Perfusion imaging in acute and evolving brain ischemia

By
Andrew Bivard BSc (Hons)

Submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy

Submitted
10 July 2012
Declarations

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Andrew Bivard

5 July 2012
ACKNOWLEDGEMENT OF AUTHORSHIP

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Andrew Bivard

5 July 2012
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Current medical practice is multidisciplinary simply because no single person can possess the vast variety of skills required to comprehensively treat a patient. Therefore, any research involving patients utilises a wide range of skill sets from many different people. For this thesis to be completed I would like to thank the radiographers and stroke nurses at the John Hunter Hospital for their input and effort. Without their patient care and record keeping, the research in this thesis would not have been possible.

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Statement of Contributions of Others

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7. Soren Christensen; Bill O'Brien; Bijoy Menon; Andrew Bivard; Bruce Campbell; Patricia Desmond; Stephen Davis; Mark Parsons. Mapping of Cerebral
Vascular Territories Using Whole Brain Perfusion CT Imaging: A New Method
*Stroke.* 2012; 43: A54

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1) Threshold selection
2) CT perfusion software – lack of standardisation
3) MR vs. CT for core and penumbra detection
4) Delay in passage of contrast to the ischemic region

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# Abbreviations

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<tr>
<td>AIF</td>
<td>Arterial Input Function</td>
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<tr>
<td>AIS</td>
<td>Acute Ischemic Stroke</td>
</tr>
<tr>
<td>ASL</td>
<td>Arterial Spin Labeling</td>
</tr>
<tr>
<td>BCD</td>
<td>Block Circulant Deconvolution</td>
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<tr>
<td>CBF</td>
<td>Cerebral Blood Flow</td>
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<tr>
<td>CBV</td>
<td>Cerebral Blood Volume</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<td>CTA</td>
<td>Computed Tomography Angiography</td>
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<td>CTP</td>
<td>Computed Tomography Perfusion</td>
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<tr>
<td>ddSVD</td>
<td>Delay and dispersion corrected Single Value Deconvolution</td>
</tr>
<tr>
<td>DT</td>
<td>Delay Time</td>
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<tr>
<td>DWI</td>
<td>Diffusion Weighted Imaging</td>
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<tr>
<td>EPI</td>
<td>Echo Plainer Imaging</td>
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<tr>
<td>FT</td>
<td>Fourier Transform</td>
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<tr>
<td>ICH</td>
<td>Intracranial Haemorrhage</td>
</tr>
<tr>
<td>INR</td>
<td>International normalized ratio</td>
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<tr>
<td>IRF</td>
<td>Input residue function</td>
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<tr>
<td>MRA</td>
<td>Magnetic Resonance Angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MTT</td>
<td>Mean Transit Time</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PWI</td>
<td>Perfusion weighted imaging</td>
</tr>
<tr>
<td>rTPA</td>
<td>Recombinant tissue plasminogen activator</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SVD</td>
<td>Single value deconvolution</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient ischemic attack</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to peak</td>
</tr>
<tr>
<td>VOF</td>
<td>Venous out flow</td>
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Thesis Abstract

**Background:** Established acute stroke treatment protocols require further investigation to identify patients who are most likely to respond to treatment. The aim of hyper-acute ischemic stroke treatment is to salvage hypoperfused tissue that would infarct soon (penumbra), thus preserving brain tissue and allowing better functional recovery of an individual patient. Penumbral salvage is achieved by removal of a cerebrovascular occlusion in the cerebral circulatory system through the use of intravenous thrombolytic therapy (iv rtPA), or mechanical intra-arterial thrombus retrieval. However, the current clinical guidelines for the treatment of ischemic stroke totally fail to measure the volume of the treatable penumbra. This thesis aims to provide the technical ability to measure the acute penumbra and infarct core, using readily available clinical imaging techniques. Furthermore, this thesis also aims to provide the clinical relevance of measures of the acute infarct core and penumbra, when compared to current treatment guidelines.

**Objectives:** This thesis investigated clinically accessible perfusion imaging techniques, such as Computed Tomography Perfusion, as well as Magnetic resonance perfusion weighted imaging and arterial spin labelling, for their utility in acute ischemic stroke. The specific aims of this thesis were:

1) Determine a method by which to investigate perfusion imaging as compared to current gold standard measurements of tissue pathophysiology.

2) Investigate the threshold cut offs to determine the acute critical tissue pathophysiology measurements of the acute penumbra and infarct core.

3) Optimise the measures of the acute penumbra and infarct core.
4) Standardise the measure of the acute penumbra and infarct core, or failing standardisation, determine the optimal thresholds for the acute infarct core and penumbra for all software post processing algorithms available.

5) Determine the clinical importance of measures of the acute penumbra and infarct core.

6) Finally, compare various perfusion techniques to one another to determine cross compatibility of different measures.

Methods: A cohort of 320 acute ischemic stroke patients who were admitted to the John Hunter hospital were enrolled in the studies for this PhD. These patients underwent an acute CTP with a 24 hour follow-up MR sequence. Sixty seven patients also underwent an additional acute MR, with perfusion and diffusion imaging. Clinical assessments were performed on all patients acutely, at 24 hours and at 90 days post stroke by a certified neurologist or neurology registrar. Clinical assessments included the National Institutes of Health Stroke Scale (NIHSS), and a modified Rankin Score.

All perfusion images underwent post processing using MiStar, a commercially available software package. MiStar generates the perfusion maps of cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT) and time to peak.

Once imaging was processed and ready for analysis, a broad range of statistical tests were used. Statistical tests included summery statistics such as linear regression, or specific statistical methods such as a receiver operator characteristic curve analysis.

Results: Acute CTP imaging was able to readily identify the volumes of the acute infarct core and penumbra. Analysis of different post processing algorithms revealed
there were obvious themes for detecting the acute tissue pathophysiology. A Time To Peak measures (or its variants of Tmax and Delay Time) were always optimal to define the acute perfusion lesion, and a CBF measure was optimal to define the acute infarct core. However, each post processing algorithm used, required a different threshold to define the acute tissue pathophysiology. Additionally, by defining the acute infarct core within the acute perfusion lesion, through restricting the volume of the infarct core, a greater level of accuracy was always achieved.

Next, the MR sequence, Arterial Spin Labelling was the only perfusion technique that is clinically available, that was able to show hyperperfusion. Hyperperfusion at 24 hours was associated with reperfusion and penumbra salvage. Therefore if a patient showed hyperperfusion in their stroke region (previously hypoperfused tissue), they ultimately had a much better clinical outcome compared to patients that did not reperfuse or hyper-perfuse.

**Conclusions:** This thesis demonstrated that it was possible to define acute ischemic tissue pathophysiology using CTP. Moreover, it was shown that measures of the acute penumbra and infarct core were directly related to clinical outcome, and likelihood of treatment success. The threshold measures of the acute infarct core and penumbra provided by this thesis can be applied to all acute clinical CTP scanning platforms in order to provide treatment relevant information. This underlines the importance of perfusion imaging in the acute clinical setting to guide treatment based decision making.
Chapter One

Acute Stroke and Imaging

Aims

The aim of this introductory chapter is to establish the concepts relevant to this thesis, including acute ischemic stroke, clinical assessments of stroke and acute neuro-imaging. A detailed explanation of the practical, technical and clinical aspects of acute stroke and imaging will be discussed as well as the rationale behind this PhDs projects.

Stroke

Stroke is the second most common cause of death in the developed world, and the leading cause of adult disability. For example, in 2006 stroke cost the Australian health system an estimated 2.14 billion dollars affecting some 50,000 people\(^1\). It has been projected that the total number of strokes per year worldwide may rise 60% within the next two decades as the proportion of elderly in the population increases\(^2\).

Of strokes in Western countries, 85% are ischemic and 15% are haemorrhagic, with a higher proportion of haemorrhage in Asia. An ischemic stroke occurs when the blood supply in or to the brain is impaired, typically due to a blood clot (figure 1.1). The cells that are solely supplied by the occluded blood vessel die within a few minutes, due to necrosis, in an area termed the infarct core. Surrounding the infarct core is a larger area of brain that is hypoperfused but does not infarct rapidly because it is receiving blood flow from unaffected collateral vessels, and is termed the
ischemic penumbra. The penumbra can be saved from infarction if it is reperfused promptly by unblocking the occluded vessel, thereby increasing blood flow to the penumbral area above the perfusion threshold for imminent cellular death. However, if the occlusion persists, the penumbra progressively dies, becoming incorporated within an expanding infarct core. Rescuing the ischemic penumbra from infarction is the primary target of acute stroke therapies, such as thrombolysis, and clot retrieval techniques\textsuperscript{3}. Currently, restoring blood flow is the only proven clinical method of rescuing the penumbra from infarction.

**Figure 1.1. Stroke.** This figure demonstrates the difference between an ischemic stroke and a haemorrhagic stroke. An ischemic stroke is caused by a blockage in a blood vessel in the brain, the grey area is the region affected by the resulting drop in regional blood flow. In a haemorrhagic stroke a blood vessel bursts, causing blood to leak into the brain.

**Treatment for acute ischemic stroke**

Apart from care in a Stroke Unit\textsuperscript{4}, there are three proven ‘therapies’ for acute ischemic stroke:

(i) Aspirin

(ii) Hemicraniectomy

(iii) Thrombolysis
Aspirin has a modest effect in reducing death and disability, most likely via prevention of stroke recurrence\textsuperscript{5,6,7}. Decompressive hemicraniectomy is a life-saving (but possibly not disability-preventing) procedure limited only to a rare subgroup of younger stroke patients with massive hemispheric infarction\textsuperscript{8}. Intravenous thrombolysis with the fibrinolytic agent, recombinant tissue plasminogen activator (rtPA, or alteplase), has one of the most powerful treatment effects in medicine, with only 7 patients needing to be treated within 3 hours of stroke onset to achieve a virtual ‘cure’ (no/minimal disability at 3 months)\textsuperscript{9,10}. Presumably, this powerful treatment effect relates to salvage of the ischemic penumbra from progression to infarction, with less consequent disability. However, the seminal study proving benefit was done in the era preceding advanced brain imaging techniques, and a three hour treatment time window after stroke onset was used as a surrogate for the presence and extent of penumbral tissue\textsuperscript{9}. Indeed, in later time windows, again without direct assessment of penumbra to select patients, there may even be a tendency towards harm with thrombolytic treatment\textsuperscript{11}. However, the recent ECASS III trial\textsuperscript{12} produced evidence justifying the extension of the time window for treatment with IV rtPA to four and a half hours after stroke onset. The treatment benefit in the group arriving to hospital 3 to 4.5 hours after symptom onset was reduced, resulting in a greater number of patients needed to be treated in order to observe any statistical benefit. The powerful effect of time since symptom onset has been investigated in a pooled analysis of patients from the ECASS, ATLANTIS, NINDS and EPITHET trials\textsuperscript{13} (figure 1.2). The combined analysis did not observe any interaction with time and haemorrhagic transformation but mortality was significantly associated with time to treatment. The reduction in the benefit from thrombotic administration in the later time window likely represents a reduction in the total volume of penumbra left to salvage and larger
infarct cores at the time of admission. The powerful penumbral salvage effect seen with early intravenous thrombolysis has also led to enthusiasm for endovascular approaches\textsuperscript{14,15}, as well as for other fibrinolytic agents to salvage penumbra due to the high rate of recanalisation with a presumed increase in penumbra salvage and improved outcome\textsuperscript{16,17}.

![Graph showing relation of onset to treatment delay with treatment effect.](image)

Figure 1.2. Relation of onset to treatment delay with treatment effect. Chance of a patient having an outcome modified Rankin score 0-1 compared to 2-6 depending on time since symptom onset to treatment (OTT). The clear trend shows that the earlier a patient gets treatment, the better they perform under current clinical guidelines.

An alternative therapeutic approach in acute ischemic stroke is to ‘preserve’ the ischemic penumbra through ‘neuroprotection’. Many animal studies have demonstrated that the penumbra can be, at least temporarily, prevented from progressing to infarction by various therapeutic agents targeted at blocking the injurious biochemical cascade that leads to cell death in the penumbra\textsuperscript{18}. Since 1957, some 1026 neuroprotective agents have been identified in animal studies but none have translated into positive human trials\textsuperscript{19}. The reasons for these failures are complex, but may relate to the ultimate need to reperfuse and salvage penumbral
tissue to prevent infarction. A scientific committee was formed following a run of negative neuroprotection trails in 1999 and was called the Stroke Therapy Academic Industry Roundtable (STAIR) which recommended streamlining and standardisation of animal experiments to facilitate their translation into human trials\textsuperscript{20}. The STAIR recommendations include: the fundamentals of good scientific inquiry should be followed by eliminating randomization and assessment bias, a priori defining inclusion/exclusion criteria, performing appropriate power and sample size calculations, and disclosing potential conflicts of interest. Neuroprotection may be helpful in preserving the penumbra and extending the time window for which reperfusion therapies can salvage the penumbra but the practical barriers that prevent patients presenting to hospital within 4.5 hours also hinder the time specific administration of neuroprotective agents.

Survival of the penumbra can vary from less than 3 hours to 48 hours from patient to patient. As a general rule, however, 90\% of patients with supratentorial large artery occlusion (typically middle cerebral artery) have a clinically significant volume of penumbral tissue at 3 hours after stroke onset\textsuperscript{11,12}. By 6 hours, 75-80\% of patients still have some penumbral tissue\textsuperscript{11,12}. Thus, at least 25\% of patients in the 3-6 hour window are unlikely to benefit from thrombolysis if the standard clinical criteria are applied, almost certainly because a significant proportion of their penumbral tissue has been converted to infarct core\textsuperscript{7}. Indeed, it was recently demonstrated in ischemic stroke patients admitted to hospital within 3-6 hours, the size of the infarct core was the strongest predictor of outcome\textsuperscript{21}, regardless of the extent of residual penumbra. Further, the volume of the infarct core varied considerably from patient to patient despite similar times after stroke onset\textsuperscript{12}. Therefore, if the volume of the acute
penumbra and infarct core can be determined acutely, this could serve as a ‘tissue clock’ aimed at treating patients with significant penumbra volumes and excluding patients with large infarct cores or no penumbral tissue. A tissue clock where the extent of both irreversible damage (infarct core) and reversible ischemia (penumbra) is determined, rather than a rigid time window for thrombolytic treatment, may seem a more rational guide to patient selection for thrombolysis. A tissue clock could also make a major difference to clinical practice when the time of stroke onset is unknown or unclear. This is a very common situation, for example, when the patient was alone at the time of stroke onset and is unable to provide a history, or in the setting of ‘wakeup’ stroke. Although such patients may actually have a favourable tissue clock, they are currently excluded from thrombolytic treatment due to the ‘uncertainty’ of onset time. A practical and accessible method of imaging the infarct core and penumbra could thus have a major impact on the use of current and investigational therapies for acute stroke. This PhD will undertake projects that will produce a tissue clock in the time window of 0-6 hours after ischemic onset, which can then be trialed to patients to see if there is indeed an increased benefit.

The Ischemic Penumbra: the ‘Holy Grail’ of acute stroke therapy

Jean-Claude Baron and Marie-Germaine Bousser\textsuperscript{22} described the limited blood flow in an ischemic region as ‘misery perfusion’, as it resulted in electrical silence but preservation of ion homeostasis. Later, Astrup, Siesjso and Symon\textsuperscript{23} experimentally described the concept of different perfusion thresholds in the brain for the preservation of cellular function, impending cellular death (penumbra), and necrotic cell death (infarct core). The concept of the penumbra followed on from this, with the observation that prompt restoration of blood flow to a hypoperfused area allowed
threatened tissue to be salvaged. Therefore penumbral tissue was defined as critically hypoperfused tissue destined for infarction, unless normal perfusion could be restored promptly.

There are differences in the cause of cellular death between the infarct core (necrosis) and penumbra (apoptosis). In the infarct core necrosis occurs within a few minutes of vessel occlusion. Necrosis is a form of traumatic cell death that results from acute cellular injury caused by external factors, such as hypoxia in ischemic stroke. However in the penumbra, there are multiple cell death mechanisms that are activated: excitotoxicity and ionic imbalance, oxidative stress, and apoptotic-like cell death may all occur and progress more slowly than necrosis.

In the infarct core there is a severe, sudden drop in blood supply that a cell cannot withstand for more than a few minutes. However, blood flow in the penumbra is higher than in the infarct core, and as such tissue can survive for longer. Additionally, there is also tissue adjacent to the penumbra that has only a mild reduction in perfusion (benign oligaeemia) and is not at risk of progressing to infarction should perfusion remain at that level. The difference between these three areas (benign oligaeemia/penumbra/core) is that the level of perfusion has dropped below the respective specific thresholds (Table 1.1). The effect of reduced perfusion is well documented from studies using Positron Emission Tomography (PET).
<table>
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<td>&lt; 55 benign oligaemia</td>
<td>Decline in protein synthesis</td>
</tr>
<tr>
<td>&lt; 35</td>
<td>Halt in protein synthesis / Increase in glucose consumption</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>Decline in glucose consumption</td>
</tr>
<tr>
<td>&lt; 20 Penumbra</td>
<td>Excitotoxicity</td>
</tr>
<tr>
<td>&lt; 15</td>
<td>Electrical Failure (resulting in neurological deficits)</td>
</tr>
<tr>
<td>&lt; 11 Infarct core</td>
<td>Loss of cellular ion homeostasis</td>
</tr>
<tr>
<td>&lt; 8</td>
<td>Irreversible cell death</td>
</tr>
</tbody>
</table>

Table 1.1. A summary of the effect of progressively reducing CBF on a cell in the brain

Using the more practical (than PET) and commonly used CT or MR perfusion imaging techniques, it has been very difficult to separate the benign oligaemic region from the penumbra. This means that measurements of penumbra often overestimate the penumbra size as they include some benign oligaemic tissue (not destined for infarction even without reperfusion). Lastly, the rate of expansion of the penumbra to infarct is influenced by other physiological events such as hyperglycaemia, temperature, age and blood pressure. These co-morbidities influence perfusion and/or metabolism, and since they are risk factors for stroke, are commonly abnormal in stroke patients.

**Effect of time since stroke onset upon the penumbra**

The minimum perfusion threshold for infarction has been found to rise depending on the time since ischemia onset as ATP stockpiles are utilised to temporarily compensate for hypoperfusion. Cerebral Blood Flow (CBF) of 13 mL/100g/min for 30 minutes, 23 mL/100g/min for 6 hours or 32 mL/100g/min for 12 hours can all cause irreversible cellular death. This is a result of the threshold for the loss of cellular function increasing with the duration after stroke onset resulting in the affected cells no longer being able to withstand hypoperfusion. Every ischemic stroke patient on arrival to the emergency room differs in the severity and duration of
cerebral hypoperfusion, meaning that a method of assessing tissue pathophysiology that can take into account severity of hypoperfusion would be ideal in determining suitability for penumbral salvage therapy. This makes treating every patient exactly the same a very flawed approach, as each patient has a different level of risk associated with them. By assessing a patient’s individual stroke, it is possible to tailor a treatment specific to them in order to increase benefit and reduce the risk of a negative outcome. Stroke specific imaging has the potential to generate a tissue clock to guide an acute stroke patient’s treatment.

**The role of acute stroke imaging**

Acute stroke imaging can be used to confirm the diagnosis, identify the site of vessel occlusion, the extent of the perfusion deficit, the age of the lesion and define the tissue pathophysiology. Initial clinical evaluations are not an accurate predicator of these features and cannot differentiate stroke sub types and imitators. Therefore acute cerebral imaging is a requirement in the management of all stroke, or potential stroke patients, although each imaging technique has its own strengths and weaknesses.

*Non-contrast Computed Tomography (NCCT)*

Computed Tomography is a quick, economical and readily available imaging technique that is used extensively in medicine. This imaging technique is used to investigate potential ischemic stroke patients and differentiate them from haemorrhagic stroke and stroke mimics such as a tumour. NCCT cannot differentiate a transient ischemic attack from an acute stroke, nor can it positively identify an acute ischemic stroke within the current treatment window where a patient would benefit
from treatment. The sensitivity of NCCT for ischemic stroke is only 58% in patients imaged within 5 hours\textsuperscript{29}. However NCCT is currently the workhorse of acute stroke imaging, as the treatment protocol relies on exclusion of stroke imitators or haemorrhage for treatment with thrombolitics.

The early NCCT changes indicative of an ischemic stroke are parenchymal hypodensity and focal swelling\textsuperscript{30}. Parenchymal hypodensity is seen as a reduction in normal tissue density, often appreciated as a loss of the usual definition between grey and white matter. This reflects accumulation of water (oedema) in intracellular and extracellular areas of the ischemic region and probably reflects irreversible injury, this is the most common stroke sign, and some hypodensity is often visible to an expert’s eye. Focal swelling is less commonly observed than hypodensity and is seen as a flattening out of cortical sulci, asymmetry between Sylvain fissures and compression of the ventricles. This has traditionally also been assumed to reflect oedema but, in the first few hours after stroke onset, it has been shown that the pathophysiology may in fact represent increased blood volume, and that these regions might not be irreversibly ischemic\textsuperscript{31}. However, the problem is that in most patients there are very subtle or no changes on non-contrast CT up to six hours post ischemic stroke onset, as evolving infarction signs like tissue hypodensity, become more pronounced after 12-24 hours, which is ironically when reperfusion therapy is unlikely to be of benefit\textsuperscript{36}. Thus, NCCT cannot reliably differentiate infarct core from penumbra (or penumbra from normal brain), and as such yields very little pathophysiologic information related to reperfusion treatment suitability.
A systematic method of assessing early ischemic change on NCCT, such as the Alberta Stroke Program Early CT Score (ASPECTS), may help improve the accuracy of acute assessment. However the overall inter-observer variability is high for early the changes on NCCT. Due to the larger area that is supplied, middle cerebral artery (MCA) or internal carotid artery occlusions usually have the most widespread changes on NCCT. However changes in the anterior cerebral artery or posterior cerebral artery territory can be harder to spot on NCCT.

The limitations of NCCT mean that treatment decisions in acute stroke are based on a negative diagnosis, rather than a positive identification of salvageable tissue, or already infarcted tissue whose fate cannot be altered (Figure 1.3). Furthermore, without accurate measurement of the infarct core volume before treatment, clinicians are further limited in their ability to predict response to thrombolytic treatment. It is also important to remember that the risk of cerebral haemorrhage as a complication of thrombolysis is 10 times greater with stroke than for myocardial infarction. The lack of a positive diagnosis, combined with fears about brain haemorrhage, contributes to physician uncertainty about patient suitability for thrombolytic treatment, and at least partially explains the very low rates of thrombolytic treatment for stroke around the world.

*CT Angiography*

Computed Tomography Angiography with IV contrast (CTA) provides information about cerebro-vascular anatomy and is highly accurate in detecting intracranial proximal large vessel occlusion, as well as extra-cranial occlusion or
stenosis. The presence of a vessel occlusion is obviously a target for thrombolytic therapy, however, occlusions distal to the M1 segment of the middle cerebral artery may not be reliably seen on CTA (or MR angiography)\textsuperscript{35}. Expert neuro-radiologists can at times identify more distal occlusions, although this often depends on accurate clinical information to allow detailed examination of a specific branch of the MCA. However, CT perfusion (or MR perfusion) may be more sensitive for these more distal occlusions, as the topography of a perfusion lesion in the territory of the vessel may be suggestive of a smaller distal occlusion beyond the resolution of the CTA\textsuperscript{37}.

Computed Tomography Angiography may also provide information on tissue status. Hypodensity on the source images used for CTA may be more accurate than hypodensity on NCCT at identifying the infarct core. Thus, the combination of NCCT and CTA is a superior imaging regime rather than CTA alone\textsuperscript{36}. However the combination of NCCT and CTA is not as accurate as CTP at identifying the volume of the infarct core and penumbra\textsuperscript{37}, as shown in Figure 1.4. However, CTA does provide some supplementary information. It has been recently shown that the presence of good collateral flow seen on CTA, distal to an occlusion correlates not only with a smaller infarct core but also predicts less infarct growth and better clinical outcomes in response to successful reperfusion\textsuperscript{38}. Thus it appears good collateral status is a marker of ‘penumbral life expectancy’. As reperfusion generally occurs some time after the initial imaging, good collaterals probably ensure penumbral viability until reperfusion (usually with thrombolytic treatment) occurs.
Figure 1.3 A comparison between acute NCCT, and CTP in acute ischemic stroke. A patient imaged 2 hours after an acute stroke. The acute NCCT shows relatively minor early ischemic change in the left MCA region (caudate, lentiform, insular and M2). The acute NCCT substantially underestimates the very large infarct core seen on CTP (red map), coinciding with extensive areas of CBV and CBF reduction. The patient was treated with IV thrombolysis but suffered major, fatal haemorrhage into the large infarct core that is apparent on the 24 hour CT.

Figure 1.4. A comparison between an acute CTP CBF map, CTA source map and an acute NCCT in a patient with a persistent right MCA occlusion. The CTP CBF map clearly delineates the extent of hypoperfusion in the right hemisphere. The CTA source image shows hypodensity in the right MCA hemisphere but its extent is poorly delineated compared to the CBF map. Similarly, the NCCT shows hypodensity in the right MCA region but clearly the CTP map provides the most information about the size of the stroke and the severity of hypoperfusion.
Magnetic Resonance Imaging (MRI)

MRI is the examination of choice for most neurological conditions. Reports on the promise of MRI in selection of stroke patients for thrombolysis have been considerable. The strengths of MRI are, high soft tissue resolution, high sensitivity to tissue oedema and easy re-orientation of images, MRI does not use radiation like CT and MRI has a higher sensitivity to cerebral and brain stem ischemia than CT. However, there are some practical disadvantages of MRI. An MRI scan can take up to 40 minutes, delaying the initiation of acute reperfusion therapy. Secondly, 10-15% patients cannot have an MRI due to the presence of pacemakers or other metallic objects, claustrophobia or an inability to lie still, which is commonly seen in strokes where the patients’ cognitive ability is compromised. Moreover, MRI scanners are more costly and availability is limited worldwide.

$T1$ and $T2$ imaging

The human body is largely composed of water molecules that have two hydrogen nuclei or protons. In MRI, a patient is placed inside a strong magnetic field, causing the magnetic dipole moments of the protons to align longitudinally to the vector of the magnetic field. Normally these magnetic dipoles are randomly aligned so that their net nuclear magnetism is zero. During a scanning sequence, a radio frequency transmitter is turned on to produce an electromagnetic field. The hydrogen nuclei (protons) of interests ($^{1}\text{H}$) can then be made to rotate about the axis of the magnetic field. The energy of the electromagnetic field that is required to selectively rotate the aligned protons is called the resonance frequency. After the radio transmitter is turned off, the rotated protons decay back to the original alignment of the magnetic field, which produces signal that the MRI receiver coils can detect. In
total there are five different tissue variables; spin density, longitudinal relaxation time $T_1$, transverse relaxation time $T_2$, perfusion and spectral shifts can be used to construct images.

The earliest morphological changes that are detectable are mostly due to oedema with shortening of cortical sculi and distortions of the ventricular areas due to brain volume changes and retention of cerebral blood volume, both of which are best viewed with $T_1$ imaging. Changes on $T_2$ imaging are a result of the development of vasogenic oedema which develops after the acute stroke phase around 6 to 8 hours after onset.

**Diffusion Weighted Magnetic Resonance Imaging**

In diffusion-weighted imaging (DWI), a pair of strong pulse gradient fields are applied to select and manipulate rapidly moving (diffusing) water (hydrogen) molecules. The first diffusion gradient de-phases the ‘spin’ of water molecules, and the second pulse re-phases their spin, and records the net movement that has occurred between the pulses. If the water molecules have been moving freely (diffusing) between the two gradients they lose signal, whereas areas of higher signal indicate restricted diffusion, as seen in Figure 1.5. Restricted diffusion is thought to occur within minutes of onset of ischemia due to cytotoxic oedema\textsuperscript{39,40} or astrocyte swelling. Cytotoxic oedema is a consequence of dysfunction of cellular metabolism\textsuperscript{41} resulting in the failure of sodium and potassium pumps causing retention of sodium and water. This is an earlier pathophysiologic process than vasogenic oedema, which occurs as a result of a breakdown of the blood brain barrier, causing a build-up of
intravascular proteins and fluid in the cerebral parenchymal extracellular space. T2-weighted MRI is a conventional MR sequence that is able to detect vasogenic oedema but not cytotoxic oedema or astrocyte swelling, so like NCCT, T2-weighted MRI is often normal in the first 6 hours after stroke onset. DWI is considered the ‘gold-standard’ method to image the acute infarct core in clinical practice (figure 1.5)\textsuperscript{42}. Indeed, recent guidelines indicate that DWI should be the preferred modality to NCCT for the diagnosis of acute ischemic stroke within 12 hours of symptom onset, and that DWI should be performed for the most accurate diagnosis of acute ischemic stroke.

