the potential for intra-venous contrast to cause serious renal impairment. We are reassured by the data produced by our colleagues in Newcastle, N.S.W., Australia. Ang et al. [16] retrospectively studied 667 patients who had baseline serum creatinine levels early (<3 days) and later follow-up levels (day 4 or later). They looked for >25% increases in creatinine or development of contrast-induced nephropathy. None of the patients developed symptomatic renal disease or required dialysis. Only 2.8% developed transient rises in creatinine and there were no cases of subsequent severe renal impairment. We agree with their conclusion that CTP can, therefore, be used safely in routine emergency care settings as long as sensible care is taken to exclude severe renal impairment based on history and examination, and maintenance of hydration during and after the procedure.

### Important Questions about the Ischaemic Penumbra

#### Does Tissue Reperfusion Improve Clinical Outcome?

While it does seem a fundamental question, this was not addressed in vivo in patients with stroke until more recently. Using single-photon emission tomography, we [17, 18] showed in patients with stroke within 48 h that there was a clear relationship between perfusion and favourable outcome. Barber et al. [19] used MR DWI/PI techniques to also make the important distinction between nutritional and non-nutritional flow. This reaffirmed that therapies and other interventions, which will be discussed later in this paper, should be directed toward vessel recanalization and tissue reperfusion [20]. Interestingly, Marchal et al. [5] were able to demonstrate that ar-

### Table 4. Imaging of the ischaemic penumbra: techniques and issues related to each

<table>
<thead>
<tr>
<th>Technique</th>
<th>Penumbra</th>
<th>Problems</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{15})O(_2)/(^{15})O(_2) PET</td>
<td>OEF</td>
<td>Poor resolution</td>
<td>Original method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technically difficult</td>
<td>Physiological parameters</td>
</tr>
<tr>
<td>(^{11})C CBF Flumazinal &amp; (^{15})O(_2) PET</td>
<td>Mismatch between neuronal integrity and CBF</td>
<td>Grey matter only</td>
<td>Neuronal marker</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technically difficult</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not validated, indirect</td>
<td></td>
</tr>
<tr>
<td>CT perfusion</td>
<td>Mismatch between core (CBV or relative CBF) and perfusion</td>
<td>Thresholds</td>
<td>Simple, accessible</td>
</tr>
<tr>
<td>MR DWI/PWI</td>
<td>Mismatch</td>
<td>Thresholds uncertain</td>
<td>Technically simple</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indirect</td>
<td></td>
</tr>
<tr>
<td>(^{18})FMISO PET</td>
<td>FMISO uptake</td>
<td>Limited access</td>
<td>Simple, direct</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No perfusion measure</td>
<td></td>
</tr>
</tbody>
</table>
eas of hyperperfusion seen on PET were associated with poorer outcomes, a phenomenon which was also noted using the xenon-133 technique [5, 21].

What Is the Duration of the Ischaemic Penumbra?
This has been one of the most important questions about the penumbra since its original description. Obviously, this has huge implications concerning potential treatment windows for therapy after stroke onset. There is reasonable consensus based on evidence using a number of in vivo imaging techniques to suggest that in some patients penumbral remnants may be present at least as late as 24 h and probably 48 h after stroke onset. For example, Darby et al. [22] reported that the proportion of all patients with DWI/PI mismatch declined steadily from the time of stroke onset from around 100% to about 50% at 24 h. Heiss et al. [23] used PET to show that mismatch tissue was present at about 48 h. Similarly, we were able to prove that significant volumes of 18FMISO imaged by PET were still present at about the same time [24].

Does Salvage of the Imaged Ischaemic Penumbra Continue to Improve Clinical Outcome at Later Time Epochs?
This is also a question of fundamental importance. Clearly, even though we may be able to demonstrate the presence of an apparent penumbral pattern using a variety of imaging techniques, it does not necessarily follow that one of the most important components of the definition of the ischaemic penumbra, viz: tissue salvage improves outcome, still holds. Indeed, as mentioned earlier, to fulfill the definition of penumbra according to our criteria, tissue salvage must be demonstrated. There is no doubt that penumbral salvage during the earlier time epochs does occur, definitely up to 4.5 h and probably up to 6 h based on post hoc analyses of the EPITHET (Echoplanar Imaging Thrombolytic Evaluation Trial) and DEFUSE (Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution) data sets [25–27].

Using 18FMISO PET, we were able to demonstrate that tissue salvage occurred fairly consistently at about 50% of penumbral volume up to 48 h after stroke [24]. Even more importantly, we were able to show that tissue salvage correlated with a better clinical outcome for both the first 12-hour epoch and for tissue salvaged between 12 and 48 h after stroke. Similarly, using MR DWI/PI mismatch, we were able to evidence how mismatch salvage remained an independent factor for a better clinical outcome up to 48 h, albeit at modest levels [28]. Another important demonstration of the efficacy of late tissue salvage comes from the DEFUSE 2 study, which showed that vessel recanalization resulted in good clinical outcomes in both the less than and greater than 6-hour epochs [29].

Does the Ischaemic Penumbra Fragment with Time?
Most classical visual descriptions of the ischaemic penumbra show a central core of infarcted tissue surrounded by an evenly distributed halo of penumbral tissue. Salvaged proportions of the infarct tissue were associated with improved clinical outcomes. However, it seems unlikely that this idealized concept of the penumbral topography continues to exist with time, and the close relationship between the penumbra and the infarct core may become more complex. If, for example, the penumbral tissue was located in a less eloquent part of the brain and the infarct core dominated the relationship to outcome, salvage of the penumbra would be less helpful. We have shown that the classical penumbral pattern breaks down with time when careful co-registration techniques are used. In a cohort of 76 patients with acute ischaemic stroke, the proportion with the intact classical pattern fell from 100% in the first 6 h after stroke onset to only 24% in the 24- to 48-hour epoch [30]. If late tissue salvage with interventions does become a reality, the issue of penumbral fragmentation and location will need to be understood more thoroughly.

Penumbral and Infarct Core Thresholds
The establishment of the thresholds at the benign oligoemia/penumbra and penumbra/infarct core is complicated by the dynamic nature of the ischaemic process and the different modalities used to image these elements. Generally, it seems that the evolution from ischaemia to infarction is a slower process in humans than in animals. An excellent summary of thresholds in various species is given by Touzani and Baron [31]. Another complicating factor is that these thresholds are often averaged over both the grey and white matter compartments of the brain. We and others have shown that white matter is more resistant to ischaemia than grey matter and, ideally, should be compartmentalized separately [32, 33].

For the more commonly used MR and CT imaging techniques, some general comments about thresholds can be made. First, in the selection of patients for clinical trials, thresholding for the benign oligoemia/penumbral interface is absolutely critical. During fairly short time windows used for most clinical trial recruitment, while much of the image-identified penumbral tissue is more likely to be salvageable, the potential also exists for the presence of large volumes of intact benign oligoaic tis-
The problem may be exacerbated when slightly longer time windows are used and patients are erroneously recruited with little or no salvageable tissue and only benign oligaemia. This may have confounded the results of a number of recent clinical trials with image-based inclusion criteria such as DIAS (Desmoteplase in Acute Ischemic Stroke Trial). From the EPITHET database, we have established that the haemodynamic parameter $T_{\text{max}}$ is the most useful for this threshold with an absolute 6-second delay [34]. This can be built-in automated imaging software programmes such as RAPID, which we have found to be the most useful programme of its type [35].

The penumbral/infarct interface can be either identified by MR DWI using absolute apparent diffusion coefficient values or CTP. For the former, particularly when visual thresholds were used, it was found that portions of the DWI appeared to be reversible. While this concept has its merits, we have shown that the proportion of tissue involved in this process is quite small when careful elimination of CSF and other artefacts is undertaken [36]. One of the advantages of CTP is the automatic co-registration between core and penumbra based on common spatially acquired haemodynamic parameters. While investigators had suggested earlier that absolute cerebral blood volume (CBV) values $<2.0 \text{ g/100 ml}$ was consistent with infarct core, we have demonstrated that relative CBF may be a more reliable predictor of infarct core: the optimal threshold was $<31\%$ of the mean contralateral hemisphere [37].