Figure 1.5. MRI scans that are used clinically to diagnose ischemic stroke. The above patient has had a large ischemic stroke of the right MCA. The DWI shows a large area of infarction, and the PWI shows the area of hypoperfused tissue. The regions of hypoperfused tissue and DWI infarction overlap, suggesting there is no penumbra to salvage. The T2 GRE is used to identify tissue breakdown, which is suggestive of a future haemorrhage. This patients T2 GRE is clear and they are unlikely to haemorrhage. The MRA shows the location of the vessel occlusion.
Measurements of perfusion

In contrast enhanced imaging, a tracer bolus is administered and travels in or with the blood throughout the brain. The passage of this tracer is then monitored and processed to produce perfusion maps. Typically, a mathematical process called deconvolution is used to quantify perfusion values with image processing requiring the selection of reference arteries, commonly the internal carotid or middle cerebral artery. Perfusion scanning techniques can be divided into diffusible or non diffusible measures of perfusion, based on the type of tracer used.

Diffusible Tracers

Perfusion measurements using diffusible tracers apply the Fick principle, or the law of conservation of material. When applied to perfusion scanning, the principle implies that the difference between the arterial input and the venous output is the amount of tracer left in the tissue. The exact utilisation of this theory depends on the scanning modality used as well as the type of tracer. With the exception of ASL, scanning with diffusible tracers is very difficult in an acute stroke patient, and as such is only done for scientific purposes rather than for medical investigations.

Nitrous Oxide

Nitrous Oxide is inhaled and rapidly exchanged between the brain and venous blood. Therefore measurements of arterial input and venous output for the concentration of Nitrous Oxide yield perfusion measurements. However this contrast requires blood sampling and only yields information on the total perfusion in the brain rather than for individual regions such as the middle cerebral artery region.
Xenon

Gamma cameras are used to monitor the concentration of this radioactive tracer inside the brain tissue which is delivered through intra-arterial injection, intravenous injection or inhalation. The use of this technique has limited spatial resolution, especially for the deep tissues of the brain.

Computed Tomography is able to image the concentration of xenon because it appears hyper-dense on scanning. As such the concentration of xenon can be measured and quantified to calculate perfusion maps. The advantage of using CT is that it can image the deep tissue of the brain. A potential weakness of using xenon is that it can affect the consciousness and the cerebral blood flow of a patient because it is a mild anaesthetic, meaning that measures using this technique may underestimate or influence perfusion measures. Overall Xenon CT is more sensitive for acute ischemia but access is very restricted due to limited availability of the tracer.

Single Photon Emission Tomography (SPECT)

SPECT uses a tracer such as technetium, iodine or Xenon to measure the spatial presence of these gamma emitting substances. SPECT is most often combined with stress testing, such as the inhalation of CO2 or with drugs such as acetazolamide to monitor changes in blood flow. This is mainly because SPECT has poor spatial resolution and limited quantification ability of the tracer.
**Positron Emission Tomography (PET)**

In PET, an injected or inhaled labelled compound emits a positron during its decay. This positron will collide with an electron soon after its emission, emitting two gamma rays that travel in opposite directions. These gamma rays are detected by scintillation crystals which convert the gamma rays into photons which are received as signal. When a photon is received on both sides of a patient, they are interpreted as signal. 3D scans of good resolution are possible using PET because the direction the gamma rays travel are opposed, which allows for correction of scatter or interference because they can be traced back to one another.

PET can measure Cerebral Blood Flow, Oxygen extraction fraction and Cerebral Blood Volume, making PET a measure of all physiological parameters of microvasculature. However due to the tracer used in PET, its availability is limited to proximity of a cyclotron, which makes the tracer.

**Arterial Spin Labelling (ASL)**

ASL uses similar principles to PET, but the contrast agent used is the proton spins on water in blood. Labelling is performed by inversion or saturation of the flowing proton spins, which are recorded as they diffuse into the brain vasculature and tissue. The labelled image is then subtracted from a baseline image without labelling. The decay of the spinning proton is shorter than that of the tracers used in PET (around 2 second in a 1.5 T scanner and 6 seconds in a 3T MRI), and as such quantification is difficult. ASL is discussed in greater detail in chapters two and seven.
Non diffusible tracers

For non diffusible tracers the indicator dilution theory is applied. This method measures the first passage of an intravascular tracer to calculate perfusion maps. The concept is that each junction of the vascular tree divides the flow of the contrast according to the flow in each vessel. As the tracer does not diffuse into the surrounding tissue, the amount of tracer in a vessel is proportional to the flow in that vessel. The use of scanning techniques employing non diffusible tracers are much more common in the clinical setting, and as such are the focus of clinical research and this thesis.

In CT, an intravascular iodinated contrast agent is injected and produces changes in the raw CT measure of intensity, (Hounsfield unit) proportional to concentration over time. Therefore, the tracking of contrast movement over time can be used to generate perfusion maps. In MRI contrast perfusion scanning, a paramagnetic tracer is used that reduces the transverse relaxation time of the surrounding tissue, which can be quantified and processed to produce perfusion maps. Quantification of contrast volume is straightforward in CT as the relationship between tracer volume and image signal is linear and the tracer movement is limited to the blood vessels. However in MRI the signal enhancement is not linear with concentration of tracer and noise is generated in the surrounding tissue. This makes the generation of MRI perfusion maps more complicated and possibly dependent on local tissue properties.

The changes in contrast concentration during a tracer bolus produce a detectable change that is relative to the volume of tracer. These changes in signal
intensity reflect contrast volume and are recorded over multiple scans that record signal intensity. The changes in signal intensity are recorded on a concentration time curve which can be used in conjunction with the indicator dilution theory to determine the perfusion values by a process called deconvolution. This is explained in greater detail in chapter two.

**Perfusion MRI**

There are two basic perfusion MR techniques, susceptibility (contrast) based methods and arterial spin labelling techniques. Susceptibility based methods (perfusion weighted imaging, PWI) are similar to CTP methods of perfusion mapping as it involves tracking the bolus of an injected contrast agent. A newer and less established method is arterial spin labelling (ASL), which turns the water in blood into a contrast agent by magnetically labelling flowing water protons in the vasculature supplying the brain. Currently, ASL is not widely used in clinical practice as the spatial resolution of the technique is suboptimal and the quantification of CBF remains problematic⁴⁶.

Perfusion weighted imaging also aims to generate a concentration time curve that can then be processed to create maps including CBF, CBV, TTP and MTT. PWI is more complicated than CTP as the contrast used relies on manipulation of the tissues’ magnetic properties rather than image intensity alone to measure changes in contrast concentration. The passage of paramagnetic contrast agents used in MR perfusion imaging alters the magnetic field of surrounding tissues and cause a reduction in signal intensity. As the contrast bolus exits the tissue, the magnetic field of the tissue returns to its natural resting state. The magnitude and duration of the
signal change is determined primarily by the volume of the contrast itself, although this relationship is not directly proportional.

Currently, the main advantage MRI has over CT is the ability to image infarct core with DWI, whereas CTP relies on a perfusion threshold for core as well as penumbra. Penumbral imaging with perfusion MRI follows similar physical principles as CTP. A ‘mismatch’ between the DWI lesion and the larger perfusion MR lesion is thought to be representative of the ischemic penumbra, but the same validation issues with perfusion imaging that relate to CTP also apply equally to perfusion MRI. Unfortunately the current clinical guidelines stop short at recommending perfusion and diffusion mismatch to select patients for thrombolytic therapy in routine clinical practice as high level evidence around the clinical benefit to measuring the acute infarct core and penumbra is lacking\textsuperscript{47}.

**Computed Tomography Perfusion**

An advance in CT imaging is CT perfusion (CTP), which is able to track the passage of an IV bolus of iodinated contrast agent through an organ. This is primarily achieved as new CT scanners have multiple detectors which are able to image a substantial volume of tissue rapidly, and repeatedly. The passage of the contrast over time is recorded via a time versus contrast concentration curve. From this curve, using the mathematical function of deconvolution, various measures of perfusion are calculated for each image pixel, and parametric maps of, Cerebral Blood Flow(CBF), Cerebral Blood Volume(CBV), Mean Transit Time(MTT), Time To Peak(TTP), and T\textsubscript{max} are generated (Figure 1.6).
Figure 1.6. A flow diagram for CTP processing. CTP imaging involves the injection of a contrast medium, and the tracking of the passage of this contrast over time. The contrast results in a change in the image intensity (or tissue density). These changes can then be plotted on a tissue contrast concentration vs time curve that is used to calculate the various perfusion maps (bottom row). This patient has a perfusion deficit in the left hemisphere, most apparent with delayed MTT (red/yellow) and TTP (red/yellow/green).

The maps that CTP generate can be used to identify the tissue pathophysiology critical to stroke treatment. Figure 1.7 demonstrates how the use of thresholds enables the identification of the penumbra and the infarct core. This is primarily achieved by combining thresholds to highlight the penumbra and infarct core – thus producing a ‘CTP mismatch’ between the penumbral and core thresholds. This concept may be an oversimplification as there is also the need to distinguish penumbra from ‘benign oligaemia’ as well as the need for a threshold to separate penumbra from core. These critical perfusion boundaries give a snap shot of the current tissue left to salvage through reperfusion therapy. Currently a mismatch between an MTT of 145% for the outer perfusion boundary and a CBV of <2mL/100g as the inner boundary are the
most commonly used, and are demonstrated in figure 1.7. Another method of tissue identification is to visually inspect the individual CTP maps for the depth, or severity of hypoperfusion and make a judgment call on the likely volume of the infarct core and penumbra. This method is somewhat unreliable as it is impossible to visually distinguish between the perfusion boundary lines mentioned above.
Figure 1.7. The CTP mismatch concept. The top row contains an acute and follow-up DWI in a patient with no reperfusion. This can be used to compare the definitions of the infarct core and penumbra in the lower perfusion CT scan maps. The acute DWI lesion is the infarct core and the 24 hour DWI lesion minus the acute DWI lesion is the penumbra. The bottom two rows show the CTP slice corresponding to the DWI with different thresholds used to describe tissue at risk of infarction (Green) and infarcted tissue (Red). The middle row shows the effects of using single thresholds to describe the penumbra (MTT >145%) and infarct core (CBV <2mL/100g) respectively. It is clear that both these thresholds overestimate the tissue they are trying to describe. The bottom row shows two different CTP mismatch definitions used to describe the penumbra and infarct core. The left CTP map combines the two thresholds used in the middle row onto one map. (penumbra threshold of MTT >145% and infarct core threshold <CBV 2mL/100g, as described by Wintermark et al[41]) This clearly increases the accuracy of the thresholds in describing the current status of the tissue but still significantly over-estimates core and misclassifies penumbral tissue. The right image on the bottom row shows two thresholds that are more accurate (i.e. mismatch between Tmax 2seconds for penumbra and a CBF <50% for the infarct core). The new penumbra and infarct core thresholds used for this map have been derived using deconvolution analysis with MIStar software (Melbourne, Australia)42.
Disadvantages of CTP include the additional radiation received by a patient (generally around double that of a non-contrast CT head), whilst this is not excessive with a single examination, this can be an issue if studies are repeated. Additionally, a small proportion of patients cannot receive the iodinated contrast material, either due to an allergy, or severe renal impairment. However, the risk of contrast nephropathy with IV contrast is extremely low, with previous literature giving artificially high rates due to inclusion of catheter-based angiography data\textsuperscript{51}. Indeed, the proportion of patients not suitable for CTP is much smaller than the proportion that are unsuitable for MRI, and, the safety screening to identify such patients is much faster for CTP\textsuperscript{52}. Therefore, many centres now use advanced or ‘multimodal’ CT to assess acute stroke patients (NCCT, then CTP, then CTA) as it provides information about tissue and vascular anatomy, and is usually more rapidly completed than a stroke MRI protocol\textsuperscript{38}.

CTP is a very promising and practical modality to image ischemic stroke pathophysiology. However, the perfusion thresholds by which infarct core and penumbral tissue defined by CTP are incompletely validated and are the focus of much investigation\textsuperscript{53}. Until recently, another potential limitation of CTP was that it could only cover a limited area of brain, as the number of slices and their thickness from a CTP acquisition depends upon the number of detectors and their width. For example, current generation 64 slice scanners can cover up to 80 mm of brain. Although most of the forebrain is covered, large perfusion lesions are not completely encompassed and small lesions, or posterior circulation lesions can be missed. This has now been circumvented by the latest generation CT scanners, which have up to 320 detectors and are capable of whole brain coverage. The poor evidence to date that
inhibits the generalisation of CTP into the acute ischemic stroke clinical setting is as a result of several key issues currently facing routine perfusion imaging.

**Key Issues to be solved with perfusion imaging before its routine use in clinical practice**

1) *Delay in passage of contrast to the ischemic region*

   Accurately quantifying the ‘true’ penumbra with perfusion imaging is hampered by the problem of ‘delay’ in arrival and dispersion of the contrast bolus to the ischemic region. This is a particular problem (but not isolated to) where there is a stenotic vessel supplying the ischemic region. It also occurs with collateral supply bypassing the blocked vessel to supply blood to the ischemic region via a more circuitous route. As a result, the arrival of blood (and hence contrast) is more spread out over time. However, the mathematical algorithm used to calculate perfusion measures (deconvolution) is modelled on a single ‘tight’ bolus over a limited time frame in order to accurately calculate the various perfusion maps (e.g. CBV, CBF, MTT and Tmax). Delay may significantly underestimate CBV and CBF, as such, the suspected size of both the infarct core and penumbra can be overestimated if one uses these perfusion measures to define core and penumbra as shown in Figure 1.8. Variations of the deconvolution algorithm are widely available in an attempt to correct for this problem\(^5^4\), although again more validation is needed.
Figure 1.8. The effect of delay and dispersion on CTP maps. A delay in the arrival of contrast can lead to an overestimation of the volume of a perfusion lesion. However the most important aspect of adding a delay correction technique is that it reduces the severity of a deficit within a perfusion lesion which would undoubtedly change the thresholds required to define acute tissue pathophysiology.

2) CT and MR perfusion software – lack of standardisation

Currently there are multiple manufacturers of stroke specific brain perfusion software that can generate the perfusion maps (CBV, CBF ect), however there are many variations on the mathematical models (mostly based upon singular value deconvolution and block circulant deconvolution) that are used to generate these maps. On a clinical level this lack of standardisation causes two important problems. Firstly, if a patient’s scan is processed with the different software, the severity, volume, and sometimes even location of perfusion lesions can change substantially. Secondly the application of penumbral selection trials across centres using different vendors’ CT and MR scanners (and hence perfusion software) is problematic. For example, if the CTP infarct core or MR/CTP penumbra has been found to correlate to a single perfusion threshold using one software package, there is very little likelihood that this threshold will translate across the software platforms. Variation in
manufacturer software is confusing for front line clinicians and frustrating for perfusion imaging researchers.

3) MR vs. CT for core and penumbra detection

Currently, the bulk of research has been carried out with MRI scanners and there is a lack of cross validation with CTP. The main focus of core detection with MRI is with DWI, as it is currently the diagnostic gold standard for acute infarct core detection. However the perfusion MR technique uses the same methods as CTP. This means that any criticism directed at CTP also applies to perfusion MR. Moreover, the currently used method of acute penumbra detection of DWI – PWI mismatch describes an area of hypoperfusion outside of the DWI infarct core as penumbra. However as described above, this perfusion lesion may also consist of a variable amount of benign oligaemia

A very important consideration when thinking about CTP vs perfusion MR for acute stroke assessment is the practical advantages of CT over MR in the emergency setting. CT scanners are a ubiquitous technology, generally much easier to access at short notice and require less pre-screening. Even advanced CT stroke protocols are much faster to complete. Although CT exposes the patient to ionising radiation, the caution over the use of iodinated CT contrast is probably unwarranted. MRI scanners on the other hand have substantial practical difficulties to overcome in the emergency setting. Firstly, patients need to be pre-screened for metallic objects (leading to delay), the length of scanning means that patient movement becomes problematic, and claustrophobia is also common, leading to patient movement and poor quality images.
4) Threshold selection.

There are a wealth of studies using MR-PWI and a few using CTP examining perfusion thresholds to define acute tissue pathophysiology. Currently, there is uncertainty over whether Tmax thresholds of 2, 4 or 6 seconds are the best to define the acute perfusion lesion (i.e. infarct core and penumbra combined). A study of only five acute stroke patients scanned using PWI and positron emission tomography found a Tmax of 4 seconds best correlated with the PET-defined penumbra, next Neumann-Haefelin and colleagues identified time to peak (TTP) delay of 4 and 6 seconds best correlated to the acute perfusion lesion in terms of tissue progressing to infarction. Most of these results have not been validated on an independent data set and the studies have limited patient numbers, generally ranging from 5-20 and there is clearly no agreement on a single threshold to define acute tissue pathophysiology using perfusion scanning.

For CTP, a penumbra threshold of MTT of 145% has been proposed to be the penumbra by Wintermark et al, however these results have not yet been validated. Clearly there is a trend that transit measures of TTP, Tmax or MTT are the best to identify the acute penumbra. It is even possible that the same thresholds for PWI and CTP could be used should the same post processing techniques be applied. However further study is required.

Conclusions

In summary, both MRI and multimodal CT have great potential for imaging tissue pathophysiology directly. Although DWI is now considered the diagnostic gold standard in hyper-acute ischemic stroke, neither MRI or multimodal CT have level 1 evidence for improving patient selection for stroke thrombolysis. There are a number
of problems that need to be solved with these penumbral imaging techniques before this is likely to occur.

This PhD thesis aims to increase the accuracy of tissue pathophysiology measures in hyper-acute ischemic stroke and relate the relevant measures of penumbra and infarct core to patient outcome. It is expected that by using a more stringent set of perfusion thresholds to define the acute stroke pathophysiology, it is possible to predict a patient’s outcome in the setting of penumbra salvage and non penumbra salvage. The ability to predict a patient’s outcome may alter the treatment decision a doctor makes. This in turn should reduce the negative effects of stroke treatment, such as haemorrhage following thrombolytic administration and increase the benefit of treatment to patients who might otherwise not be included in the current treatment protocol. Overall this PhD aims to increase the diagnostic accuracy around the selection of patients for treatment with thrombolytic therapy by implementing a specific perfusion scanning protocol.
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Chapter two:

Perfusion and Diffusion Theory and Modelling

Introduction:

In this chapter, the theoretical and mathematical processes that underlie perfusion imaging calculations will be explored. The aim of this chapter is to discuss the imaging techniques that form the core of this thesis.

Imaging types:

In acute stroke, perfusion imaging is becoming more relied upon in order to make treatment decisions. However, it is critical to access this information quickly, and ensure that it is as accurate as possible. Currently, perfusion weighted magnetic resonance imaging (PWI) has undergone the most study in the acute clinical setting. However computed perfusion tomography (CTP) has also started to be realised as a more practical and less time consuming perfusion scanning technique, although clinical applications of CTP are less thoroughly reported\(^1\). The general principles around the generation of measurements of CBF, CBV, MTT and TTP are the same for CTP as they are for PWI, therefore the clinical applications can be easily bridged\(^2\). The most important difference between CTP and PWI is the relationship between contrast concentration and signal attenuation, which in CTP, is a linear relationship and allows for quantitative measurements of CBF and CBV. In PWI the indirect T2* effect which is induced in the tissue by the gadolinium contrast agent is not linearly related to the contrast concentration which makes quantification of CBF and CBV difficult.
Perfusion Calculations

Serial imaging is used to monitor an intravenously administered contrast agent during its first circulation through the brain tissue capillary bed. This approach is known as dynamic first pass assumes that the tracer is not diffusible into the surrounding tissue through metabolism or absorption. In a brain that has sustained Blood Brain Barrier (BBB) damage such as in cases of inflammation, tumour growth or infarction, this assumption can be inaccurate as there is contrast leak into the extra-vascular space, resulting in over estimation of cerebral blood volume (CBV) calculations.3

Calculating CBV

Perfusion values are determined by comparing the arterial input and the venous outflow enhancements of contrast. Tracer kinetic theory of a non diffusible tracer states that if the input and the outflow of a contained system are known, the volume of the distribution and the clearance rate can be determined. In this case the volume distribution represents the fractional vascular volume or CBV and the clearance rate is the flow per unit of tissue volume of the Cerebral Blood Flow (CBF).4,5 The fractional vascular volume, \( f \), is defined by the following equation:

\[
  f = \frac{V_{\text{vasc}}}{V_{\text{vasc}} + V_{\text{interstitium}} + V_{\text{cells}}} = \frac{V_{\text{vasc}}}{V},
\]

\( V_{\text{vasc}}, V_{\text{interstitium}}, \) and \( V_{\text{cells}} \) are the volumes occupied by the vascular space, interstitium, and cells, respectively. Selection of the region of interest for the \( f \) is critical, as if there is no major blood vessels in the region, the measured changes as the contrast passing through the brain will reflect the tissue blood pool. The contrast
concentration in the tissue, $C_{\text{tissue}}$, is smaller than the intravascular concentration, $C_{\text{vasc}}$, by the fraction $f$.

$$C_{\text{tissue}} = f \cdot C_{\text{vasc}}.$$  

Using the conservation-of-mass principle, the total amount of contrast material delivered to the tissue must be equal to the amount leaving the tissue, that is, the product of CBF with the integral of $C_{\text{vasc}}(t)$. The total amount of contrast material delivered to the tissue via the arteries is the product of the CBF times the integral of the arterial concentration, $C_{\text{artery}}(t)$:

$$\text{CBF} \cdot \int_0^T C_{\text{vasc}}(t) \, dt = \text{CBF} \cdot \int_0^T C_{\text{artery}}(t) \, dt$$

By combining the above two equations we get:

$$f = \frac{\int_0^T C_{\text{tissue}}(t) \, dt}{\int_0^T C_{\text{artery}}(t) \, dt}.$$  

Using the above, and the brain attenuation, $p$, and a correction factor $CH$, CBV can be calculated. $CH$ is used to adjust for the difference between the arterial and capillary hematocrit. In 1970s in vivo experiments revealed a markedly lower hematocrit concentration in the capillaries compared to the arterial concentration. The hematocrit level is important because the tracer used does not penetrate into the
Red Blood Cells (RBC), and as such travels in the blood plasma. Therefore the plasma volume in capillaries and small vessels of the brain and other organs is larger than the plasma volume in large vessels of the body. With these new parameters, CBV can finally be quantified as follows:

\[
CBV = \frac{CH}{\rho} \cdot \frac{\int_0^T C_{\text{tissue}}(t) \, dt}{\int_0^T C_{\text{artery}}(t) \, dt}.
\]

**Calculating CBF**

There are two options available to calculate the CBF in CTP, using either deconvolution or non-deconvolution methods. Non deconvolution methods are simpler and less time consuming but make assumptions about the underlying vascular tree.

**Non Deconvolution methods**

Using the Fick principle of conservation of mass in an area of brain parenchyma, CBF can be calculated. This approach assumes that the CBF and the time integral of the difference between the arterio-venous contrast concentration is equal to the accumulated mass of the contrast \( Q(T) \) in a voxel of the brain during the complete inflow and outflow of the contrast bolus:

\[
Q(T) = CBF \cdot \int_0^T \left[ C_{\text{artery}}(t) - C_{\text{vein}}(t) \right] \, dt.
\]
One simplification of this equation is the Mullani-Gould formula\(^9\) which assumes that during the acquisition period that the venous concentration is zero. This only holds true for when the acquisition is <6 seconds, which is the transit time of blood through the whole brain:

\[
Q(T) = CBF \cdot \int_{0}^{T} C_{\text{artery}}(t) \, dt.
\]

This equation can again be rewritten and simplified into:

\[
\left[ \frac{dQ(t)}{dt} \right]_{t = T} = CBF \cdot C_{\text{artery}}(T),
\]

Also, if the rate of contrast accumulation will peak when the arterial concentration is maximal then another equation is used:

\[
\left[ \frac{dQ(t)}{dt} \right]_{\text{Max}} = CBF \cdot [C_{\text{artery}}(t)]_{\text{Max}}.
\]

Using the above equation, CBF is the ratio of the maximum slope of \(Q(t)\) to the maximum arterial concentration. This is known as the “maximum slope method” and is the simplest means of calculating CBF. However the maximum slope method requires a very high rate of contrast injection, typically a minimum of 10 mL/s in order to satisfy the assumption of no venous outflow\(^{10,11}\). Overall this methods assumption of no venous outflow is an over simplification of the complex hemodynamics of the brain and only yields relative rather than absolute values. This leads to large inter patient variation in results as each patient has a different baseline perfusion level.
Deconvolution based models

Using a deconvolution based model to calculate perfusion maps in CT, concentration of a bolus of contrast material in a tissue voxel of interest ($C_{\text{tissue}}(t)$) can be defined in terms of 2 functions:

1) AIF, $C_{\text{artery}}(t)$: The concentration of the contrast in a feeding vessel at time $t$.

2) Residue function, $R(t)$: the remainder of the contrast left in a voxel after a bolus injection at time $t$. $R(t)$ is unit less and is equal to 1 at $t=0$.

Using Deconvolution to calculate CBF requires measurement of the temporal shape of the arterial input curve and the tissue – time - attenuation curves. Ideally, a measurement of the input for each voxel in the brain would be taken and processed individually. However, in practice, a single arterial input function (AIF) is selected from a major artery such as the Middle Cerebral Artery (MCA). This AIF is then assumed to represent the exact input for every voxel in the brain. This method has some weaknesses, as each vessel in the brain may have a different blood flow or may receive blood sooner or later than the selected artery (Delay), or alternatively may receive blood over a longer period of time (dispersion). Delay or dispersion estimation errors in the underlying assumptions for using an AIF during the passage of the contrast through the brain will induce errors of over or underestimation of CBF depending on the severity, extent and direction of the delay or dispersion.

The primary measurement taken in perfusion scanning, the tissue – time attenuation curve is the sum of the AIF and the baseline tissue properties that are detected by the scanner. Once this curve is generated, the AIF needs to be removed through the process of deconvolution to derive $R(t)$. This function is now dependent
only on the hemodynamic properties of the individual voxel being calculated and shows a quick, tight rise, a plateau equal to the minimum transit time through the tissue of interest and then a slow decay towards baseline. Meier and Zieler\textsuperscript{16} showed that the tissue concentration curve can be represented as CBF multiplied by AIF convolved with \( R(t) \):

\[
C_{\text{tissue}}(t) = \frac{\rho}{CH} \cdot CBF \cdot [\text{AIF}(t) \otimes R(t)]
\]

\[
= \frac{\rho}{CH} \cdot CBF \cdot \int_{\tau}^{t} \text{AIF}(\tau) R(t - \tau) d\tau,
\]

Where \( \otimes \) is the convolution operator, \( \rho \) is the brain tissue attenuation, and \( CH \) is the correction factor for the capillary hematocrit levels. \( C_{\text{tissue}} \) and AIF are measured directly from the time-attenuation curve from the cine CTP source images. Now a method of calculating \( R(t) \) and CBF is required to complete the function through deconvolution. There are two broad methods to derive \( R(t) \) and CBF through deconvolution. Parametric models use a method that is able to assume the value of \( R(t) \), however this method has many pitfalls as the assumption is very weak due to individual physiological differences between patients which affect the shape of the \( R(t) \) curve\textsuperscript{17,18}. Alternatively, a non-parametric approach can be taken that does not assume the value of \( R(t) \) and can be done in one of two ways.

\textit{Transform Approach}

In this approach, the convolution theorem of the Fourier transform (FT) is used to deconvolve the above equation. This approach assumes that the FT of the convolution of two time domain functions is equivalent to the multiplication of their respective FTs\textsuperscript{19,20} and the convolution equation is rewritten as:
\[
R(t) = \frac{1}{\text{CBF}} \cdot \mathcal{F}^{-1}\left\{ \frac{\mathcal{F}[C_{tissue}(t)]}{\mathcal{F}[AIF(t)]} \right\},
\]

where \(\mathcal{F}\) is the Fourier transform. \(R(t)\) and CBF can now be determined as the inverse FT of the ratios of the 2 transforms from the sampled AIF\((t)\) and \(C_{tissue}(t)\).

**Single Value Deconvolution (SVD)**

This is the most common approach and is an algebraic reformation of the convolution integrals from the convolution equation and is written as:

\[
\Delta_t \begin{pmatrix}
AIF(t_1) & 0 & \cdots & 0 \\
AIF(t_2) & AIF(t_1) & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
AIF(t_N) & AIF(t_{N-1}) & \cdots & AIF(t_1)
\end{pmatrix}
\begin{pmatrix}
R(t_1) \\
R(t_2) \\
\vdots \\
R(t_N)
\end{pmatrix} = \begin{pmatrix}
C_{tissue}(t_1) \\
C_{tissue}(t_2) \\
\vdots \\
C_{tissue}(t_N)
\end{pmatrix},
\]

Where \(t_1, t_2, \ldots, t_n\) are evenly spaced time points where \(AIF(t)\) and \(C_{tissue}(t)\) are measured.

This can be rewritten as a matrix-vector:

\[
A \cdot b = c,
\]
Where $b$ is the vector element of $R(t)$, $c$ is the vector element of $C_{\text{tissue}}(t)$ and $A$ is the convolution matrix from the convolution equation above. The least squares solution for $b$ is given as $(A^T \cdot A)^{-1} \cdot A^T$, where $A^T$ is the transpose of the convolution matrix and $(A^T \cdot A)^{-1}$ is the inverse of the symmetric matrix $A^T \cdot A$. SVD decomposes $A$ into a product of matrices, such that the solution for $b$ can be written as the sum of terms weighted by the reciprocal of the singular values of $A$ due to $(A^T \cdot A)^{-1}$. By truncating small singular values in the sum of the solution vector $b$, oscillations from noise in both AIF($t$) and $C_{\text{tissue}}$ are avoided. This makes SVD a more stable and accurate measure than the transform approach as the noise is much reduced due to the processing method which produces more stable and reliable results.

**Calculating MTT**

Once CBV and CBF are known, MTT can be calculated as the ratio of CBV and CBF using the central volume theorem\(^\text{23}\) (figure 2.1).

\[
\text{MTT} = \frac{\text{CBF}}{\text{CBV}}
\]

Another illustration of CTP map processing is figure 2.1.
Figure 2.1. Graph illustrating a Tissue attenuation curve (TAC) plotted from perfusion CT data obtained in normal brain tissue. From this curve, per-voxel hemodynamic variables are calculated for TTP, CBF, and CBV. TTP is the time from the start of injection until maximum contrast enhancement is reached. However, some authors prefer to measure TTP from the beginning of enhancement by subtracting “time to start” (i.e., the time between the start of injection and the start of enhancement). CBF can be estimated from the “maximum slope” of the curve, or the peak height multiplied by time. CBV is calculated from the area under the normalized curve and can also be estimated from the maximum enhancement compared with that of a reference vessel (e.g., intracranial artery, superior sagittal sinus)\textsuperscript{24}.

Post Processing

Once a patient has been scanned, the data is sent to a workstation to be processed using manual, semi automated or automated software techniques that generate parametric maps. Each software package will take one of the processing methods outline above to display the perfusion maps. Pre-processing inputs of AIF and a venous outflow function (VOF) are required to be selected for processing. Some software packages can select these automatically, or present to the user with several optimal areas to choose from before processing begins. Also, some software packages undertake image segmentation that divides areas of the brain based on the Hounsfield (HU) units of image intensity. Non-cerebral tissue such as veins, arteries, suci, skull and air can be segmented out in this process. White matter has a HU around 20-30
and grey matter usually has a HU around 30-40, therefore pixels with a HU of <0 and >60 or >80 can be removed as bone, fat and air\textsuperscript{25}.

**Partial Volume Correction**

To identify an AIF, the user selects an area of interest using the Region of Interest (ROI) tool that is orthogonal to the imaging plane as to maximise volume averaging. Next an ROI is placed over a candidate VOF area. Now images can be processed using the programs method of choice. The purpose of the VOF is to correct for partial volume averaging. This is an effect of the limited area of small arteries results in a signal that is part blood bourn contrast and part vessel/extra arterial\textsuperscript{26} as seen in figure 2.2. By averaging an AIF with the VOF, a more realistic signal can be generated that corrects for this resolution error\textsuperscript{27}.

![Figure 2.2](image)

**Blood Vessel Elimination**

Blood vessels in the periphery and perforating arteries are also excluded from analysis in CTP software as they create areas of falsely high perfusion in the brain\textsuperscript{29}. Additionally vessels with a CBV over 8mL/100g are eliminated from analysis as they
also lead to overestimation of perfusion values as identified. Leptomeningeal vessels that are in the subarachnoid sulci are also eliminated from perfusion analysis. This prevents CBF overestimation\textsuperscript{30}. These steps enable much closer correlation between CTP CBF values and the gold standard PET CBF values.

**Delay and Dispersion Correction**

Problems caused by delay and dispersion have been alluded to in the previous chapter and above, and will be addressed here. Selection of an AIF for CBF calculations from a major artery assumes that the selected input represents the exact and only input to the tissue voxel of interest and has neither delay nor dispersion effects. However in a clinical setting, disease states can induce a delay of contrast arrival to a voxel through affecting the arterial or venous systems. Such extra cranial states include carotid stenosis, poor left ventricular ejection or atrial fibrillation, while intracranial causes could include a proximal intracranial obstruction. Also, the bolus of contrast forming the AIF can spread out over multiple pathways proximal to the tissue of interest and disperse. The two factors of delay and dispersion can result in an underestimated CBF and overestimated MTT perfusion values\textsuperscript{31,32,33}. Studies have identified that non delay corrected perfusion algorithms can overestimate the perfusion study results and cause pseudo reversibility of apparent stroke tissue pathophysiology, which misleads a treating physician\textsuperscript{34}. Lastly, it has been shown that if the tracer arrives earlier in other tissue than in the chosen AIF (Figure 2.3), CBF can be either over or under estimated depending on the extent of the tracer arrival difference and the underlying hemodynamics of the tissue\textsuperscript{35}. Therefore a means of correcting for these errors is needed.
There are several mathematical models that can attempt to correct for a delay or dispersion effect in SVD models of perfusion map generation. Wu et al (2003)\textsuperscript{36} proposed a block circulant decomposition matrix that is insensitive to the tracer arrival time. The principle behind this technique is to remove the causality behind the assumption of SVD that tissue of interest signal intensity cannot arrive before the AIF, as would happen in a diseased vessel. If causality is assumed for a diseased vessel the SVD equation would overestimate MTT and underestimate CBF, however if causality is not assumed the $R(t)$ would be shifted by a time delay. This is achieved by using circular as opposed to the linear deconvolution of SVD, which allows for $R(T)$ to be calculated with a circulated shift by the time delay. This means that the AIF can lag $C(t)$ by a time delay $t_d$ in practice, since the measured AIF, $C_a'(t)$, is not
necessarily the true AIF for that voxel, $C_a(t)$, thus resulting in $C_a'(t) = C_a(t-t_d)$. This delay can come about when the AIF is taken from a diseased vessel. So, $R'(t)$ should be $R(t + t_d)$ for $C(t)$, however if causality is held, $R'(t)$ cannot be properly approximated by inversion of the SVD equation.

By using circular deconvolution instead of linear deconvolution, $R'(t)$ can be represented with $R(t)$ circularly time shifted by $t_d$. Therefore, replacing matrix $A$ with a block-circulant matrix, $D$, whose elements are:

$$d_{i,j} = a_{ij} \text{ for } j \leq i, \text{ and } d_{i,j} = a_{L+i-j,0}$$

Otherwise, the SVD matrix can be reformulated as:

$$g = D \cdot f,$$

Where $g$ is the zero-padded $c$, and $f$ is the residue function scaled by $F_t$. The inverse of $D$ can be decomposed to:

$$D^{-1} = V_c \cdot W_c \cdot U.$$  

One can again make use of SVD techniques to solve for $f$ by:

$$f = F_t V_c \cdot W_c \cdot U \cdot g.$$  

When using circular deconvolution, however, due to the errors at $t = 0$ and $t=L$, leakage frequencies may be bigger, allowing spurious oscillations having a large effect on the deconvolved signal\textsuperscript{37}. However, increasing $P_{SVD}$ reduces the oscillations\textsuperscript{38}. Using a modified oscillation index from that described by Gobbel and Fike\textsuperscript{39}: 

71
where $f$ is the scaled estimated residue function, $f_{\text{max}}$ is the maximum amplitude of $f$, and $L$ is the number of sample points, $P_{\text{SVD}}$ can be varied until the estimated residue function's oscillation index falls below a user-specified value ($OI$) and CBF set to $f_{\text{max}}$ at that instance. Therefore the circular deconvolution avoids the time aliasing of linear deconvolution. The block circulant method has been demonstrated to be insensitive to tracer arrival time differences in models and clinical data and performed well when compared to SVD in healthy controls\textsuperscript{40}. Lastly, Block circulant deconvolution outperforms linear SVD in patients with a large territorial infarct at estimating the volume of the penumbra. Overall block circulant deconvolution seems to be a reliable standard for perfusion scanning in brains when there is a vascular abnormality that inhibits or disrupts normal hemodynamic operation.

Another delay correction technique is to use a local AIF from a smaller vessel close to the tissue of interest, however this technique is problematic due to the partial volume effect\textsuperscript{41}. One alternative to overcome this partial volume effect is the automated selection of an AIF, which would seek out the vessel with the highest signal in order to generate a perfusion scan\textsuperscript{42}. Alternatively, an independent component analysis can be used to identify spatially independent patterns and is based on the assumption that signals of interest can be decomposed into a linear combination of statistically independent components\textsuperscript{43}. This method has been shown to produce a higher CBV and shorter MTT when compared to a global AIF method for areas with delay and/or dispersion of contrast arrival.
Next, a curve fitting approach can be undertaken on the tissue concentration curve of the AIF to determine the relative time difference between the AIF(t) and the $C_{tissue}(t)$. This approach requires the pixel by pixel least squares fitting as a preparatory step before SVD deconvolution\textsuperscript{44}. Only data up until the AIF peak is corrected because the tail of the data reflects the tissue passage of contrast, and not the tracer arrival timing.

**AIF Selection**

The selection of an AIF in a thermodynamically compromised brain is complicated and without consensus. In a stroke, there is also no agreement in whether the AIF should be selected from the ipsi- or contralateral to the stroke affected hemisphere, however the future application of delay correct software should negate this issue altogether. At the moment however, it is still an issue of concern for studies using perfusion scanning.

Sanelli et al(2004)\textsuperscript{45} constructed CTP maps in 3 patients with a stroke, and reported that choosing large proximal intracranial artery versus a smaller distal artery ipsi- or contralateral did not affect the CBF, CBV or MTT values. Also, a study with 18 patients reported that CBF and CBV values were constant between scans using ipsi- and contralateral AIF selection\textsuperscript{46}, however the MTT values did differ between the AIF selection groups (figure 2.4). In these studies the patients were not reported as having delayed perfusion as a result of a stenosis or atrial fibrillation. However, for MTT to change, CBF and CBV values would have had to change because MTT is the ratio of CBF and CBV.
Figure 2.4 The maximum slope method of CTP post processing. CBF can be calculated from the ratio of the maximum slope (Max Slope) of Q(t) to the maximum arterial concentration. The higher maximum slope in the contralateral region of interest (ROI) (ie, the region of interest without stroke) will give a higher CBF than that for the ipsilateral region of interest, for which the CBF will be reduced.

**Arterial Spin Labelling (ASL)**

Non invasive perfusion MR can be performed using ASL where water nuclei in arterial blood are tagged magnetically, before it enters the area of interest (see figure 2.5). Firstly a control image is obtained without spin labelling, and then labelling of the arterial input starts. After a specific time, dependent on the blood transit velocity, the amount of labelled contrast is measured. A subtraction of the two images is performed and the difference in magnetization is identified due to the tagged arterial blood passage. The signal intensity is proportional to perfusion on the subtracted image and is used to calculate absolute cerebral blood flow (CBF). There are two main types of ASL, pulse ASL (PASL) and continuous ASL (CASL).
Figure 2.5. The ASL technique. The difference in magnetization between both acquisitions (obtained by subtraction) is proportional to CBF. A- Labeling of arterial blood by magnetic inversion. B- Acquisition of the labeled image. C- Repeat acquisition without labeling. D- Acquisition of the control image. By subtracting images b and d, we can generate a map that is proportional to CBF.

Originally ASL was proposed by Williams et al using a method previously used for angiography. CASL uses a continuous low intensity radio frequency pulse to tag arterial blood. Inversion of the arterial input magnetisation requires a continuous 2-4 second radiofrequency pulse that is applied to blood travelling through a magnetic field gradient. This adiabatic process relies on the labelling plane to be as close as possible to the tissue of interest and requires sampling over a longer period of time than PASL so that tagged spins can reach the region of acquisition, diffuse and exit the intravascular compartment. The continuous labelling of blood as it passes through the tagging plane results in a higher perfusion sensitivity than other ASL techniques. CASL has the advantage of good signal to noise ratio but is limited due to
a long acquisition time and labelling period. PASL however, has the advantage of reduced magnetisation transfer effect and a shorter acquisition time, but is limited by its sensitivity to transit delays and poor spatial resolution and coverage.\textsuperscript{50}

The PASL technique was proposed in 1994 by Eledman with fast echo-planar imaging (EPI).\textsuperscript{51} PASL uses short radio frequency pulses (5 – 20ms) after a delay time (TI) to measure signal difference between images before and after labelling to quantify the volume of tagged blood that reaches the area of interest after the delay time. Unlike CASL, PASL uses one pulse and then after the TI, measures selected areas for the volume of tagged blood. PASL is affected by transit time variations, which would be very common in stroke patients. The most significant issue would relate to the problems quantifying CBF values below 15-20 mL/100g/min, where PASL becomes very insensitive. Solutions to the quantification of low CBF values have been proposed with modest increases in signal from the hypoperfused areas.\textsuperscript{52}

**Acquisition and quantification**

Using ASL, a stack of images over a region are taken in order to examine tissue depth. The area where blood is labelled is called the saturation band and is placed proximal to the volume of interest. After acquisition and subtraction, the resulting image will now be perfusion weighted. The relationship between the signal difference and the CBF depends on proton density and T1 relaxation rates of the tissue imaged and the labelled blood. The traditional quantification of CBF for ASL was using the tracer clearance theory proposed by Kety and Schmidt\textsuperscript{53} below which was adapted to ASL by Detre and Williams.\textsuperscript{54}
Where $M_t$, $M_{t,0}$ and $M_a$ are the tissue-, equilibrium- and arterial-magnetizations, respectively, $\lambda$ is the blood–brain partition coefficient, and $T_{1t}$ is the longitudinal relaxation rate of the tissue.

Other derivations have been proposed by Calamante\textsuperscript{55} followed by Buxton et al\textsuperscript{56}. However, the most widely used and now accepted model is the “Standard Model” \textsuperscript{57,58,59} below:

$$\text{CBF} = \frac{\Delta M(TI_2)}{2M_{0,\text{blood}} \alpha TI_1 q_p(T_{1,\text{tissue}}, T_{1,\text{blood}}, TI_2)} e^{(TI_2/T_{1,\text{blood}})}$$

Where CBF is the cerebral blood flow, $M(TI_2)$ is the mean difference in the signal intensity between the label and control images, $M_0$, blood is the equilibrium magnetization of blood, is the tagging efficiency, TI$_1$ is the time duration of the tagging bolus, TI$_2$ is the inversion time of each section, $T_{1,\text{blood}}$ is the longitudinal relaxation time of blood, and $q_p$ is a correction factor that accounts for the difference between the TI of blood and the TI of brain tissue\textsuperscript{60}. The $M_{0,\text{blood}}$ is approximated from the $M_0$, $M_{\text{white matter}}$, which is measured directly from the $M_0$ image acquired with the perfusion-weighted images.

The principle of ASL imaging is tag input, delay for tissue arrival, and image results in a significant importance to the middle step of delay time, or the post
labelling delay (PLD). The PLD or inversion time in PASL has a critical impact on the CBF values of a perfusion map. With a short PLD the blood does not have enough time to reach the parenchyma meaning that the tagged signal is low. This results in significant overestimation of CBF and shows reduced perfusion in the watershed territories\(^61\). With a long PLD, there is more tagged blood in the image resulting in more accurate perfusion values. However a longer PLD will result in a longer acquisition time and less signal from the tagged blood due to T1 relaxation\(^62\).

**ASL in stroke**

ASL can be obtained in an acute ischemic stroke and be used in perfusion-diffusion mismatch to show penumbral and infarct core volume\(^63\). ASL rCBF lesions have been correlated to PWI perfusion lesions with some studies showing a good correlation with PWI rCBF\(^64, 65\) and other showing a better correlation with PWI MTT\(^66\). ASL has also been shown to be effective at showing hyperperfusion at 24 hours in stroke patients, in these studies patients would generally do better as it has been proposed that 24 hour hyperperfusion might be a marker for penumbral salvage\(^67,68\).

The main problem in using ASL in ischemic stroke the issue of delay arterial transit time and reduce blood flow resulting in underestimation of parenchymal perfusion levels. As PASL is the most commonly used ASL sequence, the post labelling delay (PLD) between tagging of arterial blood and sampling of a volume of interest is extremely important. The PLD will determine how much of the tagged blood is scanned and as a result determine the accuracy of the CBF perfusion map. In a short PLD, blood would not have enough time to reach the parenchyma and the
perfusion signal would be low (as seen in figure 2.6). With the short PLD the acquisition time would be low, however the image might not represent cerebral perfusion as the whole bolus of tagged blood was not sampled and slower territories would not have received the tagged blood. A longer PLD will show more of the bolus of tagged blood resulting in a more accurate global assessment of perfusion and images will appear more homogenous, however the signal would be very weak due to the T1 relaxation of the contrast itself.

Figure 2.6. Diagram on an arterial spin labelling (ASL) time course. In this graph, an example of two voxels having the same flow (60 ml/100g/min) and relaxation characteristics, but different arrival time, is presented. The measured signal at a single inversion time of 1.5 s would result in a 12% lower DM signal in a voxel with delayed arrival of 500 ms (open square) as compared with a voxel with an arrival time of 200 ms (closed square).

Diffusion Weighted Magnetic Resonance Imaging (DWI)

In DWI, the random thermal motion of water molecules when in a magnetic field gradient causes signal loss and is called the apparent diffusion coefficient (ADC). The ADC directly reflects the mobility of water molecules in tissue (figure 2.7). Molecular diffusion causing signal attenuation in a magnetic field gradient was
identified using MR spectroscopy in 1954 by Carr and Purell\textsuperscript{70} and has been used in conjunction with a pulsed gradient technique developed by Stejskal and Tanner\textsuperscript{71} in 1965 to form the basis of the DWI method.

Brownian motion cause molecular diffusion in a liquid due to thermal agitation and can been imagined as the constant wondering of individual molecules.

Figure 2.7. Relationship between ADC and DWI. The top table shows that as ADC value decreases, there is a corresponding hyperintensity in the bottom image. This diffusion lesion results from a reduced ADC score as the water molecules stop freely diffusing in the brain.
As time passes, the mean displacement of molecules in a closed system remains zero, however the individual molecules will move from their point of origin at time 0, meaning there is a non zero probability of finding a molecule at its origin after a small time. This can be expressed as the root mean squared displacement increases in proportion to the square root of time, creating a constant proportionality called the diffusion coefficient $D$. The diffusion coefficient of pure water at 25° is around $2.2 \times 10^{-1}$ mm$^2$/s. In soft tissues, the reduced mobility of the water molecules causes a decrease in the diffusion coefficient when compared to pure water, as soft tissue behaves like an aqueous protein solution which reduces the freedom of the water molecules. Boundaries of reduced permeability reduce the free diffusion of water, this decreases the diffusion coefficient, which means that each tissue type will have a different diffusion coefficient. By applying a Brownian motion model to the soft tissues of the brain we get an apparent diffusion coefficient (ADC) as there is locally restricted water molecule motion when compared to the diffusion coefficient of free water. Unfortunately in the brain, molecular mobility is not the same in all directions as the diffusion process is anisotropic and the scalar diffusion coefficient must be replaced by a tensor quantity$^{72}$. This allows diffusion imaging to provide insight on the microscopic structures and processes such as the presence and permeability of membranes or the subtle differences between tissue types, inside the imaged tissue as reflected by the motion of water molecules.
Diffusion imaging can be divided into two broad categories, Scalar diffusion models and Tensor diffusion models.

**Scalar diffusion model**

In an isotropic environment, where the Brownian motion is similar in all spatial directions, the molecular mobility can be described by a scalar diffusion coefficient. When a field gradient is switched on during a signal preparation, the motion of the free water molecules leads to spin de-phasing which cannot be undone due to the random nature of the successive trajectories of each individual molecule. The result is an exponential attenuation of the original signal:

$$S_0(N(H), T1, T2)$$

Obtained in the absence of field gradients:

$$S = S_0(N(H), T1, T2) e^{-bD},$$

Where $D$ is the (apparent) diffusion coefficient of the medium and $b$ is a scalar reflecting the properties of the gradient $G(t)$. In the absence of magnetic field gradients, the signal is unaffected by the presence of incoherent motion.

$$b = \gamma^2 \int_0^{TE} \left( \int_0^t G(t') dt' \right)^2 dt.$$

Here $G(t')$ is replaced by $-G(t')$ for the gradients switched on after $180^0$ pulse at $t=TE/2$. 
For a constant linear gradient of strength $G$ we can use\textsuperscript{74}:

$$b = \gamma^2 G^2 TE^3 / 12,$$

While for sensitization using two identical rectangular pulses (duration $d$, spacing $D$) placed on either side of the $180^\circ$ pulse \textsuperscript{75}:

$$b = \gamma^2 G^2 \delta^2 (d - \delta / 3).$$

Complete rephrasing of the static spins is achieved with both the above schemes, however the second equation is the one most commonly used. The advantage of the second equation is that there is no need for a strong gradient during the echo sampling which leads to a better signal to noise ratio. ADC values can be calculated by using two diffusion weighted images with different $b$ values and fitting the signal values to the equations above.

**Tensor diffusion model**

For many tissues the ADC values depend on the direction of the sensitising gradient in what’s called anisotropic diffusion behaviour as a result of their morphology. For these anisotropic processes a new equation must be used:

$$S = S_0(N(H), T1, T2) e^{-\sum b_u D_u},$$
Where $i$ and $j$ can be any of the three spatial directions $x$, $y$, $z$ in an orthogonal frame of reference. The $b_{ij}$ factors characterize the sensitizing gradients along the $i$ and $j$ directions$^{76}$:

$$b_{ij} = \gamma^2 \int_0^{\text{TE}} \left( \int_0^t G_i(t') \, dt' \right) \left( \int_0^t G_j(t') \, dt' \right) \, dt,$$

Where the $D_{ij}$ are elements of the apparent diffusion tensor. This tensor is symmetrical and contains only six independent elements, the determination of which needs the acquisition of images with at least two different diffusion weightings for each of six independent directions of the sensitizing gradient. The information in the diffusion tensor may be conceptualized using the ‘diffusion ellipsoid’ picture: the portion of space within which we can expect a molecule to end up due to its aleatory motion expands around the point of origin as time goes by and, in general, has the shape of a flattened cigar, reflecting anisotropic mobility$^{77}$. Diffusion tensor imaging is the most commonly used in stroke, as the acquisition is fast and has been validated to represent the acute infarct core in patients with an ischemic stroke$^{78}$.

**Conclusions**

This chapter has detailed the theory and mathematical modelling required to undertake perfusion analysis, ASL scanning and DWI map generation. The next chapter will discuss the clinical methodology and measures used for this thesis.
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Chapter Three:  

Clinical Methods

Introduction

This chapter will describe, in detail, the clinical methods used in this series of  
studies. The previous chapter has described the imaging based methods.  

Ethics approval was obtained as part of a large clinical trial being undertaken  
at the John Hunter Hospital and was approved by the Human research and Ethics  
Committees at the John Hunter Hospital and the University of Newcastle.

Patients

The analysed study group consisted of 530 patients that were admitted to the  
John Hunter Hospital between 2003 and 2011 with the acute diagnosis of an ischemic  
stroke. Patients included in the analysis had acute multimodal Computed Tomography  
(CT) scans and/or Magnetic Resonance Imaging (MRI) according to the institute’s  
stroke imaging protocols. Clinical assessments were immediately performed prior to  
imaging to rule out potential stroke imitators and assess stroke severity. After the  
clinical assessment, acute imaging was undertaken and analysed.

Inclusion and Exclusion Criteria

Only patients with a cerebral vessel occlusion as identified on acute imaging  
(CT or MR) were studied. Cerebral perfusion studies were the most useful tool to  
identify ischemic areas caused by hypoperfusion. These assessment methods are  
optimal for identifying ischemic stroke as well as excluding haemorrhagic strokes or
stroke imitators such as a tumour. Clinical diagnosis of hemispheric ischemia were made on the bases of cortical neurological deficits (such as dysphasia, anosognosia, visual or sensory inattention, dyspraxia or parietal sensory deficit) in the emergency room, as well as evidence of hemispheric hypoperfusion on acute CT scanning. However, not all patients admitted to hospital under the diagnosis of a stroke presented with all of these inclusive criteria, and were excluded from this thesis.

Exclusion criteria were; previous history of an ischemic stroke or a pre-existing neurological deficit (including but not limited to dementia or extra-pyramidal disease). Additionally, patients with the diagnosis of a transient ischemic attack (TIA) or haemorrhage, made on the bases of acute imaging, were excluded from analysis. There were no exclusion criteria based on sex, age or stroke severity. Additionally, non clinical, imaging based exclusion criteria were applied. Imaging based exclusion was due to poor acquisition either due to operator error, excessive patient movement, or possible file corruption. These patients were excluded as outliers as their data was very noisy or beyond salvage, and has been detailed in chapters four and five.

**Definition of stroke onset time**

The onset of ischemic stroke was defined as the time since a patient was last known to be without a neurological deficit. The final diagnosis was made according to the criteria above.
Clinical Assessment

Clinical Assessments were performed by a certified neurologist or neurology registrar on admission and at 24 hours post admission. Additionally, a modified Rankin Scale (mRS) was recorded at 90 days after symptom onset by a certified neurologist or neurology registrar. Assessors for neurological function were not blinded to imaging results or treatment. Patients who died prior to assessment in the study were assigned a maximum National Institutes of Health Stroke Score (NIHSS) and an mRS of 6. Demographic data was also recorded such as age, details of clinical co-morbidities and stroke risk factors such as diabetes, hypertension, age, obesity, hyperlipidemia, current medications, smoking history and atrial fibrillation.

National Institutes of Health Stroke Scale (NIHSS)

National Institutes of Health Stroke Scale (NIHSS) score was taken as soon as a patient that has been identified as a potential stroke and admitted to the stroke unit. Scores were recorded acutely, at 24 and 48 hours. The NIHSS is a simple and standardised score that assesses the neurological status in a patient without impairment to consciousness. The NIHSS assesses a patient’s cortical function (aphasia, neglect and visual field impairment), motor function (power, coordination in the upper and lower limbs), mentation (level of consciousness, orientation and cognitive ability) and sensation. The NIHSS is on a variable scale of 0- 42, identifying patients with little dysfunction to those with sever deficits and has been validated in acute stroke\(^1\).
Modified Rankin Scale (mRS)

The modified Rankin Scale (mRS) is a physical disability and independence scale that was performed at 90 days post stroke onset at the same time as outcome imaging studies. The mRS is on a scale of 0-6 as follows;

0: No disability of symptoms
1: No significant disability of symptoms
2: Slight disability but able to live independently
3: Moderate disability that requires some assistance
4: Moderately severe disability that requires assistance to attend bodily needs and mobilise
5: Severe disability requiring constant nursing care
6: Death

Image acquisition

All imaging in this study was done using a single acquisition protocol for Computed Tomography (CT) and for Magnetic Resonance Imaging (MRI). Hyper-acute CT imaging was used as the acute diagnostic tool. Where appropriate and practical, concurrent MRI was also performed.

Computed Tomography (CT)

Whole brain non-contrast CT (NCCT) was followed by perfusion CT, comprising two 45-second series (16-slice Philips Mx8000 or 64-slice Philips
Brilliance; Philips, Cleveland, Ohio). Each perfusion series covered a 24-mm axial section acquired as four adjacent six-mm slices (16 slice CT) or 40-mm section acquired as eight adjacent five mm slices (64 slice CT). The first section was at the level of the basal ganglia/internal capsule, and the second was placed 6-mm above to avoid overlap, towards the vertex. CT angiography (CTA) was performed after CTP with acquisition from the aortic arch to the top of the lateral ventricles².

Magnetic Resonance Imaging (MRI)

MR imaging was performed on a 1.5T MRI (Siemens Avanto, Erlangen, Germany). For this study the stroke MRI protocol included an axial isotropic diffusion-weighted echo planar spin-echo sequence (DWI), time of flight MR angiography (MRA), and an arterial spin labelling (ASL) sequence performed before the contrast injection bolus-tracking perfusion weighted imaging (PWI).