Enormous advances have been made in our understanding of the ischaemic penumbra and multimodal imaging to evaluate its presence is here to stay. These techniques are likely to become even simpler and more informative in the coming years. Regardless, we feel that this is becoming an essential part of the selection of patients for therapeutic intervention, the subject of the next section.

### Part 2: Using Penumbral Imaging in Trials for the Selection of Thrombolytic Therapy

The single licensed drug therapy for acute ischaemic stroke, intravenous tissue plasminogen activator (tPA) [38, 39], represents a notable example of successful

### Table 5. Trials of thrombolysis with penumbral imaging

<table>
<thead>
<tr>
<th>Trial/investigators</th>
<th>Therapy</th>
<th>Time window, h</th>
<th>Study design</th>
<th>Patients, n</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPITHET/Donnan and Davis [8]</td>
<td>tPA</td>
<td>3–6</td>
<td>Randomized controlled trial of tPA vs. placebo Patients treated without reference to MRI findings</td>
<td>101</td>
<td>Infarct growth Reperfusion in mismatch patients</td>
<td>Strong trends to limitation of growth Increased reperfusion with tPA</td>
</tr>
<tr>
<td>DEFUSE/Albers et al. [47]</td>
<td>tPA</td>
<td>3–6</td>
<td>Open-label study All patients received tPA</td>
<td>74</td>
<td>Reperfusion on PWI Clinical outcomes</td>
<td>Early reperfusion in mismatch associated with good clinical outcome</td>
</tr>
<tr>
<td>DIAS/Hacke et al. [59, 76]</td>
<td>Desmoteplase</td>
<td>3–9</td>
<td>Dose escalation trial Mismatch used for selection</td>
<td>102</td>
<td>Reperfusion on PWI</td>
<td>Identification of a safe, effective dose that enhances reperfusion and improves outcomes</td>
</tr>
<tr>
<td>DEDAS/Furlan et al. [60]</td>
<td>Desmoteplase</td>
<td>3–9</td>
<td>Dose escalation trial</td>
<td>37</td>
<td>Reperfusion on PWI</td>
<td>Similar results to DIAS</td>
</tr>
<tr>
<td>DIAS-2/Hacke et al. [59]</td>
<td>Desmoteplase</td>
<td>3–9</td>
<td>Phase III randomized, double-blind trial Patients selected with penumbra using MR and CT techniques</td>
<td>186</td>
<td>Clinical improvement score</td>
<td>Negative study Increased mortality in the 125-μg/kg group due to non-neurological causes</td>
</tr>
<tr>
<td>DEFUSE-2/Albers et al. [68]</td>
<td>IA therapy (some patients had initial IV tPA)</td>
<td>&lt;12</td>
<td>Open-label study without MRI selection Refined MRI parameters</td>
<td>138</td>
<td>Reperfusion Clinical gains</td>
<td>Favourable clinical outcomes with IA therapy in mismatch Benefits extended to 12 h</td>
</tr>
<tr>
<td>Tenecteplase/Parsons et al. [42, 43]</td>
<td>Tenecteplase vs. tPA</td>
<td>0–6</td>
<td>PROBE design Patients selected with dual target of CTP mismatch and large vessel occlusion</td>
<td>75</td>
<td>Co-primary 24-hour reperfusion and change in NIHSS</td>
<td>Superiority of tenecteplase selected with mismatch, including 90-day functional outcome</td>
</tr>
<tr>
<td>MR RESCUE/Kidwell et al. [64]</td>
<td>IA clot retrieval with MERCI or penumbra devices</td>
<td>8</td>
<td>MRI not used in selection but pre-specified hypotheses</td>
<td>120</td>
<td>90-day functional outcome</td>
<td>No benefits with clot retrieval in penumbral or non-penumbral groups</td>
</tr>
</tbody>
</table>

IA = Intra-arterial; IV = intravenous.
translation from the original preclinical model mentioned earlier and described by Symon [1] and others over 30 years ago [2]. Thrombolysis is based on the principle of arterial recanalization and rapid reperfusion of the ischaemic penumbra. However, current clinical guidelines use similar eligibility criteria to the initial National Institute of Neurological Disorders and Stroke trial in 1995, imaging with non-contrast CT to exclude patients with haemorrhage, non-vascular pathologies and, more controversially, major early ischaemic changes, with a time window of 4.5 h.