Timing of scanning and clinical assessments

Timing of scans was a critical factor in terms of the research questions that could be applied by these studies. As described in chapter one, the infarct core steadily expands into the penumbra. This dynamic even requires gold standard definitions to compare to perfusion scanning.

Hyper-acute studies

Effort was made to recruit as many patients as possible within the current 4.5 hour thrombolytic treatment time window. This time window was selected as it was likely that patients treated within 4.5 hours were more likely to benefit from treatment, due to the presence of penumbral tissue that was likely salvaged. However, we also recruited patients up to 12 hours after stroke onset (including uncertain onset).
All patients in these studies were scanned using multimodal CT, comprising of CT perfusion, CT angiography and non enhanced CT, which is the routine acute stroke imaging protocol at John Hunter Hospital. A sub-group of patients also had acute MRI, generally this was in patients outside the thrombolytic time window (>3 or >4.5 hours depending on year of enrolment). Additionally, all acute clinical information was recorded, and a detailed pre admission history taken for these studies.

Sub acute studies

After 24 to 48 hours following hospital admission, all patients were rescanned and assessed clinically. Imaging included an MR sequence comprising of Diffusion weighted imaging, Perfusion weighted imaging, T2, and in some cases Arterial Spin Labeling. A clinical examination included assessment of the NIHSS as detailed above.

Outcome studies

Imaging and clinical studies using an mRS were repeated approximately at 90 days post stroke admission. This allowed a measure of functional recovery and total infarct volume measurement.

Reperfusion status and patient grouping

Chapters 4, 5 and 6 all measure the volume of the acute penumbra and infarct core. In order to do this satisfactorily, we are required to stratify patients into one of three groups, major reperfusion, partial reperfusion and no reperfusion. Reperfusion status was measured as the change in volume of an MTT lesion (MTT lesion
threshold 145%, see previous chapter) from the acute CTP (or MR where applicable) to the sub acute MR-PWI MTT lesion.

It is important to aim for ‘pure’ reperfusion and no reperfusion groups to define the most accurate acute perfusion thresholds for critically hypoperfused tissue and infarct core. The definition of critically hypoperfused tissue is that which progresses to infarction without reperfusion. The definition of the infarct core is, tissue which infarcts despite reperfusion. Given our aim was to determine the most accurate thresholds for core and critically hypoperfused tissue, it makes biologic sense to restrict the ‘reperfusion’ group to those with near complete reperfusion (>80% reduction in MTT lesion 145%), and the ‘no reperfusion’ group to those with virtually no reperfusion (<20% reduction in MTT lesion 145%). There is little data on what is the best ‘no reperfusion’ cut point at 24 hours, so we chose <20% to be consistent with the major reperfusion cut point. Patients with partial reperfusion (between 20% and 80% reduction in MTT lesion 145%) were excluded as there is no way of knowing how much of their infarct core has expanded into the penumbra. Therefore 24 hour measurements of infarct core (DWI) would include a broad range of infarct growth, and are not likely to allow derivation of an accurate perfusion threshold for critically hypoperfused tissue.

Once patients were identified as either having major or no reperfusion, an analysis could begin:

*Hyper-acute studies – Infarct core definition*

In order to define the acute infarct core, two methods were viable. The most reliable but hardest to achieve is to acquire concurrent acute CT and MRI data with as little time between scans as possible. The acute DWI will provide information on the
volume and location of the acute infarct core that can be compared to the acute CTP in a volumetric analysis. Acute DWI is the gold standard of acute infarct core detection, making this method the best to determine the corresponding acute CTP measure and threshold as an indicator of infarct core.

*Hyper-acute and follow-up studies – Infarct core definition*

An acute CTP and 24 hour DWI in patients who showed major reperfusion 24 hours after symptom onset is another potentially useful method of determining the volume of the acute infarct core on CTP. The weakness of this approach is that there is no way of knowing the exact time of reperfusion, which allows for infarct core growth from the acute CTP time point, to the 24 hour DWI scan. This means that the 24 hour DWI might be slightly larger than the volume of the infarct core at the time of the acute CTP, as the infarct core could have grown due to persistent ischemia. One method of reducing the effect of this drawback is to only include patients who had reperfusion following thrombolytic administration, as they are more likely to have reperfused soon after thrombolytic administration. If the patient were to self recannalise, there is no way to know when reperfusion took place. Another strategy that was employed to counter any possible weaknesses of this approach, was to use the stringent reperfusion definitions (MTT 145% lesion reduction >80%). This ensured that only patients with effective reperfusion would be analysed.

*Hyper-acute and follow-up studies – The perfusion Lesion definition*

The only analytical approach that can determine the penumbral volume in a patient is to have an acute CTP, followed by an MRI at 24 hours in patients without reperfusion. This way the CTP will be an indicator of the severity of the perfusion
deficit in the acute patients, and can be volumetrically matched to volume of the
tissue that was infarcted and was destined to infarct (perfusion lesion), as it was not
salvaged, and progressed to infarction at 24 hours. The strength of this technique, as
with the first infarct core definition is that we are able to identify the whole area in
question with DWI imaging for comparison to CTP with very little variability, such as
time of reperfusion.

**Image Processing**

All imaging for these analyses was processed using MiStar. This software tool
allows for easy co-registration which was used in all studies as well as investigation
of multiple deconvolution methods (chapter 6).

**Statistical analysis**

This thesis used summary statistics and a tailored Receiver Operating
Characteristic (ROC) analysis. All analysis was undertaken on STATA (College
Station, Texas), Excel (Microsoft) and SPSS (IBM).

**Summary Statistics**

Summary statistics were used to compare lesion volumes between scans
(Chapter 7) as well as to clinical assessments (Chapter 5). Demographic and time of
scan data are presented as mean values with a standard deviation. Dependent variables
are compared using non-parametric techniques or parametric equivalents when
normality could be found and are presented as mean difference with 95% confidence
intervals. Spearman’s correlation coefficient was used to measure the strength of
association between lesion volumes and clinical measures.
Receiver Operating Characteristic (ROC) analysis

Receiver Operating Characteristic (ROC) Curve Analysis was used to test the predictive performance of CTP in relation to DWI infarct core[3]. The DWI image was considered to be the ‘true’ lesion and the pixels where the DWI lesion and CTP lesion overlapped were considered true positive. Pixels within the CTP lesion but not within the DWI lesion were assigned false positive, and pixels within the DWI lesion but not within the CTP lesion were assigned false negative. Specificity \( \frac{TN}{TN + FP} \) and sensitivity \( \frac{TP}{TP + FN} \) were calculated for each perfusion threshold.

Sensitivity and specificity were then plotted on an ROC graph, from which an area under the ROC curve (AUC) was taken. This analysis was performed for each threshold separately per case, so as to gain an AUC for each measure. The Positive Predictive Value and Negative Predictive Value as well as the volume difference between the CTP lesion and DWI lesion were also calculated for each CTP threshold.

In order to provide balance in the number of pixels being measured and prevent a very large true negative value from overwhelming the ratio to false positives in the calculation of specificity, only hemispheric (ischemic side) brain pixels were analysed rather than whole brain[11]. Without this correction the false negative values would have a much greater influence upon AUC than the false positives, producing results that would dramatically overestimate the true lesion volume.

To validate the AUC results, a lesion volume analysis was also undertaken to determine the closeness of fit between CTP- and MRI-defined infarct core and penumbra volumes for each patient. Lesion volumes were calculated for each threshold investigated and plotted against the corresponding acute and/or 24 hour DWI lesion volume for each patient. Regression analysis was used to determine the closeness of fit.
**Eligible patients**

During the study period (2005-2011) 314 stroke patients underwent the full acute multimodal CT stroke protocol and so were suitable for inclusion. Of these 144 had no reperfusion, 106 had major reperfusion, and 64 partial reperfusion.

Of the 144 patients without significant reperfusion, 124 were suitable for analysis; 7 patients were excluded due to inadequate co-registration because of very large sheer coefficients, 9 had inadequate quality CTP maps (3 due to slow arrival of contrast, 4 due to severe patient movement), and 4 had basilar artery occlusion.

Of the 106 patients studied with major reperfusion, 89 were suitable for analysis, with 17 patients being excluded; 9 due to very large sheer co efficient leading to poor co-registration, 3 due to basilar occlusion and 5 due to poor quality CTP maps (2 due to mistimed injection of contrast and 3 due to patient movement).

For the acute DWI analysis, 67 patients were eligible because they had both acute CTP and MRI within 12 hours of stroke onset. Twenty four of the 67 patients received thrombolysis (mostly in clinical trials such as EPITHET). Of these 57 patients, 23 had major reperfusion at 24 hours, of which 17 had received intravenous thrombolysis.

The median age of patients was 70 years (range 23-89), median acute NIHSS was 13 (range 5-24) and median time to end of CTP was 162 minutes (IQR 185-240 mins). Intravenous thrombolysis was administered to 174 patients according to institutional guidelines. Of the patients given thrombolysis, 82 showed major reperfusion (47%), 57 showed no significant reperfusion (32%) and 35 showed partial reperfusion on follow-up imaging (20%).
Conclusion

This chapter has detailed the general concepts that were studied and used in this thesis. More specific detail is given in each chapter as it relates to the specific study.
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Chapter Four:

*Defining the extent of irreversible brain ischemia using perfusion computed tomography.*

**Aim:** The aim of this chapter is to investigate if CTP can reliably define the acute infarct core when compared to DWI. The volume of the acute infarct core has been shown to be the most powerful acute predictor of patient outcome, and an easy to access measure of the acute infarct core would be critical to frontline staff. CTP is a more clinically assessable imaging technique than MRI, and as such has the potential to lead to better patient care through the practical and quick assessments of critically important clinical characteristics of stroke.

**Abstract**

**BACKGROUND:** Perfusion computed tomography (CTP) shows promise in acute stroke assessment. However, the accuracy of CT perfusion thresholds in defining the acute infarct core remains uncertain. **METHOD:** Concurrent CTP and MRI-DWI performed 3-6 hours after symptom onset were assessed in 57 ischemic stroke patients. CTP was compared to DWI images to define the infarct core using a pixel-based Receiver Operator Characteristic Curve analysis to calculate the Area Under the Curve (AUC) for thresholds from CTP maps that were coregistered with DWI slice location. **RESULTS:** A relative cerebral blood flow (CBF) of 45% of contralateral hemisphere was found to be the most accurate threshold for describing the infarct core (AUC 0.788), and was also by far the most frequent threshold with the highest AUC across patients. **CONCLUSION:** CBF thresholds on CTP more accurately define the
acute infarct core than other CTP thresholds, including a cerebral blood volume of 2mL/100g.

**Introduction:**

The central premise of stroke thrombolysis is to rescue the threatened yet potentially salvageable ischemic penumbra surrounding the irreversibly injured infarct core. However, current routine practice in assessing patient suitability for treatment with recombinant tissue Plasminogen Activator (rtPA) does not incorporate imaging of the penumbra or infarct core. Most centres will use a non contrast CT (NCCT) to assess the severity of an acute stroke and determine if there has been haemorrhage\(^1\). However NCCT cannot show the volume of the infarct core or the penumbra in the acute time window when treatment should be considered\(^2,3\). A more advanced form of stroke imaging may improve the selection of patients for treatment\(^4\).

Preliminary evidence using magnetic resonance imaging (MRI) suggests promise in using tissue-based selection for thrombolysis (EPITHET\(^5\) and DEFUSE\(^6\)). Computed Tomography Perfusion (CTP) is a more accessible and readily available imaging technique than MRI in the acute setting\(^7\), however there has been much less study of its role in tissue-based imaging selection for thrombolytic treatment.

Although most advanced imaging studies have focussed on the ischemic penumbra, the size of the infarct core appears to be a more important predictor of outcome than the size of residual penumbra\(^8,9\). Indeed, without an accurate measure of infarct core, the penumbra cannot be reliably measured, since it forms the inner boundary of the penumbra. Thus, it is critical to demonstrate that infarct core can be
reliably measured using CTP before this technique can be further assessed in trials of tissue-based imaging selection for thrombolysis.

Widely used CTP measures of infarct core are currently based upon absolute measures of cerebral blood volume (CBV)\textsuperscript{10} or the product of absolute CBV and cerebral blood flow (CBF)\textsuperscript{11,12}. However, we have observed in our clinical practice that absolute CBV and CBF values vary quite considerably between individual acute stroke patients, raising doubt about the generalizability of absolute CTP thresholds to identify core and penumbra. We hypothesised that relative CTP measures may be a more accurate determinant of infarct core than absolute perfusion values.

Material and Methods

We prospectively studied consecutive acute stroke patients presenting to the John Hunter Hospital between 2005 and 2007 with hemispheric ischemia within 6 hours of symptom onset. Patients underwent CTP/CT angiography (CTA) and diffusion weighted Imaging (DWI), perfusion weighted Imaging (PWI), and Magnetic Resonance Angiography (MRA) acutely, followed by repeat MRI with the same sequences at 24 hours. National Institutes of Health Stroke Scale (NIHSS) was performed immediately prior to acute and 24 hour imaging. This study was approved by our institutional Ethics Committee and individual patient consent obtained.

Imaging. Multi-modal CT was obtained with a multi-detector scanner (16-slice Philips Mx8000). Whole brain non-contrast CT (NCCT) was followed by perfusion CT, comprising two 45-second series. Each perfusion series covered a 24-mm axial section acquired as four adjacent six-mm slices. The first section was at the level of the basal ganglia/internal capsule, and the second was placed 6-mm above, towards
the vertex. CT angiography (CTA) was performed after CTP with acquisition from
the base of the skull to the top of the lateral ventricles.

MR imaging was performed on a 1.5T MRI (Siemens Avanto). The stroke
MRI protocol included an axial gradient-echo T2*-weighted series, an axial isotropic
diffusion-weighted echo planar spin-echo sequence (DWI), time of flight MR
angiography (MRA), bolus-tracking perfusion-weighted imaging (PWI) with an axial
T2*-weighted sequence, and a fluid-attenuated inversion recovery sequence.

*Image Analysis.* Perfusion CT maps were calculated by commercial software
(MiStar, Apollo Medical Imaging, Melbourne Australia)\textsuperscript{13} using deconvolution
analysis and delay correction\textsuperscript{14}. This required selection of an arterial input function
(from anterior cerebral artery) and a venous outflow function (from sagittal sinus), to
correct for partial volume averaging. The tissue enhancement curves were then
deconvolved with the arterial input function and from this residue function curve:
CBF was calculated from the peak height of the curve, CBV from the area under the
curve, time to peak of the residual function (Tmax) from the time to peak height (and
measured by delay in seconds from the peak of the AIF), and transit time (MTT) from
the ratio of CBV/CBF.

The perfusion CT maps were co-registered to the corresponding acute DWI
(b=1000 image). Images that failed to register in the first attempt using a rigid body
3D registration were subjected to a standardized sequence of alternate registration
procedures, including manual initialization as well as scaling and shear transforms to
correct for echo planar imaging artefacts. Cases that failed these co-registration attempts were excluded from this study.

The acute DWI lesion was used to define the acute infarct core. The DWI lesion was delineated based on signal intensity and highlighted using an area of interest tool to identify the contours of the DWI lesion\(^\text{15}\). Next, the DWI area of interest was transferred to the co-registered CTP maps for statistical volume analysis. A range of relative and absolute thresholds were then investigated at constant increments as shown in Table 4.1. The selection of these thresholds was based on values previously reported in the literature so as to encompass thresholds that have previously been reported as relevant to the prediction of tissue fate. Analysis was only undertaken on the DWI slices that matched the location of the CTP images after co-registration.

Co-registration of the acute CTP maps with the 24 hour DWI (\(b=1000\)) was also performed in the subset of patients who had major reperfusion and/or complete vessel recanalisation at 24 hours on MRI. Major reperfusion was defined as \(>80\%\) reduction in the acute to 24 hour MTT lesion\(^\text{15}\). In this subgroup, the same acute perfusion thresholds were applied to determine the most accurate measure of the infarct core at 24 hours (Table 4.1).

**Statistical Analysis**

*Receiver Operating Characteristic (ROC) Curve Analysis* was used to test the predictive performance of CTP in relation to the DWI infarct core, considered the gold standard for this study\(^\text{16}\). Overlapping CTP and DWI pixels were considered
“True Positive (TP)” and the CTP lesion pixels not overlapping the DWI lesion were assigned “False Positive (FP)”. Pixels not within either the CTP or DWI lesion were considered “True Negative (TN)”, and pixels within the DWI lesion, but not the CTP lesion, were assigned “False Negative (FP)”. Specificity \( \frac{TN}{TN + FP} \) and sensitivity \( \frac{TP}{TP + FN} \) were calculated for each perfusion threshold as summarised in Table 4.1. In order to provide balance in the number of pixels being measured and prevent the ratio of true negative from overwhelming the other volumes in the calculation of specificity, only hemispheric (ischemic side) brain pixels were analysed rather than whole brain. Sensitivity and specificity were then plotted on an ROC graph, from which an area under the ROC curve (AUC) was taken. This analysis was performed for each threshold separately as to gain an AUC for each threshold rather an overall AUC for each perfusion measure.

<table>
<thead>
<tr>
<th>CTP parameter</th>
<th>Range</th>
<th>Increments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative CBV</td>
<td>10 to 70%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBV</td>
<td>1 to 2.5 mL/100g</td>
<td>0.25 mL/100g</td>
</tr>
<tr>
<td>Relative CBF</td>
<td>10 to 60%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBF</td>
<td>3 to 17 mL/100g/min</td>
<td>2 mL/100g/min</td>
</tr>
<tr>
<td>Delay(Tmax)</td>
<td>2 to 10 s + baseline</td>
<td>0.5 seconds</td>
</tr>
<tr>
<td>MTT</td>
<td>150-300%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Table 4.1: Range and increments used for ROC analysis to investigate CTP thresholds to define the infarct core. Thresholds and their increments were chosen in accordance with previous reports of ischemic perfusion threshold ranges.
**Histogram Analysis:** The number of patients for whom each threshold was the most accurate (highest AUC) was analysed for each perfusion measure using histograms, to determine the level of agreement and individual patient accuracy of the optimal thresholds. This test was used as a direct measure of the reliability of a single threshold across patients as we aimed to find a single best threshold. From the AUC experiments alone, it is not known if a high AUC from the ROC test for an individual would be common for the majority of patients, and as such the histogram statistical analysis was performed.

**Post Processing Analysis: Noise Elimination Methods.** Analysis was repeated for each CTP threshold following implementation of the noise elimination technique ‘closed clustering’. The ‘clustering’ technique was used to exclude isolated ‘lesions’ or clusters of pixels below a set area, since isolated pixels with apparently abnormal perfusion values may reflect noise/artefact rather than truly hypoperfused pixels. Two minimum cluster exclusion groups were created, one where clusters of ‘hypoperfused’ pixels <5mm were excluded and one where clusters of ‘hypoperfused’ pixels <10mm were excluded. For each of these additional criteria, the entire dataset was reanalysed for each CTP threshold and measure.

All the above analyses were repeated on the 24 hour reperfusion sub-group with the 24 hour DWI as the measure of infarct core.

**Results**

In this study 65 patients had an acute CT and concurrent MR between three and six hours of symptom onset. However, in total 8 were excluded, 3 due to no acute lesion
on CTP slices (due to a lesion outside the plane of the CTP scan but present on DWI), 2 due to failed co-registration, 1 due to a basilar occlusion, and 2 due to poor quality CTP maps (mistimed injection of contrast, severe patient movement). Thus, 57 patients comprised the acute analysis, 24 of whom received thrombolysis. Of these 57 patients, 23 had major reperfusion at 24 hours, of which 17 had received intravenous thrombolysis.

The median age was 74 years (range 23-89) with a median acute NIHSS of 16 (5-24) on admission. The median time to end of CT scan was 195 minutes (IQR 185-240 mins) while the median time to start of MR scan was 225 minutes (IQR 210-270 mins). The median time between the end of CT scanning and the start MRI scanning was 28 minutes (IQR 20-40 mins)

CBF and CBV thresholds for infarct core. In the primary analysis, the CBF measures produced the consistently highest AUC with a relative CBF of 45% (AUC =0.788, confidence intervals, CI 0.68-0.90), and an absolute CBF of 9 mL/100g/min (AUC = 0.781, CI 0.66-0.90) being the best thresholds of all the measures and thresholds analysed (Figure 4.1). From the histogram frequency analysis (Figure 4.2), there was a narrow distribution around a relative CBF threshold of 45%, with 36/57 patients having the highest AUC for the relative CBF at a threshold of 45%. In contrast, despite similar overall AUC, the highest absolute CBF threshold (9 mL/100g/min) occurred in only 14/57 patients (see figure 4.2). Thus, the relative CBF infarct core threshold was more consistent across patients than the absolute CBF threshold. A relative CBV of 55% (AUC = 0.74, CI 0.62-0.86) was the next best measure of the acute infarct core. Absolute CBV was the poorest performing measure
of all the CBF and CBV measures, with the best AUC for absolute CBV being 2mL/100g, (AUC =0.69, CI 0.56-0.82).

Figure 4.1 AUC results for acute and subacute thresholds to define the infarct core using CTP. The top graph shows the acute DWI results and are compared to the 24 hour follow-up DWI data in the second graph (23 patients). CBF is shown to be the most accurate by reaching the highest points on the graph, however both Relative and Absolute thresholds perform well. The CTP threshold most accurately describing the acute infarct core in this study is a CBF of 45%, closely followed by a CBF of 9mL/100g/min.
Figure 4.2, CBF results comparison. A more detailed results from the specific measures shows the sensitivity and specificity for each CBF measure across the range of thresholds investigated (Table 1). The histogram also shows what threshold has the highest AUC for each patient, showing which threshold was best for that single patient. A narrow distribution around the CBF of 45% shows a high level of confidence compared to the absolute CBF which has a more widely distributed result. This makes a relative CBF of 45% the best threshold from this study at predicting the volume of the infarct core.

A scatter plot was also generated to compare acute DWI lesion volumes with CTP CBF 45%, CBF 9mL/100g/min and a CBV of 2mL/100g lesion volume (Figure 4.3). The figure clearly demonstrates that a CBF of 45% has a higher correlation to the DWI lesion volume ($R^2 = 0.55, P<0.001$) than CBF 9mL/100g/min ($R^2 = 0.35, P<0.004$) and a CBV of 2mL/100g ($R^2 = 0.17, P<0.02$)
Figure 4.3. Scatter plot comparing Acute DWI lesion volume with acute CTP CBF 45% ($R^2=0.55$, $P<0.001$), CBF 9mL/100g/min($R^2=0.35$, $P<0.004$) and CBV 2mL/100g ($R^2=0.17$, $P<0.02$) lesion volumes. It is clear that the relative CBF threshold stays within the confidence intervals and is closer to the linear fit line than any other measured threshold.

**MTT and Tmax thresholds for infarct core.** The most accurate thresholds for these measures were found to be at very low thresholds (Figure 4.1). The most accurate measure of infarct core was an MTT of 150% AUC of 0.74, (CI 0.68-0.80), and for Tmax the +2 seconds delay was most accurate AUC was 0.73 (CI 0.58-0.90). The transit measure results (Tmax and MTT) produced a lower AUC and were shown to be dominated by high sensitivity (0.86 for an MTT 150% and 0.81 for Tmax + 2 seconds) but unacceptably low specificity (0.61 MTT 150% and 0.58 Tmax + 2 seconds) across all thresholds tested. This demonstrates that transit measure thresholds would often overestimate the infarct core volume and not describe its location accurately.
Effectiveness of pixel clustering. The closed clustering method improved the AUC for all measures (Figure 4.4). For example, a clustering exclusion of <10mm$^2$ improved the AUC of CBF 45% from 0.74 to 0.78. These improvements in the AUC reflect a change in the specificity as small artifactual ‘lesions’ outside the DWI infarct core, probably due to noise, were eliminated by this technique. For CBF 45%, the increase in specificity was from 0.69(without clustering), to 0.79 for (clustering 10mm$^2$). Interestingly, the transit measures (MTT and Tmax) did not show improvement in AUC between clustering of 5mm and 10mm, whereas CBF and CBV did, suggesting the latter measures are more sensitive to noise.
Figure 4.4. Lesion clustering reduces the noise from a CTP scan. The cluster analysis was only applied to the CT perfusion lesion maps to exclude single or small group of ‘lesion’ pixels that are probably reflecting noise effects rather than truly hypoperfused tissue. A cluster is an isolated single pixel or a group of connected pixels. The cluster parameter is designed to limit by both linear extent and volume. For example, a cluster parameter of 5mm will exclude those clusters with either (1) a volume < the volume of a 5mm cube (i.e. 5x5x5 mm^3) or (2) a maximum linear dimension < 5mm. Another effect of the cluster analysis is to fill the holes (empty clusters) with a maximum linear dimension < 5mm. The optimum cluster parameter should exclude as much noise while still preserve the anatomical structure of the brain tissue. This is best illustrated in the figure below, where the 5 mm clustering lesion map seems closer to the DWI lesion texture. The effect of lesion clustering at 5mm. This single patient has had their perfusion data reprocessed two times using the same threshold to describe the acute infarct core but with different levels of clustering. This shows the effect of clustering on acute lesion detection, with removal of pixels clearly remote to the DWI lesion that likely reflect noise.

Comparison with 24 hour DWI in reperfused patients. Lesion volume increase between acute and 24 hour DWI in patients with major reperfusion was not significant at 1.99cm³ (standard deviation 1.83cm³). Analysis using the 24 hour follow-up DWI in the 23 patients with major reperfusion yielded very similar results to the main analysis (Figure 4.1). A relative CBF of 45% with clustering of 10mm group yielded the best AUC for infarct lesion detection (AUC = 0.785, CI 0.678-0.898).
Discussion

This study has demonstrated that CBF is the most accurate measure of infarct core on CTP, with a relative CBF of 45% being the most robust threshold for prediction of DWI-defined acute infarct core. Exclusion of small clusters of pixels with artifactual hypoperfusion (likely reflecting noise) also improved accuracy of infarct core detection (Figure 4.4). In the major reperfusion sub-group this same threshold (relative CBF <45%) was also optimal for prediction of the 24 hour DWI lesion, with an almost identical AUC to the acute DWI analysis. Although AUC for the overall patient group was similar for relative and absolute CBF, histogram analysis confirmed that the single relative CBF <45% threshold was by far the most common threshold with the highest AUC for individual patients. By contrast, the transit measures often overestimated the infarct core volume through a high specificity and as such cannot be recommended for acute infarct core detection.

Reassuringly, the 24 hour DWI data in the major reperfusion group showed exactly the same trends as the acute data. This is in keeping with contemporary thinking that early reperfusion prevents infarct core growth. There was a small decrease in the AUC, around 0.03 on average between the two groups. The most accurate threshold for predicting infarction (CBF 45%) did not change between the acute and 24 hour DWI.

The optimal CTP threshold for infarct core (CBF <45%) result differs from that of a similar, but smaller, analysis. In the previous study of 19 patients with concurrent acute CTP and DWI, showed an absolute CBV <2 mL/100g was the best method for characterising infarction\textsuperscript{10}. There are a number of methodological
differences between that study and ours which may explain this discrepancy. First, the current study used only the affected hemisphere volume rather than whole brain volumes to avoid overestimation of specificity resulting from excessively large volumes of true negative tissue. Including unaffected hemisphere pixels has a particularly strong impact (by elevating specificity, and hence AUC) on the thresholds that have high sensitivity, such as the absolute CBV and MTT results. Second, the AUC was calculated for each individual CTP threshold in our study, which allowed accurate identification of the best single threshold to detect the infarct core independent of the perfusion measure (e.g. CBV or CBF). This differs to the technique used previously of calculating the AUC by pooling all the tested thresholds for a perfusion measure. Third, the time between CT and MRI was much lower in the present study. Lastly, the calculation of perfusion measures varied somewhat, with our method faithful to the original deconvolution method, with CBV and CBF calculated directly from the area and peak height of the deconvolved tissue concentration curve (residue function)\textsuperscript{18}.

The relative perfusion values were derived from the average of the combined contralateral healthy gray and white matter perfusion level. This value was derived after blood vessels were excluded by a threshold of CBV $>9\text{mL}/100\text{mL}$ and was biased on the average of all slices. This is an optimal processing method as more slices would not alter the perfusion baseline, which allowed for comparisons between multiple scanners with varying number of slices. A potential concern is that the perfusion threshold was derived from the pooled grey and white matter perfusion levels, which vary significantly, with grey matter baseline reportedly $80\text{mL}/100\text{g/min}$ and white matter CBF being $20\text{mL}/100\text{g/min}$ with an average total CBF of
50mL/100g/min in a healthy adult\textsuperscript{24}. However the results of this study clearly show that this single threshold is optimal for clinical implementation. More research should be undertaken to examine the interaction of baseline CBF on these perfusion thresholds during ischemia and the role of perfusion modifying co-morbidities such as with hypertension or diabetes.

Limitations with our study include, firstly, the use of DWI of as a gold standard for infarct core\textsuperscript{19}. However, the group with major reperfusion had no expansion of the DWI lesion between acute and 24 hour scans, suggesting that baseline DWI is an accurate measure of the acute infarct core. Secondly, CTP did not cover the whole brain, therefore this infarct core threshold could only truly be applicable to the slices used. However the CTP coverage in this study included most of the MCA, PCA and ACA territory. We obviously cannot comment on perfusion thresholds in the brainstem. Next, there is potential for some dynamic changes in perfusion (and thus diffusion) to have occurred between acute CTP and MRI. However, time between scans was very short (median 28 minutes, IQR 20-40 mins) and the diffusion lesion change was insignificant at 24 hours follow-up in the major reperfusion group. Lastly, there is some evidence that the perfusion threshold for infarction may be time dependent\textsuperscript{20}. This study cannot adequately address this issue as the range of imaging times after stroke onset is narrow, and, though the largest of its kind, it still lacks enough patient numbers to address this concern by further dividing the dataset into sub-categories of time from stroke onset. Therefore it is still important to understand that the threshold identified in this study may only apply to the 3-6 hour time window after stroke onset.
The volume of infarct core has previously been shown to be a key predictor of both stroke outcome and risk of haemorrhagic complications\textsuperscript{19}. Hence, accurate definition of this tissue is critical for tissue based stroke imaging in general\textsuperscript{21}. This study has analysed data from the largest cohort of its kind, showing that a CBF threshold of 45\% of contralateral is the optimum predictor of infarct core as defined by either concurrent DWI, or 24 hour DWI in patients who reperfuse. The differences between our results and others (Figure 4.5) also highlight the need for more standardized methods of infarct core (and penumbra) detection using CTP. Until this occurs, CTP will not become incorporated into the routine clinical paradigm for assessment of acute stroke patients for thrombolytic therapy. The need for a better acute stroke diagnostics and training\textsuperscript{22} has become greater since it was recently shown that the current technique of Perfusion-Diffusion Mismatch visual assessment is not optimal for the selection of patients to treat with thrombolysis\textsuperscript{23}.
Figure 4.5. Effects of different thresholds at defining the acute infarct core compared to DWI. A comparison between two acute stroke patients from our dataset showing the outputs from previous studies. In the first column the current study threshold of a CBF <45% is used to characterise infarct core (Red). The 2nd column shows the threshold of 2ml/100g. The 3rd column shows a combined 1mL/100g CBV and 15ml/100g/min CBF threshold. The last column shows the acute DWI map from the same patients. It is clear from this figure that the first column is by far the most accurate predictor of infarction using acute CTP.
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Chapter Five:

**Perfusion CT: imaging and clinical validation in acute ischemic stroke**

**Aim:** Salvage of the acute penumbra from infarction is the aim of all acute stroke therapy to date. However, without identifying the presence or extent of the penumbra acutely, blinded treatment is placing a patient at risk of haemorrhagic complications. This is thought to be because the risk of haemorrhage is related to the volume of the acute infarct core. Therefore identifying patients who would benefit from treatment is critical for clinical staff, positive patient outcomes and avoiding risk. This study aims to identify the optimal CTP threshold to define the acute perfusion lesion and to optimise measures of the acute infarct core. Lastly, this study aimed to investigate correlations between imaging and clinical measures of stroke severity.

**Abstract**

**Introduction:** Computed tomography perfusion (CTP) imaging in acute stroke requires further validation. We aimed to establish the optimal CTP parameters defining the infarct core and critically hypoperfused tissue. **Methods:** Sub-6 hour CTP and 24 hour MRI were analysed from 314 consecutive ischemic stroke patients. MRI diffusion-weighted lesion volume (DWI) at 24 hours was used to define the extent of critically hypoperfused tissue (in patients without reperfusion between acute and 24 hour time points), and infarct core (in patients with major reperfusion at 24 hours). Pixel-based analysis of co-registered CTP and DWI was then used to define
the optimum CTP perfusion thresholds for critically hypoperfused at-risk tissue and infarct core. These optimised acute CTP threshold-based lesion volumes were then compared with 24 hour DWI infarct volume, as well as 24 hour and 90 day clinical outcomes for validation. **Results:** Relative Delay Time (rDT) >2 seconds was the most accurate CTP threshold in predicting the extent of critically hypoperfused tissue with both receiver operating curve analysis (AUC 0.86), and the volumetric validation (mean difference between CTP and 24 hour DWI lesions = 2 cm², 95% CI 0.5-3.2 cm²). Cerebral blood flow <40% (of contralateral) within the rDT >2 seconds perfusion lesion was the most accurate CTP threshold at defining infarct core with both ROC analysis (AUC = 0.85) and the volumetric validation. Using these thresholds, the extent of CTP mismatch tissue (the volume of ‘at-risk’ tissue between the critically hypoperfused and core thresholds) salvaged from infarction correlated with clinical improvement at 24 hours (R²=0.59 p=0.04), and 90 days (R²=0.42, p=0.02). Patients with larger baseline CTP infarct core volume (>25 mL) also had poorer recovery at day 90 (p=0.039). **Discussion:** CTP can accurately identify critically hypoperfused tissue that progresses to infarction without early reperfusion, and the CTP CBF infarct core closely predicts the final volume of infarcted tissue in patients who do reperfuse. The CTP infarct core and at-risk measures identified are also strong predictors of clinical outcome.
Introduction

An individually tailored approach to the selection of acute stroke patients for acute reperfusion therapies using tissue pathophysiology involves assessment of irreversibly injured tissue (infarct core) and salvageable brain (critically hypoperfused ‘at-risk’ tissue)\(^1\). Perfusion CT (CTP) is more widely available than MRI and has the potential to provide similar pathophysiologic information to stroke MRI more rapidly\(^2\). There is still validation needed to determine the most appropriate perfusion thresholds for identification of critically hypoperfused at-risk tissue for both modalities. However, for CTP there is the added complexity that thresholds to accurately identify both infarct core and critically hypoperfused tissue are required. We have recently shown that a relative cerebral blood flow (CBF) threshold defined the infarct core accurately with CTP\(^3\). When compared with other perfusion techniques such as PET\(^4\) and SPECT\(^5\), CTP-CBF absolute values for infarct core are underestimated, but the relative values are very similar.

The specific aims of the current study were: (i) to identify the most accurate perfusion threshold for critically hypoperfused tissue, (ii) to assess whether the application of a more stringent perfusion threshold within the critically hypoperfused region would more accurately identify the infarct core, and (iii) to clinically validate these CTP infarct core and critically hypoperfused lesion measures by correlating them with clinical recovery and tissue outcome.
Methods

Patients

We prospectively studied consecutive patients with hemispheric ischemia presenting within 6 hours of symptom onset. All patients underwent baseline multimodal CT examination and follow-up MRI at 24 hours. A subset of these patients also had acute MRI within 60 minutes of CTP. Clinical stroke severity using the National Institutes of Health Stroke Scale (NIHSS) was performed immediately prior to imaging, and level of disability at 3 months was measured with the modified Rankin Scale (mRS). If eligible, patients were treated with intravenous thrombolysis according to standard guidelines. The study was approved by the institutional ethics committee and all patients gave informed consent.

Imaging

Whole brain non-contrast CT (NCCT) was followed by perfusion CT, comprising two 60-second series (16-slice Philips Mx8000 or 64-slice Philips Brilliance; Philips, Cleveland, Ohio). CT perfusion imaging was performed with an intravenous bolus injection of contrast agent (40 mL of ultravist 370; Bayer HealthCare, Berlin, Germany) injected at a rate of 6 mL/sec, with 45 time points acquired each 1.33 seconds. Each perfusion series covered 24 to 40-mm section acquired as four to eight adjacent five to six-mm slices (depending on whether 16 or 64 slice CT). The first section was at the level of the basal ganglia/internal capsule, and the second was placed 6-mm towards the vertex to avoid overlap. CT angiography (CTA) was performed after CTP with acquisition from the aortic arch to the top of the lateral ventricles.
MR imaging was performed on a 1.5T MRI (Siemens Avanto, Erlangen, Germany). The stroke MRI protocol included an axial isotropic diffusion-weighted echo planar spin-echo sequence (DWI), time of flight MR angiography (MRA) and bolus-tracking perfusion weighted imaging (PWI).

Image Analysis

Perfusion CT maps were calculated by commercial software MIStar (Apollo Medical Imaging Technology, Melbourne, Australia). This required selection of a global arterial input function (AIF) from a normal major artery (such as the anterior cerebral artery) and a venous outflow function (VOF) from a large draining vein (such as the sagittal sinus). Deconvolution of the tissue enhancement curve and the AIF was performed using a model-free singular value decomposition (SVD) with a delay and dispersion correction. This methodology produces Delay Time (DT) maps, rather than the more widely known Tmax map, as well as maps of CBF, cerebral blood volume (CBV), and mean transit time (MTT). For more technical details on the difference between DT and Tmax see Appendix 5.1. Areas of no blood flow, chronic infarction or CSF regions, were masked from the perfusion maps: no blood flow pixels were removed by eliminating areas where CBF=0 and, CSF/ventricle and skull pixels were removed using a HU threshold and geometric analysis.

Before perfusion post-processing, each perfusion CT slab (source image data) were individually co-registered to the corresponding acute and 24 hour DWI (b=1000 image) anatomical location. Images that failed to register in the first attempt using a rigid body 3D registration (n=42) were subjected to a standardized sequence of alternate registration procedures, including manual initialization as well as scaling and
shear transforms to correct for echo planar imaging artefacts. Cases that failed these co-registration attempts were excluded (n=7).

Acute and 24 hour DWI lesions were delineated based on signal intensity and highlighted using an area of interest tool. Next, the areas of interest were transferred to the co-registered acute CTP maps for statistical volume analysis. A range of relative and absolute thresholds were then investigated at constant increments as shown in Table 5.1. Both relative thresholds (as a percentage of perfusion parameters in contralateral hemisphere, excluding large vessels), and absolute thresholds were tested. For the DT thresholds, absolute measures of delay time were used, as well as a relative measure where absolute delay was added to the average delay time in normal tissue (‘baseline delay’). Normal tissue baseline was defined as mean perfusion values from tissue in the unaffected hemisphere, with large vessel voxels being excluded from analysis. Thresholds tested also included those reported in previous studies as predictive of tissue fate 8,9.

Table 5.1 Range and increments of CTP thresholds tested for the study

<table>
<thead>
<tr>
<th>CTP parameter</th>
<th>Range</th>
<th>Increments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative CBV</td>
<td>0 to 100%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBV</td>
<td>1 to 5 mL/100g</td>
<td>0.5 mL/100g</td>
</tr>
<tr>
<td>Relative CBF</td>
<td>0 to 100%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBF</td>
<td>1 to 20 mL/100g/min</td>
<td>1 mL/100g/min</td>
</tr>
<tr>
<td>Relative DT</td>
<td>0 to 10 s + baseline</td>
<td>0.5 seconds</td>
</tr>
<tr>
<td>Absolute DT</td>
<td>0 to 10 seconds</td>
<td>0.5 seconds</td>
</tr>
<tr>
<td>Relative MTT</td>
<td>100-500%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Table 5.1: Range and increments used for ROC analysis to investigate CTP thresholds to define critically hypoperfused tissue.
Patient grouping based upon reperfusion status

Patients were divided into three groups: major reperfusion (>80% at 24 hours), no reperfusion (<20% reperfusion at 24 hours) and partial reperfusion (between 20% and 80%) based on change between acute CT and 24 hour MR perfusion lesion volume\textsuperscript{10}. As per previous studies, the same perfusion map (MTT) was used for both acute CTP and MRI, with the same threshold applied to define lesion volume (the most commonly used for CT perfusion lesion determination: MTT>145% of normal tissue)\textsuperscript{11}.

Although we have previously used acute CTP-MTT and 24 hour MR-MTT to measure reperfusion, we performed an analysis in the sub-group of patients with acute MRI to further assess the validity of CTP- versus MR-MTT lesion comparisons. This involved a volumetric correlation between the acute CTP-MTT lesion (>145% threshold) with the acute MR-MTT lesion (>145% threshold).

Analysis 1: Defining critically hypoperfused tissue in patients with no reperfusion using 24 hour DWI

The 24 hour DWI lesions in the no reperfusion group were used as the reference to define the critically hypoperfused tissue CTP threshold using ROC analysis.

Analysis 2a: Defining the infarct core within critically hypoperfused tissue in patients with major reperfusion using 24 hour DWI
Once the most accurate CTP critically hypoperfused at-risk threshold for the perfusion lesion had been identified from the non-reperfused group (Analysis 1), two analyses were performed to derive infarct core thresholds. Firstly the 24 hour DWI lesion was used as the reference in patients with major reperfusion to define the CTP infarct core perfusion threshold using ROC analysis (analysis 2a).

**Analysis 2b: Defining the infarct core within critically hypoperfused tissue using acute DWI**

In the sub-group of patients who had concurrent acute MRI the acute DWI lesion was used as the reference to define the CTP infarct core threshold using ROC analysis (analysis 2b).

For Analyses 2a and 2b we hypothesised that the infarct core thresholds would be more accurate when restricted within pre-defined critically hypoperfused lesion thresholds. This was tested using the most accurate critically hypoperfused thresholds from the three best performing perfusion maps in Analysis 1.

**Statistical Analysis**

Receiver Operating Characteristic (ROC) Curve Analysis was used to test the predictive performance of CTP in relation to the DWI infarct core. The DWI image was considered to be the ‘true’ lesion and the pixels where the DWI lesion and CTP lesion overlapped were considered ‘true positive’ (TP). DWI pixels not within the CTP lesion were considered ‘true negative’. Pixels within the CTP lesion but not within the DWI lesion were assigned ‘false positive’ (FP), and pixels within the DWI lesion but not within the CTP lesion were assigned ‘false negative’ (FN). Specificity
(TN/(TN + FP)) and sensitivity (TP/(TP + FN)) were calculated for each perfusion map. Results presented are AUC (and 95% CIs) for the whole ROC curve for a specific perfusion map. Specificity, sensitivity, positive predictive value, and negative predictive value were calculated for each threshold increment (eg. CBF or MTT).

In order to provide balance in the number of pixels being measured and prevent a very large true negative value from overwhelming the ratio to false positives in the calculation of specificity, only hemispheric (ischemic side) brain pixels were analysed rather than whole brain. Without this correction the false negative values would have a much greater influence upon AUC than the false positives, producing results that could substantially overestimate the true lesion volume.

**Volumetric validation.** To internally validate the ROC results, a lesion volume analysis was also undertaken to determine the closeness of fit between the CTP threshold-derived infarct core and 24 hour DWI lesion volumes in the major reperfusion group; and CTP threshold- derived critically hypoperfused lesion volumes versus 24 hour DWI lesion volumes in the no reperfusion group. For each patient, lesion volumes were calculated from the relevant CTP threshold and plotted against the corresponding 24 hour DWI lesion volume.

**Clinical validation.** Finally, there were statistical analyses performed to test the clinical validity of the core and critically hypoperfused at-risk thresholds. The perfusion lesion defined by the ‘best’ CTP threshold for critically hypoperfused tissue was correlated with both acute and 24 hour NIHSS. Infarct core volumes determined from the most accurate CTP core threshold were correlated with change in acute-24
hour NIHSS, and day 90 mRS, as recent studies have suggested baseline infarct core volume is an important predictor of ultimate clinical outcome. Additionally, patients were dichotomised on the basis of acute infarct core volume to determine whether a larger CTP defined infarct core (>25mL) was predictive of poor clinical outcome, as has recently been found for acute DWI-defined infarct core. Early and late clinical recovery was also correlated with the volume of CTP ‘mismatch’ tissue (defined as tissue between the critically hypoperfused and core thresholds) salvaged from infarction in all patients.

**Results**

During the study period (2005-2010) 314 ischemic stroke patients underwent CTP within 6 hours of symptom onset and a 24 hour diffusion and perfusion MRI. The median age was 70 years (range 23-89), median acute NIHSS was 13 (range 5-24) and median time from symptom onset to end of CTP was 162 minutes (IQR 185-240 mins). 144 patients had no reperfusion, 106 had major reperfusion, and 64 patients had partial reperfusion. Intravenous thrombolysis was administered to 61 patients with no reperfusion (39%), 82 patients with major reperfusion (77%), and 35 with partial reperfusion (55%). In the 67 patients with additional acute MRI, the median time to start of acute MR scan was 240 minutes from stroke onset (IQR 210-270 mins). The median time between the end of CT scanning and the start MRI scanning was 19 minutes (IQR 15-40 mins).
Validation of the reperfusion measures and classification system

In the 67 patients with acute CTP and perfusion MRI (supplementary figure 5.1), the CTP and MR-MTT >145% lesion volumes were highly correlated ($R^2 = 0.81, P=0.01$). Also confirming the effectiveness of major reperfusion at preventing infarct growth, there was a no significant difference between the acute and 24 hour DWI in patients with major reperfusion (mean lesion growth 1.5 cm$^3$ CI -0.3 – 2.2 cm$^3$ p=0.24). Furthermore, none of these patients showed significant lesion reduction between the acute and 24 hour DWI time points. In contrast, there was a significant increase in lesion size in the no reperfusion group (mean lesion growth 12cm$^3$, p=0.01) and the partial reperfusion group (mean lesion growth 8 cm$^3$, p=0.005). Infarct growth in the no reperfusion group was significantly greater than in the major reperfusion group (p=0.035), but the partial reperfusion group had a wide range of infarct growth, overlapping with the other two groups (supplementary figure 5.2).

Analysis 1: Defining critically hypoperfused tissue in patients with no reperfusion: ROC analysis and volumetric validation with 24 hour DWI

Of the 144 patients without significant (<20%) reperfusion, 124 were suitable for analysis; 7 patients were excluded due to inadequate coregistration from very large shear coefficients, 9 had inadequate quality CTP maps (3 due to slow arrival of contrast, 4 due to severe patient movement), and 4 had basilar artery occlusion.

The most accurate map using ROC analysis to predict subsequent infarction in this group was relative Delay Time (AUC 0.86, CI 0.84-0.88). The ‘best’ relative Delay Time threshold was rDT > 2s (specificity 0.89, CI 0.86-0.92; sensitivity 0.85, CI 0.81-0.90). The absolute Delay Time map was also very accurate at predicting
infarction at 24 hours (AUC 0.85, CI 0.83-0.87). The most accurate absolute Delay Time threshold was DT >2s (specificity 0.86, CI 0.80 -0.89; sensitivity 0.85, CI 0.79-0.89). These two rDT and absolute DT thresholds were also the most closely correlated with the 24 hour DWI lesion in the volumetric analysis (Figure 5.1). The relative DT > 2s threshold overestimated the DWI infarct by a small amount, mean 2 cm² (95% CI = 0.5- 3.2 cm²), whilst the absolute DT > 2s threshold was slightly less accurate, overestimating infarction by a mean of 3cm² (95% CI = 1.7cm²-4.6cm²).

Of note, the relative DT >3s threshold was also very accurate in predicting 24 hour infarction (specificity 0.93, sensitivity 0.72). However, as the rDT >3s threshold was more specific but less sensitive than rDT >2s (Table 5.2), it tended to underestimate the subsequent infarct (mean difference -3.2 cm³;95% CI = -5.1 cm² - 0.3cm².).

<table>
<thead>
<tr>
<th>rDT Threshold (s)</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
<th>CTP-DWI volume mean difference (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>0.57</td>
<td>0.94</td>
<td>0.63</td>
<td>0.92</td>
<td>3.1cm² ( 0.1 – 5.3)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>0.91</td>
<td>0.85</td>
<td>0.79</td>
<td>0.88</td>
<td>2.1cm² (-0.5 – 3.2)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>0.93</td>
<td>0.72</td>
<td>0.85</td>
<td>0.82</td>
<td>-3.2cm² (-5.1 -- 0.3)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>0.94</td>
<td>0.59</td>
<td>0.88</td>
<td>0.77</td>
<td>-4.7cm² (-1.2 --6.1)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0.96</td>
<td>0.51</td>
<td>0.91</td>
<td>0.74</td>
<td>-5.38cm² (-2.1 --8.7)</td>
</tr>
</tbody>
</table>

Table 5.2 Relative Delay Time (rDT) thresholds: accuracy at defining critically hypoperfused tissue in the ROC analysis and in lesion volume correlation with 24 h DWI. Note: These analyses were performed in patients with no reperfusion.
### Table 5.3, Accuracy of thresholds to define the acute perfusion lesion

<table>
<thead>
<tr>
<th>Best threshold per map</th>
<th>AUC</th>
<th>CI</th>
<th>PPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rDT &gt;2 s</td>
<td>0.86</td>
<td>0.89;0.81</td>
<td>0.8</td>
<td>0.82</td>
<td>0.9</td>
<td>0.81*</td>
</tr>
<tr>
<td>CBF &lt;50%</td>
<td>0.72</td>
<td>0.74;0.71</td>
<td>0.66</td>
<td>0.7</td>
<td>0.74</td>
<td>0.71</td>
</tr>
<tr>
<td>CBV &lt;55%</td>
<td>0.63</td>
<td>0.65;0.61</td>
<td>0.6</td>
<td>0.76</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td>MTT &gt;140%</td>
<td>0.78</td>
<td>0.79;0.77</td>
<td>0.74</td>
<td>0.77</td>
<td>0.79</td>
<td>0.62</td>
</tr>
<tr>
<td>aDT &gt;2s</td>
<td>0.83</td>
<td>0.87;0.79</td>
<td>0.79</td>
<td>0.82</td>
<td>0.83</td>
<td>0.8*</td>
</tr>
<tr>
<td>CBF &lt;10 mL/100g/min</td>
<td>0.74</td>
<td>0.76;0.73</td>
<td>0.5</td>
<td>0.67</td>
<td>0.83</td>
<td>0.61</td>
</tr>
<tr>
<td>CBV &lt;2 mL/100g</td>
<td>0.63</td>
<td>0.6;0.66</td>
<td>0.69</td>
<td>0.82</td>
<td>0.44</td>
<td>0.6</td>
</tr>
<tr>
<td>MTT &gt;8s</td>
<td>0.71</td>
<td>0.74;0.68</td>
<td>0.64</td>
<td>0.63</td>
<td>0.76</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Table 5.3, Accuracy of thresholds to define the acute perfusion lesion. This is the characterisation of the perfusion lesion showing the most accurate thresholds for each perfusion map tested in the study. The AUC and 95 CI refer to the perfusion map and the PPV (positive predictive value), sensitivity, and specificity refer to the best threshold for that particular map. $R^2$ and p-value relate to the CTP threshold generated lesion volume correlation with 24 hour DWI (in patients with no reperfusion). *refers to p value <0.05 for the regression equation.

**Analysis 2a: Defining the infarct core within critically hypoperfused tissue in patients with major reperfusion: ROC analysis and volumetric validation with 24 hour DWI**

Of the 106 patients studied with major reperfusion, 89 were suitable for analysis, with 17 patents being excluded; 9 due to very large sheer co efficient leading to poor co-registration, 3 due to basilar occlusion and 5 due to poor quality CTP maps (2 due to mistimed injection of contrast and 3 due to patient movement).
From Analysis 1 (Table 5.3), the three most accurate perfusion maps and their respective best thresholds to define critically hypoperfused tissue were rDT (>2s), absolute DT (>2 s), and MTT (>140%). Relative CBF (rCBF) was the most accurate CTP map to define the infarct core within all three critically hypoperfused tissue thresholds (Table 5.3), with rCBF <40% being the best performing threshold on each occasion (Table 5.4). Using rCBF, infarct core was defined most accurately within the critically hypoperfused tissue threshold rDT >2s (AUC 0.86, CI 0.83- 0.89; rCBF <40% specificity 0.78, specificity 0.93). Relative CBF also defined the infarct core very accurately within absolute DT >2 s tissue (AUC 0.85, 95% CI 0.8 – 0.87; rCBF <40% specificity 0.92, specificity 0.71). Relative CBF defined infarct core less accurately within critically hypoperfused tissue defined by the MTT >140% threshold, (AUC 0.8, CI 0.76 – 0.83; rCBF <40% specificity0.86, specificity 0.63).

The rCBF <40% infarct core threshold (within rDT >2 secs critically hypoperfused tissue) was also the most closely correlated with the 24 hour DWI lesion in the volumetric analysis (Figure 5.1 and Table 5.4). Volumes obtained from this threshold overestimated the DWI lesion by a small amount, mean 3.6 cm³ (95% CI = -0.7 – 5.4 cm³), with few outliers (figure 5.1).

**Analysis 2b: Defining the infarct core within critically hypoperfused tissue in patients with acute DWI: ROC analysis and volumetric validation**

There were 67 patients comprised the analysis with acute DWI, 24 of whom received thrombolysis. Of these 67 patients, 23 had major reperfusion at 24 hours, of which 17 received intravenous thrombolysis.
<table>
<thead>
<tr>
<th>Perfusion lesion threshold</th>
<th>Infarct core threshold</th>
<th>AUC</th>
<th>CI</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>rDT &gt;2s</td>
<td>CBF &lt;40%</td>
<td>0.86</td>
<td>0.92;0.8</td>
<td>0.93</td>
<td>0.78</td>
<td>0.77</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>CBF &lt;50%</td>
<td>0.86</td>
<td>0.92;0.8</td>
<td>0.96</td>
<td>0.62</td>
<td>0.73</td>
<td>0.62</td>
</tr>
<tr>
<td>MTT &gt;140%</td>
<td>CBF &lt;4%</td>
<td>0.8</td>
<td>0.83;0.7</td>
<td>0.86</td>
<td>0.63</td>
<td>0.65</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>CBF &lt;50%</td>
<td>0.8</td>
<td>0.83;0.7</td>
<td>0.84</td>
<td>0.55</td>
<td>0.56</td>
<td>0.68</td>
</tr>
<tr>
<td>aDT &gt;2s sec</td>
<td>CBF &lt;40%</td>
<td>0.86</td>
<td>0.89;0.8</td>
<td>0.92</td>
<td>0.71</td>
<td>0.72</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>CBV &lt;90%</td>
<td>0.8</td>
<td>0.84;0.7</td>
<td>0.92</td>
<td>0.71</td>
<td>0.65</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Table 5.4. Infarct core threshold results. This is the characterisation of the infarct core within the perfusion lesion showing the most accurate thresholds for every measure tested across three perfusion lesion measures. The AUC and 95 CI refers to the perfusion map and the PPV (positive predictive value), sensitivity, and specificity refer to the best threshold for that particular map. R² and p-value relate to the CTP threshold generated lesion volume correlation with 24 hour DWI (in patients with major reperfusion) *refers to p value <0.05 for the regression equation

The AUC results and lesion volume analysis yielded virtually identical results to analysis 2a. Relative CBF within rDT >2s critically hypoperfused tissue again had the highest AUC (AUC 0.86, 95% CI 0.81- 0.88), with rCBF <40% being the best performing threshold in the ROC and volumetric analyses.
Figure 5.1. Scatter plot results for lesion volume variation between imaging modalities. Four graphs plotting the volumes derived from the two most accurate acute CTP thresholds for critically hypoperfused tissue (top pair) and the infarct core (bottom pair) against the volume of the 24 hour DWI. Note that for both the perfusion lesion and infarct core threshold correlations with 24 hour DWI, rDT performs slightly better than aDT with less outliers outside the 95% CI lines (light blue lines).

Clinical validation of CTP thresholds

The acute NIHSS score was highly correlated with the volume of the total acute rDT >2 s lesion ($R^2 =0.71$, $p=0.01$). The volume of a duel CTP threshold (i.e. tissue critically hypoperfused, with rDT >2s, but above the infarct core threshold of rCBF <40%) salvaged from infarction at 24 hours was highly correlated with acute to 24 hour NIHSS improvement ($R^2 =0.59$ $p=0.04$), as well as with 90 day mRS ($R^2 =0.42$, $p=0.02$). Patients with a larger baseline (>25mL) infarct core (tissue rDT >2s and with rCBF <40%), appeared to have less early clinical improvement (median
acute to 24 hour NIHSS change = 4, IQR -1-6) compared to those with a smaller baseline infarct core (median acute to 24 hour NIHSS change = 8, IQR 5-11). This difference was not statistically significant (p=0.27). However, patients with a large baseline CTP-defined infarct core (>25 mL) clearly had poorer 3 month outcome than those with a small baseline infarct core (median mRS=5 versus 2 respectively, p=0.039).