We have argued that the penumbra is still present in a substantial proportion of patients at delayed time windows beyond 4.5 h. This will include many patients with ‘wake-up stroke’ and patients with unknown time of onset [22, 28]. Perfusion/diffusion mismatch with MRI or CTP as signatures of the penumbra, therefore, is appealing in the potential selection of therapy in such patients, as well as in those who have a poor treatment response to intravenous tPA and candidates for endovascular therapy (table 5). We found that, in the absence of reperfusion, there is typically expansion of the DWI lesion into the hypoperfused boundary in the mismatch region [19, 40]. Mismatch typically (but not always) reflects the presence of large artery occlusion [41].

As previously mentioned, use of mismatch in patient selection for delayed interventions is being increasingly applied with CTP-based technology. Some thrombolytic trials suggest that the use of CTP in patient selection can identify treatment responders [42, 43].

**Proof of Principle – The EPITHET Trial**

Our early work led to the hypothesis that patients with MR mismatch would respond to thrombolytic therapy at delayed times [40]. We then planned the EPITHET trial [44] based on our pilot studies suggesting that the benefits of tPA related to the presence of MRI mismatch [45]. We conducted a randomized, controlled phase II trial in which 101 acute stroke patients were randomized to tPA versus placebo in the 3- to 6-hour time window; patients were imaged with multimodal MRI before treatment, and 3–5 and 90 days after stroke onset. When we planned EPITHET, 3 h was the clinically defined time window for the use of intravenous tPA (table 5). The patients were not selected according to the presence of mismatch and the primary hypothesis was that infarct growth would be attenuated by tPA in patients with mismatch due to increased reperfusion. Conversely, we hypothesized that failure to reperfuse the penumbra would lead to major growth of the ischaemic core and a poor neurological outcome (fig. 1). We used a perfusion parameter of $T_{\text{max}} \geq 2$ s and mismatch of at least 20% (ratio 1.2) as representing significant mismatch.

This mismatch signature was seen in 86% of the patients in EPITHET. There was a strong trend towards attenuation of infarct volumes with tPA using a suite of growth definitions ($p$ values ranging between 0.24 and 0.054, depending on various pre-specified definitions of infarct growth) and a highly significant increase in reperfusion [44]. Further post hoc analyses of infarct growth in EPITHET with image co-registration showed a positive result using baseline PWI/DWI co-registration (for example geometric mean ratio 0.58, CI 0.33–0.99, $p < 0.05$) [26]. Reperfusion and infarct growth were strongly correlated with clinical outcomes, further supporting the biological validity of these biomarkers [44].
The EPITHET trial was not powered for a clinical outcome with a sample size of only 101 patients, but did show a non-significant 15% absolute risk reduction for those achieving a modified Rankin Scale score of 0, 1 at 90 days in the mismatch group treated with tPA. We were, therefore, able to power our current phase III EXTEND (Extending Time for Thrombolysis in Emergency Neurological Deficits) trial for a 2-sided $\alpha$ at 0.05 and power of 90%, with 200 patients in each arm [46].