**Discussion**

This study has shown that acute CTP can accurately identify the acute infarct core and critically hypoperfused tissue. Additionally, the volume of baseline CTP infarct core and the volume of duel CTP threshold tissue (delay time 2 seconds) subsequently salvaged from infarction had a major influence on early and late clinical outcome. Although there are also important implications in terms of perfusion post-processing methods, our study primarily has major clinical implications, demonstrating that CTP can accurately measure irreversible and reversibly ischemic tissue. As CTP is widely available, the stage is set to finally have a truly generalisable tissue-based acute stroke imaging technique.

This study provides strong evidence for the clinical validity of CTP in acute ischemic stroke. The acute CTP critically hypoperfused lesion volume was closely correlated with the baseline clinical deficit (NIHSS), indicating significant functional impairment of this tissue, consistent with many other studies using different perfusion modalities\(^{13,14}\). The similarly strong relationship of clinical recovery with duel CTP threshold tissue (core) salvaged from infarction by reperfusion emphasises the potential clinical importance of using this information to predict outcome (particularly
with thrombolysis). Conversely, the high rate of progression of duel CTP threshold tissue to infarction in patients without reperfusion, and the strong association with poor clinical outcome in this group, is also extremely valuable clinical information. Another novel finding of this study is the crucial influence of the volume of the baseline CTP infarct core upon clinical outcome, paralleling recent data from studies using DWI measures of acute infarct core\textsuperscript{15,16,17,18}.

Although the clinical impact of our results is very important, the current study also describes a number of advances in CTP post-processing methodology to define infarct core and critically hypoperfused tissue. CTP accurately defined hypoperfused “at risk” tissue by closely predicting subsequent infarction in patients who did not reperfuse using a single perfusion threshold (\(r\text{DT} > 2\) s). Additionally, use of a relative CBF threshold within critically hypoperfused tissue (\(r\text{CBF} < 40\%\) of normal and within tissue with \(r\text{DT} > 2\)s) accurately defined the acute infarct core (figure 5.4). Notably, this is the first CTP study to demonstrate that a physiological delay time threshold provides an accurate definition of tissue at risk. This is in keeping with the perfusion MR field where a similar measure to Delay Time, Tmax is the most commonly used perfusion definition of critically hypoperfused tissue\textsuperscript{19,20}. Currently, the most widely used CT perfusion measure to identify critically hypoperfused tissue is MTT. MTT maps were also reasonably accurate in our study, but did not perform as well as the Delay Time perfusion maps (Table 5.4 and Figure 5.1).

Whilst our CTP study is the first to parallel the MR literature in finding that delay time is more accurate than MTT, it is important to understand that although Delay Time and Tmax are related, they are not exactly the same measure (see
Appendix 5.1). Notably, previous MR perfusion studies have identified critically hypoperfused tissue thresholds of Tmax between 5 and 6 seconds, whereas we have shown that DT between 2 and 3 seconds is the most accurate to identify critically hypoperfused tissue. A comparison of the accuracy of Tmax and delay time at identifying the ischemic penumbra is given in chapter 6. Delay Time is derived from a vascular transport model correcting for both arterial delay and dispersion effects. Thus, DT is expected to more precisely reflect the physiological process of contrast transit than Tmax, and possibly more accurately identify critically hypoperfused tissue. Because of these corrections for arterial delay and dispersion, our physiologically modelled Delay Time threshold is expected to be less than the Tmax threshold for infarction. Whether Delay Time is more accurate at identifying critically hypoperfused tissue than Tmax warrants further study.

Some previous CTP studies have also found that CBV, or combined CBF and CBV, thresholds can predict the infarct core. The current study also showed that relative CBV maps were reasonably accurate in AUC analysis at defining the infarct core (figure 5.4). However, the most accurate rCBV threshold (<90% of normal tissue) is too high to be clinically useful. Such a high rCBV threshold in infarct core most likely relates to the fact that the infarct core threshold was calculated within critically hypoperfused tissue. This suggests that virtually the entire infarct core has (at least) a small reduction in CBV, in combination with significant Delay (e.g. >2s). As such, tissue with rCBV <90% and DT >2s was highly sensitive for detecting infarct core. However, we found that tissue with rCBF <40% and DT >2s was as sensitive but a more specific marker of infarct core (Table 5.2).
Despite the large dataset, some limitations should be acknowledged. These relate particularly to the reperfusion definitions. We decided to aim for as ‘pure’ reperfusion and no reperfusion groups as possible to define the most accurate acute perfusion thresholds for infarct core and critically hypoperfused tissue. The cut points (>80% and <20%) we chose were based on previous work\textsuperscript{22}. We also made the assumption that patients with partial reperfusion (between 20% and 80%) would have a broader range of infarct growth, and thereby not allow derivation of an accurate perfusion threshold for critically hypoperfused tissue. Reassuringly, our reperfusion definitions seem valid, based on the data from the patients with acute and 24 hour DWI. There was no significant infarct growth in the major reperfusion group, those in the no reperfusion group had extensive infarct growth, and those with partial reperfusion did have a broad range of infarct growth (supplementary figure 5.2). Notably, we also found the different reperfusion sub-groups had a gradient in clinical outcomes as well as for infarct growth, further indicating the validity of the reperfusion classification. Our reperfusion definition could also be criticised as it compared acute CTP with 24 hour MR perfusion lesion volume. In fact, we have demonstrated that using the same threshold to define lesion volume (MTT >145%), acute CT-MTT and acute MR-MTT lesions were extremely closely correlated. Others have suggested performing follow-up CTP to measure reperfusion, but we now consider that it is very difficult to ethically justify exposing the patients to a doubling of radiation dose when we have an alternative technique (MRI) that can measure reperfusion accurately and, at the same time point, can also determine infarct volume more precisely than CT\textsuperscript{23}. 
Figure 5.2 Practical outcome of study results at determining patients who are eligible for acute treatment. Two patients (the left hand columns are from one patient, and the right hand columns are from the other) in whom there was no reperfusion at 24 hours. The acute and 24 hr DWI were ‘re-sliced’ after co-registration, with the bottom images showing the respective CTP core/penumbra map overlaid on the CTP source image from the same slice location as the DWI. Note the green and red masks reflect acute CTP penumbra and core maps respectively. Note these maps are ‘smoothed’ and generated by cluster analysis to remove isolated ‘noisy’ artifactual pixels typically observed on CTP maps. The examples show that it is possible to accurately predict the acute infarct core and the critically hypoperfused at-risk tissue using acute CTP. Note in both patients the CTP infarct core is very similar to the acute DWI lesion, while the 24 hour DWI closely matches the full extent of acute CTP perfusion lesion (green + red) in these two patients with no reperfusion. These two patients were both imaged within 4.5 hours of symptom onset. The baseline CTP data strongly suggests that the left patient would not benefit from reperfusion treatment (having a large infarct core at baseline – in red), while the second patient would be highly likely to benefit should treatment result in early reperfusion as the at-risk (green) tissue has a very high probability of being salvaged. The CTP core and critically hypoperfused at-risk tissue maps were generated using the ‘best’ thresholds of rDT of 2 seconds for critically hypoperfused tissue and rCBF <40% for the infarct core.

Whilst we believe that our results have widespread applicability in terms of the potential for CTP to accurately predict tissue and clinical outcome, we must
emphasise that the specific thresholds used in our study only apply to the particular software used. It is widely recognized that the absolute values of perfusion maps derived from the same data can be dependent on the post processing algorithms and corrections implemented by different software\textsuperscript{24}. The novel post-processing methodology used in the current study has produced accurate (and clinically valid) measures of CTP infarct core and penumbra. However alternative post-processing algorithms may be equally valid, and our study provides a template for how CT perfusion post-processing methods can be validated for use in the future.

In conclusion, CTP provides rapid and accurate information on stroke tissue pathophysiology. This information has been shown to be critical for prediction of tissue and clinical outcomes. Given the major influence of subsequent reperfusion (or lack thereof) on the baseline CTP measures of core and penumbra, our results have extremely important implications in the use of CTP to select patients for acute reperfusion therapies.
Supplementary figure 5.1 A comparison between CTP and PWI derived MTT 145% lesion map volumes in 67 patients. There is a very strong correlation between CTP and PWI maps ($R^2 = 0.81$, $p=0.001$).
Supplementary Figure 5.2, characterisation of lesion growth between the three study sub groups in patients with acute and 24 hour DWI. The group with major reperfusion at 24 hours shows minimal lesion growth at 24 hours and reflects how accurately the 24 hour DWI lesion reflects infarct core restricted to patients with this reperfusion definition. The group with partial reperfusion (20-80%) showed a very broad range of DWI lesion growth, reflecting the wide range of reperfusion in these patients. These patients were excluded from the infarct core and critically hypoperfused perfusion threshold analysis due to the uncertainty over the contribution of the infarct core and penumbra to their total lesion volume. Finally, the group with no reperfusion showed a large amount of lesion growth at 24 hours. The 24 hour DWI lesion in the no reperfusion group is thus likely to be an accurate representation of the full extent of the critically hypoperfused tissue.
Appendix 5.1

Deconvolution of the AIF and a tissue profile produces a tissue impulse residue function (IRF), where the maximum of the IRF appears at a certain time point, Tmax, where Tmax=0 reflects normal blood supply in normal tissue without delay. In contrast, Tmax>0 is often associated with an acute ischemic lesion due to arterial delay and dispersion effect. However, Tmax is a mathematical parameter derived from a single deconvolution. While the physiological meaning of Tmax is unclear, its value could be dependent on various factors including (but not limited to) arterial delay and dispersion, tissue transit time and dispersion, as well as the cut-off threshold for the SVD deconvolution algorithm. The arterial delay and dispersion effects can be taken into account by a physiological model involving an arterial transport function with a delay time and a relative dispersion. A constant relative dispersion value of 0.35 is used in this study. The modelled delay time value is generally less than the corresponding Tmax value. The method used in this study uses a modified SVD deconvolution approach by looping through a series of delay time values, DTi, ranging from 0 to Tmax. For each delay time, a modelled arterial transport function is convolved with the measured global AIF to produce an AIFi, which is used for SVD deconvolution of the tissue curve to generate an IRFi with its maximum appearing at Tmax(i). The actual delay time, DT, is determined as the minimum DTi value which produces Tmax(i)=0. Subsequently, CBF and CBV can be determined by the maximum and integral of IFRi respectively, with MTT=CBV/CBF.

For Areas of no blood flow, chronic lesion or CSF regions, were removed from the critically hypoperfused at-risk analysis ( No blood flow were removed by eliminating areas where CBF=0 and, CSF/ventricle and skull were removed using a HU threshold and geometric analysis.)
References


Chapter Six:

Perfusion CT in acute ischemic stroke: A comprehensive analysis of deconvolution variation on infarct core and penumbra definition

Aims: There are multiple brand names that produce CT scanners, and just as many different ways to post process computed tomography perfusion (CTP) data. In order to fully validate CTP for clinical use, it is important to understand and quantify variability caused by using different post processing methods. The aim of this project was to investigate all the methods of CTP post processing and assess their accuracy at defining the acute infarct core and penumbra on a human data set. We hypothesise that post processing methods that use a form of deconvolution will be more accurate at defining stroke pathophysiology and that there would be considerable variability between methods.
Abstract

Aim: Despite the potential of perfusion CT (CTP) in acute stroke, the lack of standardized infarct core and penumbral assessment limits its broader implementation. Our aim was to compare the accuracy of the commonly used CTP post processing method. Methods: 314 patients with hemispheric ischemia were imaged with multimodal CT within 6 hours of onset and MRI at 24 hours. Perfusion CT maps were generated using six different post processing methods. Pixel-based receiver operator characteristic curve analysis (ROC) of acute CTP and 24 hour diffusion-weighted-MRI (DWI) was used to define the optimum perfusion thresholds for the penumbra and infarct core with each post processing method. Results: Delay corrected Single Value Deconvolution (ddSVD) using delay time (DT) >2 seconds most accurately defined the penumbra (area under the ROC curve (AUC) 0.86, p=0.046, mean volume difference between acute CTP and 24h DWI = 1.7 mL). A ‘double core threshold’ with DT > 2seconds, and cerebral blood flow (CBF) < 40% provided the most accurate definition of the infarct core (AUC = 0.86, p=0.038). The other SVD measures (block circulant, non-delay corrected) were more accurate than non-SVD methods, and CBV was a poor marker for acute infarct core. Discussion: This is the first study to systematically compare all commonly used CTP post-processing techniques. It has shown marked variability in penumbra and infarct prediction between techniques, and provides the necessary evidence on which much-needed improvements in accuracy and standardization of this promising modality can occur.
Introduction

Perfusion CT (CTP) can readily identify critically hypoperfused tissue including the infarct core and penumbra\(^1\), is fast, and widely available.\(^2\) So why has it not been incorporated into routine clinico-radiological algorithms to select patients for acute reperfusion therapy?\(^3\) One major reason is the variability in accuracy and reliability of the technique due to the different methods used transform the raw perfusion data into volumetric measures of infarct core and penumbra.\(^4\) This lack of standardisation occurs in both clinical and research settings. Limited studies to date have attempted to compare techniques.\(^5\) The aim of this study was to perform the first large-scale systematic comparison of the accuracy of all commonly used CTP data post processing methods to define infarct core and penumbra in acute stroke.

Methods

Patients

A cohort of 314 patients presenting with hemispheric ischemia between 2005 and 2011 were studied. These patients had imaging and clinical data prospectively and systematically collected at set time points after stroke onset as part of our institutional acute stroke perfusion imaging database. Patients were scanned using multimodal CT as a baseline examination (within 6 hours of symptom onset) and with a follow-up MRI stroke sequence at 24 hours. A sub-group of these patients also underwent an acute MRI within an hour of the initial CTP. Clinical stroke severity using the National Institutes of Health Stroke Scale (NIHSS) was performed immediately prior to the two imaging time points. Eligible patients were treated with intravenous thrombolysis according to standard guidelines. Other analyses using
subsets of this dataset have previously been performed.\textsuperscript{1,6} The collection of data for this study as approved by the institutional ethics committee and all patients gave informed consent.

**Imaging**

Whole brain non-contrast CT (NCCT) was followed by perfusion CT, comprising two 60-second series (64-slice Philips Brilliance; Philips, Cleveland, Ohio). CT perfusion imaging was performed with an intravenous bolus injection of contrast agent (40 mL of ultravist 370; Bayer HealthCare, Berlin, Germany) injected at a rate of 6 mL/sec, with 45 time points acquired each 1.33 seconds. Each perfusion series covered a 24 to 40-mm section acquired as four adjacent six-mm. The first section was at the level of the basal ganglia/internal capsule, and the second was placed 6-mm towards the vertex to avoid overlap. CT angiography (CTA) was performed after CTP with acquisition from the aortic arch to the top of the lateral ventricles.\textsuperscript{7}

MR imaging was performed on a 1.5T MRI (Siemens Avanto, Erlangen, Germany). The stroke MRI protocol included an axial isotropic diffusion-weighted echoplanar spin-echo sequence (DWI), time of flight MR angiography (MRA) and bolus-tracking perfusion weighted imaging (PWI).\textsuperscript{8}

**Perfusion Models**

All perfusion CT maps were calculated by the commercial software MIStar (Apollo Medical Imaging Technology, Melbourne, Australia).\textsuperscript{3} The software
automatically performs motion correction and selects an arterial input function (AIF) from an unaffected artery (most often the anterior cerebral artery) and a venous output function (VOF) from a large draining vein (the sagittal sinus). These were checked manually for accuracy and adjusted if necessary. Chronic infarcts/gliosis and CSF regions were automatically detected by a Hounsfield unit threshold and removed from the analysis. Areas of no blood flow were assigned maximal delay time (DT) and mean transit time (MTT) values. The perfusion lesion segmentation algorithm also uses a cluster analysis technique to remove small clusters of noisy pixels\(^6\). Perfusion maps were then calculated respectively using six different methods; Maximum slope (Peters) model\(^{14}\), Partial Deconvolution\(^{16}\), Singular Value Decomposition (SVD)\(^{17}\), Single Value Deconvolution with Delay Correction (ddSVD)\(^{18}\), Block Circulant Deconvolution (BCD)\(^{20}\), and a Stroke-Stenosis Model (sSVD). The theoretical basis and assumptions in each model are detailed in Supplementary Appendix One.

**Patient grouping**

Patients were divided into three groups based on change between acute CT and 24 hour MR mean transit time (MTT) 145% lesion volume\(^7,9\): major reperfusion (>80% at 24 hours), no reperfusion (<20% reperfusion at 24 hours) and partial reperfusion (between 20% and 80%). We have previously shown that patients with major reperfusion do not have significant infarct growth at 24 hours, thus the acute infarct core accurately represents the 24 hour DWI lesion.\(^6\) Patients with no reperfusion (<20%) have considerable acute-24 hour DWI infarct growth, and this volume difference is a reliable estimate of the extent of penumbra.\(^6\) The partial reperfusion group were excluded from further analyses because of wide variability in
acute infarct core to 24h DWI growth. Lastly, patients with acute DWI (67) were pooled with the patients who showed major reperfusion at 24 hours.

As a secondary analysis of acute infarct core, DWI from a subset of patients who had MRI within 1 hour of the acute CTP was used for comparative analyses.

**Image analysis**

Perfusion CT maps were coregistered to the corresponding 24 hour ± acute DWI\(^1,10\). Acute and 24 hour DWI lesions were automatically delineated based on signal intensity thresholds of \(b=1000\) to rule out noise and false infarct. Next, the area of interest was transferred to the co-registered acute CTP maps for analysis (see below). A range of relative (as a percentage of contralateral hemisphere) and absolute thresholds were then investigated at constant increments as shown in Table 6.1. Thresholds tested included those reported in previous studies as predictive of tissue fate\(^11\).

<table>
<thead>
<tr>
<th>CTP parameter</th>
<th>Range</th>
<th>Increments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative CBV</td>
<td>10 to 70%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBV</td>
<td>1 to 2.5 mL/100g</td>
<td>0.25 mL/100g</td>
</tr>
<tr>
<td>Relative CBF</td>
<td>10 to 60%</td>
<td>5%</td>
</tr>
<tr>
<td>Absolute CBF</td>
<td>3 to 17 mL/100g/min</td>
<td>2 mL/100g/min</td>
</tr>
<tr>
<td>Delay(Tmax)</td>
<td>2 to 10 s + baseline</td>
<td>2 seconds</td>
</tr>
<tr>
<td>MTT</td>
<td>150-300%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Table 6.1: Range and increments used for ROC analysis to investigate CTP thresholds to define the infarct core.
Statistical Analysis

The acute infarct core was defined as the volume of the 24 hour DWI volume in patients with major reperfusion by 24 hours (or patients with acute DWI). The acute perfusion lesion (core + penumbra) was defined by the volume of the 24 hour DWI in patients without major reperfusion. Subtracting the acute infarct core volume from the acute perfusion lesion volume defines the volume of the acute penumbra.

Receiver Operating Characteristic (ROC) Curve Analysis was used to test the predictive performance of CTP in relation to the DWI infarct core. The DWI image was considered to be the ‘true’ lesion and the pixels falling within both the DWI lesion and CTP lesions were considered ‘true positive’ (TP). DWI b=1000 threshold not within the CTP lesion were considered ‘true negative’. Pixels within the CTP lesion but not within the DWI lesion were assigned ‘false positive’ (FP), and pixels within the DWI lesion but not within the CTP lesion were assigned ‘false negative’ (FN). Sensitivity (TP/(TP + FN)) was plotted against 1 - Specificity (TN/(TN + FP)) to generate the ROC curve for each perfusion map. Results presented are area under the curve (AUC, and 95% CIs) for the ROC curve for each perfusion map.

In order to provide balance in the number of pixels being measured and prevent a very large true negative value from overwhelming the ratio to false positives in the calculation of specificity, only hemispheric (ischemic side) brain pixels were analysed rather than whole brain.\textsuperscript{1}

Once the most accurate CTP perfusion lesion threshold had been identified from the no reperfusion group, two analyses were performed to derive infarct core
thresholds: (i) the 24 hour DWI was compared with acute CTP in all patients with major reperfusion, and (ii) the acute CTP was compared with the acute DWI in the sub-group of patients who had concurrent acute MRI.

*Double-threshold infarct core analysis.* We have previously shown that infarct core thresholds are more accurate when restricted to within a pre-defined outer perfusion lesion threshold\(^1\). Thus, analyses were undertaken to compare accuracy of restricting the infarct core thresholds within the most accurate outer perfusion lesion thresholds (derived from the above ‘no reperfusion’ threshold analysis), versus single core threshold, for all six post-processing methods.

To internally validate the AUC results, a lesion volume analysis was also undertaken to determine the closeness of fit between the CTP-threshold infarct core and total perfusion lesion volumes with 24 hour DWI volumes. Lesion volumes were calculated for each threshold and plotted against the corresponding 24 hour DWI lesion volume (or acute DWI lesion volume, in the secondary analysis). Regression analysis was used to determine the closeness of fit (see Table 1 for thresholds assessed). The same major reperfusion and no reperfusion patient classifications were used for the correlation with 24 hour DWI. Thus, the most accurate acute CT perfusion thresholds for infarct core were used to generate lesion volumes, and correlated with the volume of the DWI lesion in the major reperfusion group. In the no reperfusion group, the most accurate acute CT perfusion lesion thresholds were used to generate total perfusion lesion volumes, and correlated with the final DWI lesion volume. Each analysis was repeated for the six different deconvolution techniques using the same patient cohort.
Clinical and volumetric data was also analysed using paired $t$-tests, corresponding Pearson’s correlation coefficients and summary statistics.

**Results**

From the total patient cohort of 316 patients, 146 had no significant reperfusion at 24 hours and were used to define the outer perfusion lesion threshold. There were 106 patients with major reperfusion at 24 hours, used to define the acute infarct core thresholds. Also, 64 patients showed partial reperfusion and were excluded from analysis. Sixty-seven patients received concurrent acute DWI (within one hour of acute CTP) and were used to define the acute infarct core in the secondary analysis.

The median age of patients was 70 years (range 23-89), median acute NIHSS was 13 (range 5-24) and median time to end of CTP was 162 minutes (IQR 185-240 mins). Intravenous thrombolysis was administered to 174 patients according to institutional guidelines. Of the patients given thrombolysis, 82 showed major reperfusion (47%), 57 showed no significant reperfusion (32%) and 35 showed partial reperfusion on follow-up imaging (20%).

A comprehensive analysis of the results are presented in table two and summarised here:
1. **Maximum slope model**

Relative cerebral blood flow (CBF) <45% (AUC 0.68, CI 0.64-0.72) was the best single threshold to describe the acute infarct core. MTT >145% (AUC 0.71, CI 0.67-0.75) was the best threshold to describe the acute perfusion lesion. Using the double threshold infarct core approach, relative CBF <40% within the MTT >145% perfusion lesion (AUC 0.63, CI 0.61-0.65) did not improve the accuracy of the single infarct core threshold (CBF <45%, table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.1.

2. **Partial Deconvolution**

Relative CBF <20% (AUC 0.71, CI 0.65-0.77) and absolute CBF <10 mL/100g/min were the equal best descriptors of the acute infarct core. The acute perfusion lesion was most accurately described by MTT >155% (AUC 0.73, CI 0.69-0.77) or TTP >4s (AUC 0.74, CI 0.7-0.78). A double threshold approach did not improve accuracy for infarct core (table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.2.

3. **Singular Value Deconvolution (SVD)**

Absolute CBF <20 mL/100g/min (AUC 0.7, CI 0.75-0.85) best described infarct core. The acute perfusion lesion was best defined by Tmax of >6 seconds (AUC 0.77, CI 0.73-0.81). A double threshold approach using a CBF of 7.5 mL/100g/min within the Tmax >6s lesion was more accurate at defining the acute infarct core than the best single threshold (AUC 0.74, CI 0.7-0.78, table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.3.
4. Single Value Deconvolution with Delay Correction (ddSVD)

Using SVD with delay correction, CBF <45% (AUC 0.77, CI 0.73-0.81) was the most accurate single threshold for acute infarct core. A delay time >2s (AUC 0.86, CI 0.84-0.88) most accurately defined the acute perfusion lesion. A double threshold approach, CBF <40% within a delay time lesion >2s (AUC 0.86, CI 0.83-0.89) more accurately defined the acute infarct core than any single threshold (table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.4.

5. Block Circulant Deconvolution (BCD)

Using block circulant deconvolution, absolute CBF <15mL/100g/min (AUC 0.73, CI 0.7-0.76) provided the best single threshold definition of the acute infarct core. Relative Tmax >4s (AUC 0.72) or an absolute Tmax >5s (AUC 0.71, CI 0.66-0.76) was the best descriptor of the acute perfusion lesion. The double threshold of CBF <15mL/100g/min within Tmax >5s most accurately defined the acute infarct core (AUC 0.79, CI 0.75-0.83) (table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.5.

6. Stenosis Model (sSVD)

Using the stroke stenosis model, absolute CBF <10mL/100g/min was the best threshold to define the acute infarct core (AUC 0.72, CI 0.69-0.75). The acute perfusion lesion was best defined by absolute CBF <20mL/100g/min (AUC 0.72, CI 0.68-0.76). Restricting the best infarct core threshold (absolute CBF <10 mL/100g/min) within the acute perfusion threshold did not improve the definition of the acute infarct core (AUC 0.72, CI 0.69-0.75) (table 6.2). A comprehensive analysis of the results are presented in supplementary table 6.6.
<table>
<thead>
<tr>
<th>Maximum slope model</th>
<th>Threshold</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
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<tbody>
<tr>
<td>Infarct Core</td>
<td>CBF 45%</td>
<td>0.69</td>
<td>0.46</td>
<td>0.89</td>
<td>-0.8(-5.1 – 7.3)</td>
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<td>0.1(-4.3-4.5)</td>
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<td>Double core model</td>
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<td>0.77</td>
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<td>0.74</td>
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<tr>
<td>Double core model</td>
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<td>0.66</td>
<td>0.84</td>
<td>1.6 (1.1-2.5)</td>
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<tr>
<th>Singular Value Decomposition (SVD)</th>
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<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
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<tbody>
<tr>
<td>Infarct Core</td>
<td>CBF 20 mL/100g/min</td>
<td>0.7</td>
<td>0.7</td>
<td>0.69</td>
<td>-3.1 (-5.3-0.7)</td>
<td>0.72</td>
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<td>0.75</td>
<td>0.72</td>
<td>0.4 (-0.6-1.1)</td>
<td>0.74*</td>
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<tr>
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<td>0.56</td>
<td>0.92</td>
<td>0.5 (-0.3-0.9)</td>
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<table>
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<tr>
<th>Single Value Deconvolution with Delay Correction (ddSVD)</th>
<th>Threshold</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
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<tbody>
<tr>
<td>Infarct Core</td>
<td>CBF 45%</td>
<td>0.77</td>
<td>0.75</td>
<td>0.79</td>
<td>1.99 (0.3-3.6)</td>
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<td>Perfusion lesion</td>
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<td>0.82</td>
<td>1.7 (0.3-2.6)</td>
<td>0.76*</td>
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<tr>
<th>Block Circulant Deconvolution (BCD)</th>
<th>Threshold</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
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<td>0.73</td>
<td>0.73</td>
<td>0.77</td>
<td>4.7 (0.9-8.7)</td>
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</tr>
<tr>
<td>Perfusion lesion</td>
<td>bTmax +4</td>
<td>0.72</td>
<td>0.82</td>
<td>0.62</td>
<td>2.2 (-0.5-4.9)</td>
<td>0.72*</td>
</tr>
<tr>
<td>Double core model</td>
<td>CBF 15 mL/100g/min + bTmax +4</td>
<td>0.79</td>
<td>0.75</td>
<td>0.79</td>
<td>1.1 (-0.5-2.3)</td>
<td>0.71</td>
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<tr>
<th>Stenosis Model</th>
<th>Threshold</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
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<tbody>
<tr>
<td>Infarct Core</td>
<td>CBF 10 mL/100g/min</td>
<td>0.75</td>
<td>0.84</td>
<td>0.65</td>
<td>3.6 (0.6-5.4)</td>
<td>0.67</td>
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<tr>
<td>Perfusion lesion</td>
<td>CBF 20 mL/100g/min</td>
<td>0.72</td>
<td>0.78</td>
<td>0.66</td>
<td>3.7 (0.9-4.9)</td>
<td>0.75</td>
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Table 6.2. Results for each of the six deconvolution analysis at defining the acute infarct core, perfusion lesion and the double perfusion threshold approach to improve the definition of the acute infarct core. Each deconvolution method has an optimal threshold, however it is a consistent finding that CBF best defines the acute infarct core. Also, when delay correction is applied delay measures (Tmax and delay time) are optimal at defining the acute perfusion lesion. When delay correction is not used, MTT is the optimal measure to define the acute perfusion lesion, but this is less accurate. *p<0.05

Volumetric analysis

The volumetric analysis results paralleled the ROC analysis (Table 6.2). Thus, ddSVD derived acute infarct core volumes had the strongest correlation with the acute DWI volume and 24 hour (major reperfusion group) analyses ($r^2=0.72$, $p=0.045$). Also, ddSVD derived acute perfusion lesion volume ($r^2=0.76$, $p<0.035$), and double threshold infarct core definition ($r^2=0.79$, $p<0.042$) had the strongest relationship with DWI lesion volume.