**The DEFUSE Trial and Collaboration between Stanford and Melbourne**

The EPITHET trial was designed in liaison with our Stanford colleagues, led by Greg Albers, who were conducting the DEFUSE trial, an open-label intravenous tPA trial (without controls) in 74 patients with ischaemic stroke treated 3–6 h after stroke onset, repeating the imaging protocol within 24 h [47]. As in EPITHET, the patients were not selected based on the MRI patterns, but there was a pre-specified hypothesis that MRI mismatch profiles would predict treatment responses with reperfusion. Similar to EPITHET, mismatch was defined as a 20% difference between PWI and DWI volumes; PWI calculated using a $T_{\text{max}} \geq 2$-second threshold. A 'target population’ was defined, namely those with mismatch but without the ‘malignant profile’, who would best respond to intravenous tPA at this delayed time. The malignant profile was defined as a baseline DWI and/or PWI lesion $\geq 100$ ml, with a $T_{\text{max}} \geq 8$ s. These patients with large ischaemic cores were hypothesized to be at high risk of symptomatic intracerebral haemorrhage (sICH; fig. 2). Mismatch was identified in 54% of patients and those with mismatch and early reperfusion (particularly the target mismatch) had favourable clinical outcome. Patients without mismatch did not benefit from early reperfusion. Patients with a malignant profile had a substantially increased risk of sICH.

**Further EPITHET Analyses and Pooling of the EPITHET and DEFUSE Cohorts**

We prospectively planned to pool our DEFUSE and EPITHET data sets to further evaluate our hypotheses.
Two major findings included the validation of the DWI lesion as an accurate representation of the ischaemic core and a more stringent definition of the perfusion lesion, using thresholding to exclude benign oligaemia, namely regions of mild hypoperfusion not at risk of infarction. Although examples of DWI reversal had been identified using intra-arterial therapy, these changes are often transient [48]. The EPITHET analysis and pooled EPITHET-DEFUSE trials showed that the DWI lesion is rarely reversible, certainly when intraveneous thrombolysis is used more than 3 h after stroke onset, and is therefore a valid assessment of the lesion core in most cases. Adjustment for possible reversal in fact rarely alters the penumbral (PWI > DWI) mismatch signature for possible reversal in fact rarely alters the penumbral (PWI > DWI) mismatch signature [49, 50]. Thresholding provides a more accurate measure of the more severely hypoperfused tissue at risk in the ischaemic penumbra. Several lines of research indicated that thresholding in the range of 5–6 s (with T_max) is a reliable approximation of this boundary [34, 51].

The Stanford group had originally proposed that patients with large ischaemic cores or large regions of severe hypoperfusion would not benefit from thrombolysis. Further analysis of the EPITHET results indicated that patients rarely responded if they had an initial DWI lesion >25 ml, while those >70 ml were at high risk of haemorrhagic transformation [52]. Using MRI, very intense hypoperfusion measured by very low CBV was shown to be an even better predictor of sICH than either DWI or PWI baseline volumes [53]. Neither EPITHET nor DEFUSE were adequately powered to test the mismatch hypothesis, but the results of these trials suggested that an enriched population focusing on target mismatch patients would select responders to delayed thrombolysis.

The clinical utility of using MRI mismatch has been greatly enhanced by the advent of automated techniques. We showed that ‘eyeball assessment’ of mismatch at the MRI console is inaccurate [54]. A number of these software automations are now in use. The RAPID methodology allows rapid online estimation of thresholded PWI, DWI and PWI/DWI mismatch, and similar mismatch with CTP techniques (fig. 3) [55]. An analysis of the pooled data from EPITHET and DEFUSE using the automated RAPID methodology [55] showed that the benefits of reperfusion were restricted to those with target mismatch. Neither those without mismatch nor those with the malignant profile benefitted from reperfusion [56]. These and other studies have shown a lack of growth in non-mismatch patients [40]. Patients with benign oligaemia could be better excluded by the use of T_max 6 s rather than the T_max 2 s used in EPITHET and DEFUSE.

Patients with large proximal occlusions, such as carotid T occlusion, rarely respond to intravenous tPA, generating the hypothesis that this group should ideally be triaged for clot retrieval.

These studies helped to validate MRI mismatch as a useful clinical signature of the ischaemic penumbra. For example, measurement of the DWI volume at 24 h and 3 days [57, 58] correlated well with the final infarct volume at 90 days, the traditional time for the clinical endpoint in stroke trials. Reperfusion correlated well with clinical gains and inversely with infarct growth.

**Desmoteplase Trials**

The newer intravenous fibrinolytic strategies (desmoteplase/tenecteplase) have been based on the evidence that these agents are more fibrin specific and might be more potent recanalizing agents, with a lower degree of haemorrhagic transformation, particularly when selected with advanced neuroimaging. The desmoteplase trials were the first to use penumbral imaging to select patients for inclusion. In the initial DIAS and DEDAS (Dose Escalation of Desmoteplase for Acute Ischemic Stroke) trials, intravenous desmoteplase 90 or 125 μg/kg in the 3- to 9-hour window was associated with increased reperfusion and potential clinical benefits, although these were small phase IIa trials [59, 60].

A subsequent small phase III trial of 186 patients treated 3–9 h after stroke onset, DIAS-2, using placebo and desmoteplase doses of either 90 or 120 μg/kg [59], was negative. There was an unexpected increase in mortality in the higher-dose (120 μg/kg) group, although the majority of these deaths were late and not associated with sICH. Issues with the trial included a milder patient population, which was selected with ‘on-console’ estimations of MRI and CT mismatch by the investigator, without standardized processing, thresholding of perfusion or automation. A relatively low proportion of the patients had vessel occlusion. Arterial occlusion appeared a better determinant of treatment response than mismatch in this trial and the subsequent DIAS-3 and -4 trials of intravenous desmoteplase are using this parameter in patient selection (NCT00790920 and NCT00856661).

**Meta-Analysis of Mismatch-Based Delayed Thrombolysis**

A meta-analysis based on the use of mismatch imaging in the DIAS, DEDAS, DIAS-2, DEFUSE and EPITHET trials showed that delayed thrombolysis in patients selected according to mismatch resulted in increased re-
canalization and reperfusion, which in turn were associated with improved clinical outcomes. However, delayed thrombolysis in mismatch patients was not directly confirmed to improve clinical outcome [61]. Given the relatively small sample size, validation of the mismatch selection paradigm required a phase III trial. With the exception of DIAS-2 [and more recently MR RESCUE (Mechanical Retrieval and Recanalization of Stroke Clots using Embolectomy)], these trials supported the physiological basis of the selection of patients with mismatch. More recently, we reported the benefits of tPA beyond 4.5 h in limiting infarct growth with improved outcomes using the EPITHET data [62].

**Fig. 3.** A 69-year-old patient with CTP with mismatch, successful reperfusion after tPA and endovascular clot retrieval 2 h after onset of large right MCA syndrome (left hemiparesis, hemianopia, inattention, NIHSS 17). a Non-contrast CT showing subtle loss of grey-white differentiation in the striatum. CT perfusion showing reduced CBF in the striatum (b) and delayed T_max throughout the right MCA territory (c). d CT angiogram confirming distal right M1 MCA occlusion. MRI 24 h after thrombolysis and thrombectomy showing no growth in the diffusion lesion (e) due to full reperfusion shown on T_max (NIHSS 1; f).

**EXTEND – Phase III Trial Using Mismatch to Select Patients for Delayed Intravenous tPA**

Our EXTEND trial is a phase III randomized trial of 200 patients testing tPA versus placebo in the 4.5- to 9-hour time window and in those with wake-up stroke [46]. The design was based on the EPITHET trial and subsequent refinements in mismatch definition. These include use of a $T_{\text{max}} > 6$-second threshold (vs. $T_{\text{max}} \geq 2$ s in EPITHET), excluding patients with baseline DWI $\geq 70$ ml. The mismatch ratio of $>1.2$ and absolute mismatch volume $>10$ ml remain unchanged. A key difference is that patients are prospectively selected on the basis of MRI or CT mismatch using the RAPID system. The EXTEND tri-
al is successfully recruiting in Australia, New Zealand and Taiwan. The patients are being predominantly selected using CTP rather than MRI, with similar thresholds for core and penumbra. A similar trial has commenced in Europe, ECASS4-EXTEND (Principal Investigator: Hacke, 2012), and we plan to conduct a pooled analysis of the results to enable a sample size of at least 400 patients.

**Penumbral Imaging in the Selection of Patients for Endovascular Clot Retrieval**

Despite some recent negative results [63–65], delayed intervention with clot retrieval remains an attractive strategy given the strong correlations between recanalization, reperfusion and favourable clinical outcome. The early trials used the MERCI device with more recent studies using the more effective stentriever devices [66]. The Solitaire stentriever has a recanalization rate of over 80% in registries and was shown to be superior to the MERCI device in a randomized controlled trial with a clinical endpoint [67].