Overall, ddSVD achieved the highest AUC compared to all other methods and showed the least variation between acute CTP and 24 hour lesion volumes. Thus, ddSVD was the most accurate post processing method to describe the acute infarct core (figure 6.1) and the acute perfusion lesion (figure 6.2).

A comparison between patients with acute DWI volume with 24 hour DWI volume in reperfused patients showed only a small lesion volume increase of 1.99cm$^3$ (standard deviation 1.83cm$^3$ $p=0.43$). Analysis using the 24 hour follow-up DWI in the 23 patients with acute DWI and major reperfusion yielded very similar results to the main analysis ($p=0.62$).
Figure 6.1 Acute perfusion lesion AUC results using Delay Time measures. Variation in AUC for delay time measures (DT for ddSVD, Tmax for SVD, BCD and stenosis modal, or TTP for non-SVD methods) thresholds to define the acute perfusion lesion. We see that ddSVD (red) reaches the highest point on the AUC graph, and represents the most accurate measure and threshold to determine the volume of the acute perfusion lesion. Other methods, while with a lower AUC, also show a single threshold that is optimal.

Figure 6.2 acute infarct core AUC results using the double threshold CBF and delay time measure. Variation in AUC across CBF (%) thresholds to define the acute infarct core using a
double threshold approach. The outer threshold was the optimal delay time measure threshold from figure 2. We see that ddSVD (red) reaches the highest point on the AUC graph, and represents the most accurate measure and threshold to determine the volume of the acute infarct core using a double threshold.

**Discussion**

In a well characterised and large dataset we have found considerable variation in the accuracy of core and penumbra detection, and the perfusion thresholds used to determine them, with the commonly used perfusion algorithms. Single value deconvolution with a delay and dispersion correction (ddSVD) was consistently the most accurate method, showing the best combination of sensitivity and specificity, as measured by ROC curve analysis. This was confirmed in the volumetric analysis, which showed a very small difference in lesion volume between acute CTP-defined and 24 hour DWI lesions. Although all but the maximum slope method demonstrated AUCs to define infarct core and penumbra >0.7 (Table 6.2, figure 6.1), the best threshold value varied considerably depending on the deconvolution method used. However, no matter which perfusion algorithm was used, a CBF measure was the most accurate to define the acute infarct core. This is in contrast to previously published data which has lead to CBV being a widely used core threshold clinically to define infarct core\(^\text{10}\). To define critically hypoperfused tissue (the outer perfusion threshold), the measures of delay (DT, Tmax) were the most accurate using all deconvolution algorithms (figure 6.3).

An important result of the study is the increased accuracy of the double threshold approach for infarct core detection with all SVD methods. By restricting the infarct core threshold to within the acute perfusion lesion, the accuracy, as well as
variability of the infarct core definition was improved for all deconvolution techniques. We therefore recommend this approach. However for the non-SVD algorithms, (partial deconvolution and the stenosis model), a double threshold approach did not improve the accuracy for detecting the acute infarct core. These two methods were clearly less accurate at defining both the acute perfusion lesion and infarct core. Therefore, these methods should not be used in acute stroke.

These findings mark a departure from previous widely accepted dogma that CBV is best used to define infarct core and MTT to define the acute perfusion lesion.\(^9\) The current study found that no matter the post processing method used, CBF was the best infarct core measure and delay measures (DT, Tmax; depending on method) were the most accurate for the acute perfusion lesion. The higher CBF threshold for infarct core and lower delay time threshold for hypoperfused tissue (DT >2s) seen with ddSVD is a result of the CBF and delay time values being less affected by delay and dispersion. Lack of correction results in underestimated CBF and overestimated MTT and Tmax perfusion values.\(^{12,13,14}\) For example, the commonly used Tmax >6s threshold in stroke MRI to define the acute perfusion lesion is identical to that seen with our SVD (uncorrected) method for CTP, but is much greater than the DT >2s for the acute perfusion lesion with ddSVD. The different optimal thresholds seen with varying post processing options highlight the requirement of multicentre trials to use standardized infarct core and penumbra thresholds.

The analysis comparing acute and 24 hour DWI volumes in patients with major reperfusion showed no significant difference. This validates our use of the
reperfusion measure as there was minimal infarct growth between the two scans. Therefore CTP used with different deconvolution methods would have the same results if compared to an acute DWI. This has been shown previously, and is confirmed in this study.\textsuperscript{1,6,10}

A potential limitation of this study is the use of DWI as a gold standard comparison. However recent studies have found that DWI is indeed an accurate measure of the infarct core when the false positive lesion is ruled out\textsuperscript{15}, as such a threshold of $b=1000$ was applied, both acutely and sub-acutely. Moreover, we have shown minimal infarct growth between acute and 24 hour DWI in patients with major reperfusion, confirming the validity of DWI as a measure of the acute infarct core. Another possible limitation is that the algorithms used in the current study are based on the original published methods, however CT vendors may have adjusted their post processing algorithms from the original versions (e.g. additional noise correction or 'smoothing'). Such alterations are not available to us due proprietary concerns.
Figure 6.3. One patient’s acute CTP processed with 6 different perfusion algorithms. In the top left is the patient’s acute and 24 hour DWI imaging showing the acute infarct core and the total perfusion lesion following failed reperfusion therapy. This figure allows a visual comparison between all deconvolution techniques maps of CBF, CBV, MTT and TTP (or Tmax, bcTmax, Delay time). The last map on the right is the core/penumbra map with the optimal thresholds describing acute infarct core (red) and penumbra (green) from each method. It can be seen there is dramatic variability in the extent of the infarct core and perfusion lesion with differing methodology.
This is the first study that compares all the commonly used deconvolution methods to define acute stroke pathophysiology (and in a large dataset). The main problem with stroke CTP currently is the inability to apply the results of studies using different perfusion algorithms to generate core and penumbra to one’s own clinical practice, let alone decide which of the many algorithms to use in multicentre trials to select patients for acute therapy where standardization of infarct core and penumbral volumes is crucial.\textsuperscript{16,17,18,19} The large variations seen in different post processing methods to describe acute infarct core and penumbra may explain why various centres have different experiences with CTP. Moreover, we found that the commonly used CBV measure\textsuperscript{11} of the acute infarct core is inaccurate for all methods used. Our study provides the necessary evidence on which much-needed improvements in accuracy and standardisation of this promising modality can occur. With standardization, CTP has the potential to be a far more accurate and reliable tool than many have experienced to date.
Appendix 6.1 – Deconvolution calculation methods used.

**Maximum slope (Peters) model**

Under the assumption of no venous outflow, CBF can be calculated as the maximum initial slope of the time enhancement curve in tissue divided by the maximum enhancement within the brain supplying artery\(^\text{20}\).

CBV is defined as the fractional vascular volume within a tissue voxel. The CBV is calculated as the maximum enhancement of the time enhancement curve in tissue divided by the maximum enhancement in blood\(^\text{21}\).

MTT is calculated as the ratio of CBV/CBF for each pixel according to the central volume principle.

TTP is calculated in seconds as the time from the start of contrast arrival in the AIF to the peak of the time enhancement curve for each voxel.

**Partial Deconvolution**

The MTT map is calculated using a closed-form (non-iterative) deconvolution approach\(^\text{22}\). A box-shaped residue function of certain width can be convolved with the AIF to produce a simulated time enhancement curve. A series of width values are evaluated by least-mean-squares fitting of the simulated curves against the measured time enhancement curve, and the MTT is determined as the width value corresponding to the best fit.
The CBV map is calculated from the area under the time enhancement curves (AUC) divided by the AUC of the scaled AIF. In order to remove contrast recirculation effect on the calculation of CBV, a gamma-variant curve fitting is applied to the time attenuation curves of tissue and AIF respectively. The gamma-validated fitted curves are used for CBV calculation in this study.

CBF is calculated as the ratio of CBV/MTT for each pixel according to the central volume principle.

TTP is calculated in the same way as the maximum slope method. That is, the TTP is calculated in seconds as the time from the start of contrast arrival in the AIF to the peak of the time enhancement curve for each voxel.

**Singular Value Decomposition (SVD) Deconvolution**

The tissue time enhancement curves are deconvolved with the AIF using the SVD method\textsuperscript{23} to produce a impulse residue function (IRF), and various perfusion maps can be calculated from this IRF. CBF is calculated from the peak height of the IRF curve, CBV is calculated from the area under the IRF curve, and MTT is calculated as the ratio of CBV/CBF according to the central volume principle.

In addition, the peak of the IRF curve may not always occur at zero time point, particularly for pixels with abnormal perfusion. A parameter, termed as Tmax, is calculated from the time to peak of the IRF curve, where Tmax=0 reflects normal blood supply in normal tissue without delay. In contrast, Tmax>0 is often associated
with an acute ischemic lesion due to arterial delay and dispersion effect. A global SVD threshold of $P_{svd}=0.2$ was used in this study.

**Single Value Deconvolution with Delay Correction**

In order to compensate for arterial delay and dispersion effects, a vascular transport model involving an arterial transport function with a delay time and a relative dispersion has been proposed\(^{24}\). The effect of the arterial transport function is to shift and broaden the AIF profile, in an attempt to more realistically model the physiology of acute stroke. This method uses a delay-corrected SVD deconvolution approach by applying a series of delay time values, $D_{Ti}$, ranging from 0 to $T_{max}$. For each delay time, a modelled arterial transport function is convolved with the measured global AIF to produce an $AIF_{i}$, which is used for SVD deconvolution of the tissue curve to generate an $IRFi$ with its maximum appearing at $T_{max(i)}$. The actual delay time, $DT$, is determined as the minimum $D_{Ti}$ value which produces $T_{max(i)}=0$. Subsequently, CBF and CBV can be determined by the peak height and AUC of $IRFi$ respectively, with $MTT = CBV/CBF$. A constant relative dispersion value of 0.35 is used in this study. It should be pointed out that $DT$ is different from $T_{max}$.

**Block Circulant Deconvolution (BSD)**

In the case of major vessel disease, such as acute stroke or carotid artery stenosis, the measured AIF is often associated with a delay and dispersion before it reaches the tissue of interest, and causes overestimation of the MTT and underestimation of the CBF\(^{25}\). The block-circulant deconvolution method\(^{26}\) was proposed as a delay-insensitive deconvolution technique to avoid CBF underestimation. This approach
extends the conventional SVD method by using a by the use of a block-circulant matrix. As the block-circulant method may produce spurious oscillations dominating the deconvolved IRF, it uses an optimization approach by minimizing an oscillation index with adjustable pixel-dependent Psvd threshold. Using delay time calculated by BSD was labeled as dTmax to reduce confusion with other measures of TTP/Tmax/delay.

**Stroke-Stenosis Model**

In contrast to the delay-corrected SVD method, this model is based on a forward-deconvolution method using least-square-fitting (LSF) deconvolution involving the vascular transport model as described above. Since all model-free deconvolution methods (such as SVD) are sensitive to noise, some mathematical cut-off threshold (Psvd) has to be assumed in order to derive meaningful tissue IRF. In contrast, the LSF deconvolution method uses the distributed-parameters model (TY Lee) to attempt to describe a more realistic tissue IRF, where all perfusion parameters (CBV, CBF, MTT, DT, arterial dispersion and tissue dispersion) can be determined by LSF21. For the purpose of comparison in this study, a constant arterial relative dispersion value of 0.35 is used (not a fitting parameter).

To minimize noise effect from CT perfusion imaging data, the time enhancement curve is smoothed by a gamma-variates least-mean-square fitting method. The fitted curves are used for the calculation of the above perfusion maps.
References


S Perfusion-ct assessment of infarct core and penumbra: Receiver operating characteristic curve analysis in 130 patients suspected of acute hemispheric stroke. Stroke(2006);37:979-985


Appendix 6.2 – details of best three thresholds for infarct core and perfusion lesion for each method

### Maximum slope model

**Infarct Core Definition**

<table>
<thead>
<tr>
<th>CBF</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) mL³</th>
<th>r²</th>
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<tr>
<td>40%</td>
<td>0.69</td>
<td>0.4</td>
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<td>0.61</td>
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<tr>
<td>45%</td>
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<td><strong>0.89</strong></td>
<td><strong>0.45</strong></td>
<td><strong>0.89</strong></td>
<td>-0.8(-5.1 – 7.3)</td>
<td><strong>0.65</strong></td>
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<td>50%</td>
<td>0.69</td>
<td>0.52</td>
<td>0.86</td>
<td>0.42</td>
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**Perfusion lesion Definition**

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<thead>
<tr>
<th>MTT</th>
<th>AUC (Standard deviation)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>140%</td>
<td>0.71 (0.05)</td>
<td>0.67</td>
<td>0.74</td>
<td>0.62</td>
<td>0.78</td>
<td>1.5(-2.4 – 5.2)</td>
<td>0.73</td>
</tr>
<tr>
<td>145%</td>
<td><strong>0.71 (0.05)</strong></td>
<td><strong>0.63</strong></td>
<td><strong>0.78</strong></td>
<td><strong>0.65</strong></td>
<td><strong>0.77</strong></td>
<td><strong>0.1(-4.3-4.5)</strong></td>
<td><strong>0.76</strong>*</td>
</tr>
<tr>
<td>150%</td>
<td>0.71 (0.05)</td>
<td>0.6</td>
<td>0.81</td>
<td>0.66</td>
<td>0.76</td>
<td>-1.4(-5.3 – 6.5)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Double core threshold**

<table>
<thead>
<tr>
<th>MTT 145%</th>
<th>+</th>
<th>CBF 40%</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm³</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>0.67</strong></td>
<td><strong>0.53</strong></td>
<td><strong>0.77</strong></td>
<td><strong>0.96</strong></td>
<td><strong>0.088</strong></td>
<td></td>
<td><strong>-3.9(-6.4--2.5)</strong></td>
<td><strong>0.69</strong>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.63</td>
<td>0.32</td>
<td>0.92</td>
<td>0.98</td>
<td>0.8</td>
<td></td>
<td>2.8( 2 – 3.6)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Table 6.3.1. Maximum slope model results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. An MTT of 145% is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 45%, and when combined with the perfusion lesion threshold of MTT 145%, a CBF of 40% is the most accurate to define the acute infarct core. *p=0.05
Table 6.3.2. Partial deconvolution results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. An MTT of 155% is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 20%, and when combined with the perfusion lesion threshold a CBF of 20% is the most accurate to define the acute infarct core. *p=0.05
Table 6.3.3. The SVD results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. A Tmax of 6 seconds is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 25%, and when combined with the perfusion lesion threshold a CBF of 7.5 mL/100g/min is the most accurate to define the acute infarct core. *p=0.05

<table>
<thead>
<tr>
<th>Infarct Core Definition</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm$^3$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF 25%</td>
<td>0.66</td>
<td>0.41</td>
<td>0.86</td>
<td>0.43</td>
<td>0.9</td>
<td>0.6 (-0.9-2.4)</td>
<td>0.68</td>
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<tr>
<td>CBF 20 mL/100g/min</td>
<td>0.7</td>
<td>0.7</td>
<td>0.69</td>
<td>0.94</td>
<td>0.57</td>
<td>-3.1 (-5.3--0.7)</td>
<td>0.72</td>
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<table>
<thead>
<tr>
<th>Perfusion lesion Definition</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm$^3$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT 150%</td>
<td>0.72</td>
<td>0.69</td>
<td>0.75</td>
<td>0.73</td>
<td>0.55</td>
<td>-1.7(2.4-0.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Tmax 6</td>
<td>0.77</td>
<td>0.75</td>
<td>0.72</td>
<td>0.81</td>
<td>0.53</td>
<td>0.4 (-0.6-1.1)</td>
<td>0.74*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Double core threshold</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm$^3$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT 155% + CBF 30%</td>
<td>0.7</td>
<td>0.52</td>
<td>0.88</td>
<td>0.9</td>
<td>0.66</td>
<td>0.6(-0.4-1.2)</td>
<td>0.73</td>
</tr>
<tr>
<td>CBF 7.5 mL/100g/min</td>
<td>0.72</td>
<td>0.54</td>
<td>0.9</td>
<td>0.91</td>
<td>0.64</td>
<td>0.8(-0.9-1.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Tmax 6 + CBF 30%</td>
<td>0.73</td>
<td>0.53</td>
<td>0.89</td>
<td>0.91</td>
<td>0.68</td>
<td>0.6(-0.4-1.2)</td>
<td>0.74</td>
</tr>
<tr>
<td>CBF 7.5 mL/100g/min</td>
<td>0.74</td>
<td>0.56</td>
<td>0.92</td>
<td>0.92</td>
<td>0.69</td>
<td>0.5(-0.3-0.9)</td>
<td>0.74*</td>
</tr>
</tbody>
</table>

Table 6.3.3. The SVD results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. A Tmax of 6 seconds is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 25%, and when combined with the perfusion lesion threshold a CBF of 7.5 mL/100g/min is the most accurate to define the acute infarct core. *p=0.05
Table 6.3.4. The SVD with delay correction results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. A rDelay of 2 seconds is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 45%, and when combined with the perfusion lesion threshold a CBF of 40% is the most accurate to define the acute infarct core. *p=0.05
<table>
<thead>
<tr>
<th>Block Circulant Deconvolution</th>
</tr>
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<tbody>
<tr>
<td>Infarct Core Definition</td>
</tr>
<tr>
<td>AUC</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>CBF 45%</td>
</tr>
<tr>
<td>CBF 15 mL/100g/min</td>
</tr>
<tr>
<td><strong>Perfusion lesion Definition</strong></td>
</tr>
<tr>
<td>AUC</td>
</tr>
<tr>
<td>-----------------</td>
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<tr>
<td>CBF 55%</td>
</tr>
<tr>
<td>bTmax +4</td>
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<td>bTmax 5</td>
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<td><strong>Double core threshold</strong></td>
</tr>
<tr>
<td>AUC</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Tmax +4</td>
</tr>
<tr>
<td>CBF 40%</td>
</tr>
<tr>
<td>CBV 50%</td>
</tr>
<tr>
<td>CBF 15 mL/100g/min</td>
</tr>
<tr>
<td>Tmax 5 seconds</td>
</tr>
<tr>
<td>CBF 40%</td>
</tr>
<tr>
<td>CBV 50%</td>
</tr>
<tr>
<td>CBF 15 mL/100g/min</td>
</tr>
</tbody>
</table>

Table 6.3.5. The circulant SVD with delay correction results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. A bTmax of 4 seconds is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 15 mL/100g/min, and when combined with the perfusion lesion threshold a CBF of 15 mL/100g/min is the most accurate to define the acute infarct core. *p=0.05
### Stenosis Model

#### Infarct Core Definition

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>r&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF 30%</td>
<td>0.72</td>
<td>0.85</td>
<td>0.6</td>
<td>0.56</td>
<td>0.88</td>
<td>3 (-0.3-6.4)</td>
<td>0.66</td>
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<tr>
<td>CBF 10 mL/100g/min</td>
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<td>0.48</td>
<td>0.9</td>
<td>3.6 (0.6-5.4)</td>
<td>0.67</td>
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#### Perfusion lesion Definition

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<tr>
<th></th>
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<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>r&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF 35%</td>
<td>0.69</td>
<td>0.72</td>
<td>0.66</td>
<td>0.57</td>
<td>0.79</td>
<td>4.5 (0.5-9)</td>
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</tr>
<tr>
<td>CBF 20 mL/100g/min</td>
<td>0.72</td>
<td>0.78</td>
<td>0.66</td>
<td>0.56</td>
<td>0.84</td>
<td>3.7 (0.9-4.9)</td>
<td>0.75</td>
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#### Double core threshold

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Mean Volume Error (95% CI) cm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>r&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF 20 mL/100g/min</td>
<td>0.72</td>
<td>0.82</td>
<td>0.63</td>
<td>0.49</td>
<td>0.89</td>
<td>1.6 (-0.3-2.6)</td>
<td>0.73</td>
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</table>

Table 6.3.6. The Stroke Stenosis with delay correction results for the detection of the acute infarct core, perfusion lesion and the combination of results required for an effective mismatch in order to improve the accuracy of acute infarct core. A CBF of 20 mL/100g/min is the most accurate when defining the acute perfusion lesion. The infarct core is best defined by a CBF of 10 mL/100g/min, and when combined with the perfusion lesion threshold a CBF of 10 mL/100g/min is the most accurate to define the acute infarct core. *p=0.05
Chapter Seven:

Arterial Spin Labeling identifies tissue salvage and good clinical recovery in acute ischemic stroke

Aim: Perfusion imaging requires the injection of a contrast agent into a patient’s blood stream, however there are some patients who suffer an allergic reaction to the contrast agent, and some centres do now allow the administration of contrast outside of work hours due to concerns over these allergic reactions. Therefore, in order to implement perfusion imaging routinely, it would need to be assessable at all hours and to all patients. A contrast free form of perfusion imaging is the Magnetic resonance imaging technique, arterial spin labeling (ASL). The aim of this study was to investigate the ASL perfusion imaging technique and gain an understanding of how it might be used in ischemic stroke patients.

Abstract:

Introduction: Arterial spin labeling (ASL) is a relatively new MR perfusion technique that requires validation. Methods: One hundred patients with an acute hemispheric ischemic stroke were imaged within 6 hours of symptom onset with perfusion CT (CTP), and at 24 hours with MRI including ASL and susceptibility weighted perfusion imaging (PWI). Baseline CTP was used to define tissue at risk. This was used to determine persistent hypoperfusion, or hyperperfusion, on 24 hour ASL maps. Results: Using 24 hour ASL, 48 of 100 patients showed hyperperfusion, and 41 showed persistent hypoperfusion. None of the PWI maps identified hyperperfusion. Compared to patients with persistent hypoperfusion on ASL, patients
with hyperperfusion had less progression of acute CTP mismatch tissue to infarction at 24 hours (p<0.05). ASL hyperperfusion was also associated with improved early clinical improvement: mean reduction in acute to 24h National Institutes of Health Stroke Scale = 12, vs 4 for ASL hypoperfusion group (p < 0.05), as well as 90 day modified Rankin Score (mean 2, vs 4 for hypoperfusion group, p<0.01). **Discussion:** Hyperperfusion of the initially ischemic area identified on ASL at 24 hours post stroke identifies patients with better tissue and clinical outcomes.
Introduction:

Perfusion MRI using gadolinium based contrast agents (PWI) is commonly used in acute ischemic stroke to identify hypoperfused tissue\textsuperscript{1,2}. The rare, but potentially fatal, reaction to gadolinium-based contrast agents that occurs with severe renal impairment, nephrogenic systemic fibrosis\textsuperscript{3}, means that many MRI units now prohibit gadolinium administration unless the renal function is known, or if the glomerular filtration rate is \textless 30 mL/min\textsuperscript{4}. This excludes a proportion of stroke patients, and in the hyper-acute setting and leads to unacceptable delays in stroke MRI\textsuperscript{5}.

Arterial spin labeling (ASL) is a non-invasive MRI technique that does not require contrast administration. The ASL technique labels the blood flowing to the brain by inverting or saturating the spins of water molecules within the blood vessels supplying the brain. Upon reaching the capillary bed, these labeled spins are exchanged with tissue water giving rise to a perfusion-weighted signal. The subtraction of control (i.e. non-labelled) from labelled images, yields an image that directly reflects cerebral tissue perfusion, with images being proportional to cerebral blood flow (CBF)\textsuperscript{6,7}. Previous work has shown that ASL produces reliable perfusion quantification in ischemic stroke\textsuperscript{8,9}, yet has not been validated in quantifying the perfusion lesion. Additionally, the clinical relevance between ASL derived perfusion values and patient outcome remains unclear. Currently, ASL is not used clinical due to the lack of validation of the technique and poor clinical accessibility of MRI. ASL maps cannot be processed on a scanner consol and so must be transferred offline. The reason for this is that there are no clinically validated situations where ASL has been shown to be equal to or outperform PWI. Moreover ASL is still in the development
stage, with more accurate (lower signal to noise ratios) and quicker acquisitions being developed\textsuperscript{10}. Additional research is required to investigate the clinical meaning of ASL examinations in order to identify situations where ASL can be used as a clinical tool.

We aimed to determine if ASL provided equivalent information to PWI in terms of defining the perfusion lesion as an essential first step in the process of assessing whether ASL could ultimately replace PWI in acute stroke MRI protocols. To do this, we compared ASL to PWI at 24 hours after acute ischemic stroke. We hypothesized that ASL would be equivalent to PWI in terms of defining successful reperfusion or persistent hypoperfusion at 24 hours after stroke. We also hypothesized that patients with successful reperfusion would have evidence of hyperperfusion on 24 hour ASL, and that these patients would have much better tissue and clinical outcomes than patients with persistent hypoperfusion.

\textbf{Materials and Methods:}

\textbf{Patients}

We prospectively studied consecutive acute stroke patients with hemispheric ischemia. Patients were seen acutely by an on call neurologist and diagnosed with an acute stroke. All patients underwent baseline multimodal CT examination within 6 hours of symptom onset and follow-up MRI at 24 hours\textsuperscript{1}. Clinical stroke severity using the National Institutes of Health Stroke Scale (NIHSS) was determined immediately prior to imaging at \textless 6h and 24h time points. If eligible, patients were treated with intravenous thrombolysis according to standard guidelines. Level of disability at 3 months was measured with the modified Rankin Scale (mRS). The
study was approved by the institutional ethics committee and all patients gave informed consent. This study was registered with the local ethics committee.

**Imaging**

Whole brain non-contrast CT (NCCT) was followed by perfusion CT, comprising two 60-second series (64-slice Philips Brilliance; Philips, Cleveland, Ohio). CT perfusion imaging was performed with an intravenous bolus injection of contrast agent (40 mL of ultravist 370; Bayer HealthCare, Berlin, Germany) injected at a rate of 6 mL/sec, with 45 time points acquired every 1.33 seconds. Each perfusion series covered 40-mm acquired as 6 adjacent 6- mm slices. The first section was at the level of the basal ganglia/internal capsule, and the second was placed 6-mm towards the vertex to avoid overlap.

MR imaging was performed on a 3T MRI (Siemens Verio, Erlangen, Germany) with a 32-channel receive-only head coil. The stroke MRI protocol included an axial isotropic diffusion-weighted imaging (DWI) spin-echo echo-planar imaging (SE-EPI) sequence, time-of-flight MR angiography (TOF-MRA) and whole brain perfusion imaging with bolus-tracking perfusion-weighted imaging (PWI). ASL data was collected using quantitative imaging of perfusion with a single subtraction, with thin-slice T1 periodic saturation (Q2TIPS) technique. This allowed for the collection of 9 slices with a thickness of 7mm placed 5mm apart. Image parameters were: TR 3000 ms, TI 500 ms; TI 1500 ms; inversion time (TI2) 1700 ms; FOV 192 × 192 mm, matrix 64 × 64.
Image Analysis

Bolus-tracking perfusion maps (CTP and PWI) were calculated by commercial software MiStar (Apollo Medical Imaging Technology, Melbourne, Australia)\textsuperscript{12,13}. This required selection of a global arterial input function (AIF) from an unaffected anterior cerebral artery and a venous outflow function (VOF) from a large draining vein (sagittal sinus). Deconvolution of the tissue enhancement curve and the AIF was performed using model-free singular value decomposition (SVD) with a delay and dispersion correction\textsuperscript{14}. This methodology produces a Delay Time/Tmax map\textsuperscript{15}, as well as maps of CBF, cerebral blood volume (CBV), and mean transit time (MTT).

ASL data was also processed using MIStar. The raw images in each scan were separated into label and control pairs and then pair-wise subtracted and CBF was calculated\textsuperscript{16}. The subtracted images for ASL were separated and corrected for motion using a 3D rigid registration algorithm. Images were spatially smoothed with a 2D 1.5 voxel Gaussian kernel.

CTP and DWI (b=1000 seconds/mm\textsuperscript{2}) lesions were determined by an automated threshold based analysis\textsuperscript{1}. PWI lesions were delineated based upon signal intensity and outlined using a semi-automated threshold based region of interest tool\textsuperscript{1} for maps of CBF, CBV, MTT and Tmax. Using PWI, lesion volumes were recorded at threshold intervals of 5% on a range of 0-200% for all maps. For ASL, a single large ROI was placed on the contralateral to stroke hemisphere, after blood vessels had been excluded. From this normal tissue ROI, mean pixel intensity was recorded. A threshold of mean ±2 standard deviation (SD)\textsuperscript{17} of normal pixels from the healthy hemisphere was used to assign pixels as hyper- or hypoperfusion respectively. The
mean ±2 SD lesion threshold was chosen based on previous physiological studies\textsuperscript{18}. This analysis was performed by two experts independently. Once a lesion had been identified, a clustering of 5mm method was applied in order to remove noisy pixels from the data analysis. Only homologues regions were compared in the ROI analysis between imaging modalities and time points with consideration taken to include equal volumes of grey and white matter between similar ROIs. ROI placements was of hemispheric grey, white and combined regions in the stroke effected and unaffected regions. Secondly, a specific ROI was generated that was the ischemic penumbra and infarct core, as determined by CTP and PWI thresholds of Tmax>6 seconds, and compared to the matching ASL region.

Patients were assessed for infarct core growth, using the difference between the acute CTP infarct core lesion (CBF< 40% of normal\textsuperscript{9}) and the 24 hour DWI lesion volume (on b=1000 images) following co-registration.

Penumbral salvage was assessed as the difference between the acute CTP perfusion lesion volume (tissue with delay time >2 seconds compared to normal contralateral to ischemic tissue delay, but above the infarct core threshold of 40% relative CBF\textsuperscript{9}) and the 24 hour DWI lesion volume. Patients with a smaller 24 hour DWI lesion volume than the acute perfusion lesion volume were said to have penumbra salvage.

Grey and white matter separation analysis was also undertaken for each imaging modality. Tissue segmentation was undertaken on all modalities using signal intensity thresholds on MRI and CT (HU) and confirmed by an expert viewer.
Segmentation was performed within MiStar on anatomical maps and regions of interest exported to perfusion scans for analysis. Following segmentation, all imaging based analysis was performed again.

Paired baseline CTA and 24 hour MRA were graded using adapted Thrombolysis in Myocardial Infarction criteria, with vessel occlusion status classified as complete (TIMI 0), minimal flow (TIMI 1), partial flow (TIMI 2), or normal (TIMI 3). Complete recanalization was defined as TIMI 3 at 24 hours, partial recanalization was defined as any TIMI grade increase (excepting return to TIMI 3)\textsuperscript{13}.

For outcome analyses, patients were divided into three groups according to reperfusion status between the acute CTP and 24 hour PWI. Perfusion status was defined as a change in the MTT 145% lesion over a 24 hour period as follows: major reperfusion (>80% reduction in MTT 145% lesion at 24 hours), no reperfusion (<20% reduction in MTT 145% lesion at 24 hours) and partial reperfusion (between 20% and 80% reduction in MTT 145% lesion), based on change between acute CTP and 24 hour PWI-MR MTT 145% lesion volume, as per previous work\textsuperscript{19}. The group with partial reperfusion were excluded from tissue and clinical outcome analyses because of the large variability seen in infarct growth between the acute and 24 hour time points in such patients in a prior study\textsuperscript{9}. Maps of CTP and PWI have previously been compared and provide similar information, giving weight to this cross modality analysis\textsuperscript{11}.

**Statistical analysis**

Hypoperfusion and hyperperfusion lesion volumes of PWI-CBV, CBF, MTT and Delay Time (Tmax) were also statistically compared to ASL hyperperfusion and
hypoperfusion lesions using paired t-tests, corresponding Pearson’s correlation coefficients and summary statistics.

Receiver Operating Characteristic (ROC) Curve Analysis was used to compare the ASL lesion (hypoperfused regions) to concurrent PWI maps using a pixel based analysis. Separate analysis was done for each hemisphere. The PWI lesion was considered the ‘true’ lesion, meaning any ASL lesion overlapping the PWI lesion was considered true positive, and any PWI lesion not covered by an ASL lesion was false negative. Finally any ASL lesion outside of the PWI lesion was considered false positive and any area that was not covered by either perfusion map was assigned true negative.

Results:

Patients

One hundred patients with hemispheric ischemic stroke were imaged within 6 hours of symptom onset and enrolled in this study. Patients were aged 26-91 (mean 72); 42 were female and 58 male. Forty seven patients were treated with thrombolysis. Of the 100 patients in the study, 53 patients had major reperfusion at 24 hours, 11 had partial reperfusion, and 36 had no reperfusion at 24 hours as defined by a reduction in the MTT 145% lesion described in the methods. The 11 partial reperfusion patients were excluded from outcome analyses. Four patients had a perfusion lesion on the ASL maps at 24 hours that was not identified on baseline CTP due to limited slice coverage. Five patients had no perfusion lesion on ASL, PWI or CTP and in all 5 patients, 24 hour DWI was consistent with single perforator vessel (lacunar) infarction. Six acute stroke patients were not recruited into this study due to severe renal impairment. All 95 patients with a PWI lesion had an ASL lesion in a
corresponding location. The average DWI lesion at 24 hours in the patients enrolled in the study was 21mL (range 9-39mL) (table 7.1). There was minimal error in the acquisition caused by patient motion, while ASL is very sensitive to movement, this sequence was added very early in the acquisition to avoid motion artefacts and resulted in no patients being excluded due to motion artefact. The inclusion of ASL into the standard clinical follow-up was simple and only added a total of less than five minutes to a scan as no preparation was required.

<table>
<thead>
<tr>
<th>Patients with hyperperfusion at 24 hours on ASL</th>
<th>Patients with hypoperfusion at 24 hours on ASL</th>
<th>Significance between ASL groups</th>
<th>Patients with major reperfusion at 24 hours on PWI</th>
<th>Patients with no reperfusion at 24 hours on PWI</th>
<th>Significance between reperfusion groups</th>
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<td></td>
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<tr>
<td>Mean acute NIHSS</td>
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<tr>
<td>Mean 24h NIHSS</td>
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<tr>
<td>Mean NIHSS improvement</td>
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<tr>
<td>Mean 90 day mRS</td>
<td></td>
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<td>Patients treated with thrombolysis</td>
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<tr>
<td>Mean penumbra l salvage</td>
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</table>

Table 7.1. The clinical and imaging findings of patients in this study. Data comparing the four groups of patients with hyper- or hypoperfusion at 24 hours on ASL and patients with either major reperfusion or no reperfusion at 24 hours on PWI. The same patients were used for the hyper/hypo perfusion group as the group comparing reperfusion status.
**ASL perfusion characteristics**

At 24 hours, 43 patients showed only hyperperfusion on ASL (figure 7.1), and 46 showed only hypoperfusion on ASL (figure 7.2). Six patients had both hyper and hypoperfusion at 24 hours, these patients had partial reperfusion on 24 hour PWI. Five patients with major reperfusion on PWI did not have hyperperfusion on ASL at 24 hours, however, these 5 patients did not have any residual hypoperfusion on ASL, indicating that reperfusion had occurred in these patients but without reaching the threshold for hyperperfusion on ASL.

<table>
<thead>
<tr>
<th>Major Reperfusion at 24 hours on PWI</th>
<th>Hyperperfusion at 24 hours on ASL</th>
<th>Hypoperfusion at 24 hours on ASL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean lesion volume: 22mL (SD 9 mL)</td>
<td>Mean lesion volume: 9mL (SD 4 mL)</td>
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<tr>
<td>Mean NIHSS change: (14 SD 5)</td>
<td>Mean NIHSS change: 6 (SD 3)</td>
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<tr>
<td>Mean penumbra salvage: (19mL SD 7)</td>
<td>Mean penumbra salvage: (7mL SD 3)</td>
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<tr>
<td>N= 36</td>
<td>N= 17</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>No reperfusion at 24 hours on PWI</th>
<th>Mean lesion volume: 8mL (SD 5mL)</th>
<th>Mean lesion volume: 41mL (SD 12mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean NIHSS change: 9 (SD 4)</td>
<td>Mean NIHSS change: 1 (SD 2)</td>
<td></td>
</tr>
<tr>
<td>Mean penumbra salvage: (13mL SD 5)</td>
<td>Mean penumbra salvage: 4mL (SD 4)</td>
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<tr>
<td>N= 7</td>
<td>N= 29</td>
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</tbody>
</table>

Table 7.2. Characteristics of patients with combinations of either hyperperfusion or hypoperfusion and either major reperfusion or no reperfusion at 24 hours.

**Hypoperfusion Lesion Volume Analysis**

The ASL hypoperfusion lesion volume was most closely matched to the PWI Tmax perfusion lesion (AUC 0.86). Next, the ASL lesion was also a good match to
the MTT lesion (AUC= 0.73) and less well matched to the CBV (AUC= 0.63) and CBF lesions (AUC= 0.76).

**Perfusion and clinical outcome**

The differences in clinical outcomes and their relationship to hyperperfusion and hypoperfusion on ASL and reperfusion on PWI are summarized in table 7.1. Patients treated with tPA were more likely to have hyperperfusion on ASL and major reperfusion on PWI. Of the patients with hyperperfusion on ASL, the mean improvement in acute-24 h NIHSS was 12 (SD = 5) compared to only 4 (SD = 2) in the group with hypoperfusion on ASL (p<0.05). Patients with ASL hyperperfusion showed penumbral salvage in 36/43 of cases, with a mean 59% of the acute penumbra tissue salvaged from infarction. In contrast, only 7/46 patients in the hypoperfusion group showed any penumbral salvage (p<0.01). The hyperperfusion group also had less infarct growth from acute to 24 hours, mean 6 ± 3 mL compared to the hypoperfusion group 13 ± 4 mL (p<0.05). With respect to discriminating between improved tissue and clinical outcomes, hyperperfusion on ASL or major reperfusion on PWI were similar for identifying greater penumbral salvage and better 3 month clinical recovery, with ASL being slightly better. However, only ASL hyperperfusion correlated with early clinical improvement (Table 7.1).

There was very high correlation between ASL hyperperfusion and major reperfusion on PWI, and between ASL hypoperfusion and no reperfusion on PWI (Table 7.2). However, patients that did have both major reperfusion on PWI and hyperperfusion on ASL had a larger mean ASL hyperperfusion lesion volume of 22mL compared to patients without major reperfusion on PWI but with
hyperperfusion on ASL (mean hyperperfusion lesion volume 8 mL, p<0.05). Patients with concordance between hypoperfusion on ASL and no reperfusion on PWI had a larger mean hypoperfusion lesion volume of 41mL, compared to patients with hypoperfusion on ASL but with major reperfusion on PWI (mean hypoperfusion lesion 9mL, p<0.05).

Patients with reperfusion, hyperperfusion and penumbra salvage demonstrated a greater early recovery (mean NIHSS improvement 11) than patients with reperfusion but no hyperperfusion or penumbra salvage (mean NIHSS improvement 6, p<0.05). However patients without reperfusion, hyperperfusion or penumbra salvage showed very little improvement (mean NIHSS improvement 2). At three months, the difference between patients with hyperperfusion and penumbra salvage in the major reperfusion group became much less clear. Patients with reperfusion, hyperperfusion and penumbra salvage showed similar 90 day outcome (mRS 2) compared to patients with reperfusion, but not hyperperfusion or penumbra salvage (mRS 3, p<0.5).

Of note, all patients that demonstrated hyperperfusion on ASL maps also had complete recanalisation on MRA at 24 hours. Further, no patients with persistent hypoperfusion on ASL maps had complete recanalisation on 24 hour MRA.

**Grey and white matter segmentation**

Analysis following tissue segmentation did not yield significantly different results from above. Overall there was a 45-150% difference in the perfusion level between grey and white matter segmented regions in all patients.


**Discussion:**

Twenty four hours after an ischemic stroke, ASL accurately predicts early and late clinical recovery, and identifies patients with penumbral salvage from reperfusion, rather than patients with reperfusion that does not result in penumbra salvage. Furthermore, hyperperfusion on ASL provided improved, information about effective tissue reperfusion compared to the commonly used method, bolus-tracking PWI. Hyperperfusion was a better marker of early clinical recovery than PWI-defined reperfusion. Hyperperfusion also showed a greater association with penumbral salvage than did PWI-defined reperfusion (figure 7.1).

Figure 7.1

Figure 7.1. A patient with hyperperfusion at 24 hours. Patient with an ischemic stroke with concurrent acute CTP, ASL and PWI imaging show hypoperfusion (this patient underwent acute MR as well as at 24 hours). Following the administration of thrombolysis the patient reperfused and at 24 hours. ASL shows that the previously hypoperfused area is now hyperperfused. Hyperperfusion is not clearly evident on PWI (MTT).
Whilst reduction in the size of the perfusion lesion on PWI is commonly used to assess reperfusion, our study has demonstrated that hyperperfusion on ASL is also a clinically significant marker of effective tissue reperfusion in acute ischemic stroke (Figure 7.1). The correlation with vessel recanalisation also confirms the validity of this new finding. Hyperperfusion was not observed on PWI. Previous positron emission tomography (PET) studies have shown that increased CBF levels corresponding to hyperperfusion representing reperfusion\textsuperscript{20,21,22}. Hyperperfusion is thought to occur following successful vessel recanalisation and tissue reperfusion, as local autoregulation may take some days to compensate for the increased blood volume in tissue with maximum vasodilatation as a response to the initial acute ischemia\textsuperscript{23}. Conversely, if reperfusion is not achieved, then hypoperfusion is expected to persist, resulting in infarction of most of the perfusion lesion (figure 7.2). This was seen in our study where patients with persistent hypoperfusion had progression of virtually the entire acute penumbra to infarct (Table 7.1). Conversely, hyperperfusion on ASL at 24 hours clearly correlated with major penumbral salvage. Previous PET studies have demonstrated ‘luxury perfusion’, or hyperperfusion in tissue already infarcted, but typically several days after stroke onset. This is probably a more subacute process than the hyperperfusion seen in our cohort. This paper has shown the benefit of hyperperfusion in chronic stroke, as even though a perfusion lesion may be reperfused, there is no guarantee that penumbral tissue was salvaged. By being able to detect effective penumbral salvage in patients it is possible to further study the factors contributing the outcome. Five patients with major reperfusion on PWI did not have hyperperfusion on ASL at 24 hours, however, these 5 patients did not have any residual hypoperfusion on ASL, indicating that
reperfusion had occurred in these patients but without reaching the threshold for hyperperfusion on ASL. The possible reason for this is that even tho patients showed recanalisation of angiography, there was no penumbra salvage. Therefore we hypothesise that hyperperfusion only takes place when penumbral tissue is salvaged

Figure 7.2. A patient with persistent hypoperfusion. This patient had a right ICA occlusion that did not recanalize at 24 hours. The 24 hour ASL map shows persistent hypoperfusion, as does the 24 hour PWI Tmax map. These correspond to extensive infarction on 24 hour DWI. The patient’s baseline NIHSS 19 deteriorated to 21 at 24 hours.

The penumbra is thought to survive due to an increase in collateral blood flow, preventing immediate infarction. From PET studies, patients with effective auto-regulation and viable penumbra supplied through collateral circulation would be expected to have increase perfusion temporarily, as a result of successful reperfusion. However PWI did not observe hyperperfusion in our study. This may be because ASL is more sensitive to such changes post-reperfusion than PWI. An additional explanation may relate to ASL using water as a contrast media rather than parametric contrast used with PWI. Hyperperfusion on ASL may partially represent an increase in the intravascular water content in the originally ischemic region post reperfusion. An alternate hypothesis for why PWI does not show hyperperfusion, is that firstly, the hyperperfusion threshold was not very high, and is around CBF 120%.
Post processing of PWI involves the selection of an arterial input function from a single blood vessel. The bolus passage of contrast through the AIF vessel is then used to standardise all pixels in the brain against this AIF to assess for changes off the baseline. However selection of the AIF is not standardised, and CBF through different vessels in the brain varies significantly. Therefore, if the internal carotid artery (ICA) were selected as the AIF, the high baseline CBF of the ICA may washout any tissue hyperperfusion signal on PWI. ASL post processing does not use deconvolution or an AIF to measure perfusion, and as such is not subjected to such a user depended output and is a significant strength.

As a direct comparison, the volume of persistent hypoperfusion on ASL was highly correlated with PWI, demonstrating the similarity between the modalities at measuring perfusion deficits. However, because no hyperperfusion was observed on PWI imaging we cannot compare hyperperfusion on ASL to PWI directly.

A limitation may be that patients from this study represent the typical patient population and as such are heterogeneous. Further, a single delay time (dt) was selected for this study, however various centres may use a different dt setting, which would alter the perfusion results. Therefore we can only validate a single dt.

Can ASL be used instead of PWI in an acute stroke MRI protocol? Our study provides strong evidence that ASL can replace PWI at the 24 hour time-point, particularly in assessing response to thrombolysis; being a better marker of successful tissue reperfusion and predictor of clinical recovery than PWI\textsuperscript{24}. This is of considerable clinical impact, as the ASL sequence not only is a better prognostic
marker than PWI, but does not expose patients to the risk of contrast induced complications which may be particularly pertinent where repeat studies are required in a short time frame. Lastly, the ASL acquisition takes less than five minutes, which may be less time than establishing IV access, setting up contrast administration and performing a PWI sequence. However, the ASL maps studied were of a lower spatial resolution when compared with their PWI counterparts. This reduced resolution may not be significant in the stroke cases studied, as in the majority of cases, hyper or hypo-perfusion was detected with similar volumes between modalities. We therefore conclude that ASL is a practical and reliable sequence to use in stroke

References


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Chapter Eight:

Conclusions

In every chapter, the specific implications of each study are given in the discussion sections. The aim of this chapter is to respond to the key issues facing perfusion imaging in stroke from chapter one, fit each study together for a broad measure of the impact of this PhD and give insight into the future directions of the role of imaging in acute stroke.

Key issues addressed by this thesis:

1) Threshold selection

This thesis has identified the measures and thresholds required to define the acute infarct core (CBF of 45%) and penumbra (delay time of 2 seconds). By firstly identifying a method by which critical thresholds can be found (chapter four), it was then possible to discover the critical components necessary to measure both the acute infarct core and penumbra (chapter five). That is, CBF was the best measure to describe the acute infarct core. Also, the penumbra was best measured using time to peak variations such as delay time. These two discoveries allowed for the combination of cerebral perfusion measures to be used in the improvement for the identification of the acute infarct core. That is, by restricting the volume of the acute infarct core to within the acute perfusion lesion, it was possible to greatly increase the accuracy of thresholds describing the acute infarct core. This thesis provided the first evidence that a mismatch of perfusion measures on CT allowed for an accurate infarct core measure.
2) CT perfusion software – lack of standardisation

Chapter six directly addresses this critical perfusion scanning issue. This thesis analysed a very large dataset using six different forms of deconvolution. The six post processing options were; Maximum slope method, Partial deconvolution, Single value deconvolution, Delay corrected single value deconvolution, block circulant deconvolution and a stroke stenosis model that is unique to this thesis. The results from this study showed that each post processing method had an optimal perfusion measure and threshold to define key acute stroke pathophysiology. Also, there was much consistency between each post processing method, in that a CBF was always the best measure of the acute infarct core, using either a threshold of 20% or 40-45%. Additionally, when defining the acute penumbra, if a form of delay correction was used, TTP measures (or its derivatives of Tmax and delay time) were optimal to define the acute penumbra. However, if there was no delay correction, an MTT measure was the best acute penumbra detection method. Therefore, this thesis demonstrates that for each given perfusion calculation technique used, the measures to define the acute infarct core are the same, however the actual threshold, and accuracy of these thresholds change. This detailed analysis provided strong data to recommend the use of Single Value Deconvolution with delay and dispersion correction, because this form of post processing allowed for the most accurate description of the acute penumbra and infarct core.

3) MR vs. CT for core and penumbra detection

Chapters five and seven each compare CTP and MR derived perfusion values. In chapter five we see a direct comparison between CTP and PWI MTT 145% lesion volumes. This analysis demonstrated that each technique produced extremely similar
lesion volumes. Also, chapter seven compared PWI, CTP and ASL lesion locations and volumes in patients with a perfusion lesion. This analysis showed, again, that each technique used to derive these maps produced extremely similar values. This is important, yet expected, as there are currently no publications directly comparing these extremely similar perfusion maps. It was expected that CTP and PWI images were very similar as they are measuring the same event. In both CTP and PWI, a contrast agent is injected into the body at a constant rate, and the flow of this contrast is monitored in the brain over a period of time. The variation in these two imaging techniques is that, in MR, the ratio of contrast concentration to signal enhancement is not linear. Additionally, the methods of post processing used by either CTP or PWI are often different, with no explanation as to why. On the first point, the contrast concentration to signal enhancement ratio is well studied and understood, and as a result should not be considered a barrier to comparing CTP and PWI. Next, the methodological convention behind the post processing used to derive the CTP and PWI maps is different, and depends on the vendor. Should a study be able to compare SVD in CTP and PWI, they would undoubtedly show that the output of the two different modalities is the same. This is what has occurred in this PhD (chapters five and six). Overall this PhD has shown that when comparing like post processing options, CTP and PWI produce the same valuable ischemic stroke information.

4) How important is delay in passage of contrast to the ischemic region in perfusion calculations?

This issue was addressed by chapter six in CTP post processing variability. This chapter analysed various post processing algorithms, as well as different forms of delay and dispersion correction. This came about through analysis of delay
corrected single value deconvolution, block circulant deconvolution and the stroke stenosis model (chapter 6). These forms of post processing employ various methods that attempt to correct for delay and dispersion that result in an underestimation of CBF and overestimation of MTT. The results of this thesis clearly demonstrate that a delay corrected CBF of 40-45% accurately defined the acute infarct core, however without delay correction, a CBF of 20-25% defines the acute infarct core. Furthermore, the results show that with delay correction, the thresholds used were more accurate and showed less variability. Overall, due to the accuracy achieved with delay and dispersion correction, we highly recommend its implementation in perfusion imaging.

**Future directions**

*Further refinement of thresholds for core and penumbra*

This thesis undertook projects to determine the critical thresholds to define the acute infarct core and penumbra. The results show a high level of accuracy at deriving the acute penumbra. This thesis then used the precise acute penumbra definition, to more accurately define the acute infarct core through the use of perfusion mismatch. This demonstrated that a single perfusion threshold could only reach a limited accuracy for detecting the acute infarct core. This thesis did not examine whether variation in stroke topography, lesion volume and collateral status makes a difference to the thresholds. Variations in perfusion lesion topography for example, will have different collateral supply due to variation in capillary density which might influence both core and penumbral thresholds. This thesis did not examine angiographic status in detail to test whether knowledge of collateral status and location (e.g. on CTA)
would have influenced acute perfusion lesion thresholds, lesion location, or influence on clinical outcome. This is an important area of future study. It is possible that collateral status is best quantified in a rapid manner by assessment of perfusion (especially delay measures) but this requires further validation.

**Refining measures of the infarct core and penumbra**

This thesis showed that a standalone (single) perfusion measure was suboptimal to define the acute infarct core because the AUC never exceeded 0.8. However by employing a duel CTP threshold approach (defining core threshold within a more lenient outer perfusion threshold), a significant improvement in the accuracy of the acute infarct core detection was made. There may still be room for improvement in defining infarct core with CTP. Variables that may influence measures of the infarct core include the extent and severity of hypoperfusion and the volume of hypoperfusion. The extent and severity of hypoperfusion relates to the effect of collateralisation in the ischemic area providing an alternate source of blood flow. Logically, if there is an extremely severe area of hypoperfusion (especially delay) within what appears to be penumbral based thresholds, this could be a guide to the likelihood (or not) of successful reperfusion would result in penumbral salvage or haemorrhage.

**How do measures of the infarct core and penumbra influence clinical outcome and response to therapy?**

The volume of hypoperfusion, or the size of the ischemic area could also be a very important tool in determining a patients suitability for reperfusion therapy. If there is a large stroke resulting in severe hypoperfusion throughout a large vascular
territory (MCA), it is possible that very little tissue will benefit from reperfusion therapy, even if it is still within the penumbral threshold. This might reflect the delay between imaging and when reperfusion occurs, such that the penumbral survival time of this tissue is limited. Another issue that needs to be investigated is where the cut-off for infarct core volume predicts no benefit (or even harm) from reperfusion therapy. There appear to be patients in this dataset, who despite considerable penumbral volumes, had no hope of a good outcome despite successful reperfusion with treatment. These patients generally have a large infarct core at baseline.

These methods attempt to find a fast and reliable measure for the acute infarct core, and guide the physician to make the best clinical decision to improve the quality of post stroke life of a patient. The author of this thesis believes that the final decision lies in the hands of a treating physician, and it is important to understand what information they are most comfortable with. By understanding what information a person makes their decisions from, we can tailor how to derive this information, and how to deliver it to a treating physician, in order to guide decision making. Such critical information would include, the volume of the acute perfusion lesion, penumbra and infarct core volumes, as well as clinical information such as time since ischemic stroke onset, current medications and current chronic illnesses. This thesis provides the information required for the first step on decision tree that a physician will use to treat an ischemic stroke. It appears from the current body of work in this thesis that the relationship between baseline core and penumbra volumes might well be the most powerful predictor of response to reperfusion treatment and even overpower some of the common clinical predictors, but this needs to be confirmed in further analysis.
Could ASL replace bolus tracking perfusion to define penumbra in the future?

In its clinical prospects, ASL is superior to PWI imaging. PWI requires patients to undergo pre-screening for GFR levels, as well as having access to crash team in case of an allergic reaction. Such pre-screening and standby resources delay the initiation of imaging, as well as restrict imaging to within working hours. However ASL requires no blood pre-screening and as such does not require a standby emergency team. Therefore ASL can be used out of hours and on any patient, even if a treating physician is unsure about the diagnosis. As a result there is now great interest in ASL clinically, however it first requires validation. The study using ASL in this thesis found ASL perfusion values to be of greater clinical relevance at 24 hours post ischemic stroke than PWI. However ASL in the acute phase has shown some weakness. ASL image quality is poor at perfusion values below a CBF of 15mL/100g/min, the baseline blood flow of white matter. This results in ischemic hypoperfusion including normal white matter. This weakness can be overcome by improving the ASL acquisition technique. Improved ASL acquisition techniques have been proposed, but they require study in a clinical sample. Therefore, ASL is not currently ready for clinical implementation, but with newer acquisition techniques, ASL may replace PWI within 5 years.

Automation

This thesis provides compelling evidence that baseline infarct core and penumbral volumes are predictive of response to reperfusion and ultimately clinical outcome. At the moment the treating physicians (apart from a few highly specialised centres) do not have timely access to such information. Thus, the next step is to increase the ease
with which acute staff are able to access this crucial information reliably and rapidly. Currently, in perfusion imaging, it is necessary to select an arterial input function and a venous outflow function. Additionally, some programs require further input such as selection of baseline images and selection of deconvolution method. An ideal program would be more sophisticated and be able to automatically select all the information that is required to post process the perfusion maps, and present frontline staff with penumbra and infarct core volumes, perfusion maps and possibly a recommendation on whether to treat an acute ischemic stroke with thrombolysis, or not. A system where a patient is treated based upon highly researched and validated criteria, such as imaging and clinical information, would be an optimal situation. This system would be able to reduce the uncertainty from decision making, and reduce the ‘errors’ from people who are either too cautious, or too enthusiastic, in order to ensure a patient receives the most beneficial treatment to them. An automated decision making system would be highly individualised and based on a patient’s specific acute ischemic stroke characteristics, such as the volume of the infarct core and penumbra as well as clinical criteria such as age, co morbidities and current medications.

**Clinical trials**

Using the results from this thesis, it is possible to commence large scale clinical trials for the concept of tissue based treatment decision making. This thesis has identified the critical thresholds to define the acute penumbra and acute infarct core, and as such this information can be utilised in the treatment of acute stroke patients. Such a trial would involve two stages. Firstly, large scale data collection from multiple sites would be required, as another prospective study validating the findings of this thesis, particularly investigating whether the core and penumbra
thresholds are reliable across different CT scanners and acquisition protocols. This study could also investigate the criteria, from which patients would be treated, and identify the exact volumes, or ratios of acute infarct core and penumbra, that are optimal in order to treat a patient.

A second trial could then test the benefit of a tissue based decision making process against the current time based process, in a randomised study of NCCT in the <4.5 hour time window vs multimodal CT selection with a extended time window. This second trial would use the perfusion imaging thresholds from this thesis, and confirmed from the first study, and critical treatment criteria in order to select suitable patients for treatment. This study would require multiple hospitals, ideally located all over the world, and involve randomising patients to either standard, time based thrombolysis administration, or to a tissue based decision making process involving perfusion imaging. The hypotheses would be that the extended time and tissue selection would lead to more patients treated with better outcomes than with standard time window and NCCT selection.

Closing remarks

This thesis provides an important backbone to allow a move to tissue-based selection of acute stroke patients for treatment. Ultimately this may lead to more patients being offered treatment resulting in better patient outcome. However large multi centre trials are required to test the implementation of such an approach.