The DEFUSE 2 trial from our Stanford colleagues explored MRI predictors of therapeutic response to delayed intervention using various intra-arterial therapies in an open-label design [68]. Patients with the target mismatch pattern with reperfusion were shown to be the clinical responders. There were better clinical outcomes with higher degrees of reperfusion. Patients without target mismatch did not benefit and those with the malignant profile exhibited worse outcomes with reperfusion.

However, negative results were shown by two other endovascular trials. The IMS3 (Interventional Management of Stroke Trial 3) compared standard-dose intravenous tPA versus a lower dose (0.6 vs. 0.3 mg/kg) followed by angiography and clot retrieval [63]. However, advanced imaging was not employed in patient selection and patients with large infarcts may have received hazardous and futile reperfusion. The MR RESCUE trial [64] enrolled 120 patients, performing MRI before clot retrieval (MERCI or penumbra) or standard care. This trial was negative and without benefits for endovascular therapy in either penumbral or non-penumbral groups. However, there was a strong correlation between reperfusion and clinical outcomes, which was also seen in IMS3. Criticisms of both trials have included the low rates of effective recanalization with the older devices employed and treatment of patients with large ischaemic cores.

These earlier trials helped us to design our EXTEND-IA trial, a phase II design in 100 patients treated with standard sub-4.5-hour tPA based on evidence of mismatch on CTP or MRI and an occluded vessel, in which patients were randomized (1:1) to clot retrieval with the Solitaire FR device or current standard of care [69]. Our hypothesis is that careful patient selection using a triple target (occluded vessel, sizeable penumbra and small core) with early delivery of therapy with an optimal device is most likely to produce a positive result (fig. 4). This trial is being conducted in 15 Australian and New Zealand centres. We are using a co-primary endpoint of reperfusion at 24 h and favourable neurological response [improvement of ≥8 points on the National Institutes of Health Stroke (NIHSS) Scale or recovery to a modified Rankin Scale score of 0, 1] at 3 days.

**Using Penumbral Imaging to Enrich Trial Populations for New Thrombolytic Strategies**

Penumbral imaging is being used to identify responder populations to new thrombolytic strategies. Our Newcastle colleagues led a phase II randomized trial comparing the standard dose of intravenous tPA with 2 different doses of tenecteplase within 6 h of the onset of ischaemic
stroke. The methodology used CTP and CT angiography to select patients most likely to respond to early reperfusion. Inclusion criteria were large vessel occlusion and a large perfusion lesion in the absence of a large infarct core [43]. The pooled tenecteplase groups had greater reperfusion and clinical improvement at 24 h than the tPA group. The higher dose of tenecteplase was superior to the lower dose and to tPA for all efficacy outcomes, including a good functional outcome at 90 days.

This trial has informed the design of the current phase III TASTE (Tenecteplase vs. Alteplase for Acute Ischemic Stroke) trial (ACTRN12613000243718). This trial of 1,024 patients involves a head-to-head comparison of intravenous tenecteplase 0.25 mg/kg versus tPA 0.9 mg/kg in patients with either MRI or CTP penumbral mismatch within the current 4.5-hour time window.

Conclusions

A large body of evidence has indicated that MR and CTP mismatch provide a valid representation of the ischaemic penumbra and that penumbral imaging can be utilized in real time to select patients for clinical trials of reperfusion therapies. There is a strong correlation between reperfusion in mismatch patients and clinical benefits provided that patients with large ischaemic cores are excluded. Our current trials are utilizing these penumbral selection techniques to extend the time window for intravenous tPA to 9 h and to test the benefits of endovascular therapy in patients who have already received intravenous tPA but have a penumbra and occluded vessel and to identify an enriched population for the testing of intravenous tenecteplase versus intravenous tPA. Confirmation of our hypotheses will validate the original prediction that penumbral imaging will become a routine part of acute stroke therapy.

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Multimodal Imaging of the Stroke Penumbra


