Lumbar Drainage Following Aneurysmal Subarachnoid Haemorrhage and the Role of Cytokine and Adhesion Molecules in the Pathogenesis of Delayed Ischaemic Neurological Deficit

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been given to the work of others.

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She has watched over me during life and since her death. If this research results in the saviour of one future mother or sister with a subarachnoid haemorrhage, it will have served its purpose.

Contents

Table of abbreviations

ANOVA	Analysis of variance	
ATP	Adenosine Tri-Phosphate	y
aSAH	Aneurysmal subarachnoid haemorrhage	y
BBB	Blood brain barrier	y
BOXes	Bilirubin oxidative products	y
CBF	Cerebral blood flow	y
CBV	Cerebral blood volume	y
CD	Cluster of differentiation	y
CI	Confidence interval	y
cm	Centimetre	y
CNS	Central nervous system	y
CPP	Cerebral perfusion pressure	y
CSD	Cortical spreading depolarisation	y
CSF	Cerebrospinal fluid	y
CSI	Cortical spreading ischaemia	y
CT	Computerised tomography	y
CT-A	Computerised tomography angiogram	y
CT-P	Computerised tomography perfusion	y
DIND	Delayed ischaemic neurological deficit	y
DSA	Digital subtraction angiography	y
ECF	Extracellular fluid	y
EDTA	Ethylenediaminetetraacetic acid	
ELISA	Enzyme-linked immunosorbent assay	y
eNOS	Endothelial nitric oxide synthase	y
ET-1	Endothelin-1	y
GABA	γ-aminoburyric acid	y

GCS	Glasgow Coma Score	y
GOS	Glasgow Outcome Scale	y
HMGCoA	3-hydroxy-3-methylglutaryl coenzyme A	y
ICAM	Intercellular adhesion molecules	y
ICP	Intracranial pressure	y
IL	Interleukin	y
IL-1ra	Interleukin 1 receptor antagonist	y
iNOS	Induced nitric oxide synthase	y
MAP	Mean arterial pressure	y
MAPK	Mitogen-activated protein kinase	y
MCP-1	Monocyte chemoattractant protein-1	y
MFI	Median Fluorescence Intensity	
ml	Millilitre	
mm	Millimetre	y
MMP	Matrix metallopeptidase	y
mRNA	Messenger ribonucleic acid	y
MRS	Modified Rankin Scale	y
NF-kB	Nuclear factor-kB	y
NK	Natural killer	y
nm	Nanometre	y
NMDA	N-Methyl-D-aspartate	y
nNOS	Neuronal nitric oxide synthase	y
NO	Nitric oxide	y
NOS	Nitric oxide synthase	y
NPRI	Nicardipine prolonged-release implants	y
OR	Odds ratio	y
PE	Phycoerythrin	y
PET	Positron emission tomography	y
PMT	Photomultiplier tube	y

$PtiO_2$	Brain tissue oxygen	y
pg	Picograms	
p53	Tumour protein 53	y
RCT	Randomised controlled trial	y
SAH	Subarachnoid haemorrhage	y
SIRS	Systemic inflammatory response syndrome	y
SPC	Sphingosylphosphorylcholine	y
SRC	Sarcoma	y
SPSS	Statistical Package for the Social Sciences	y
TBA	Transluminal balloon angioplasty	y
TBI	Traumatic brain injury	y
TCD	Transcranial doppler	y
TH	T helper cell 1	y
TLR	Toll-like receptors	y
TNF-α	Tumour necrosis factor-α	y
VEGF	Vascular endothelial growth factor	y
vWF	Von Willebrand Factor	y
WFNS	World Federation of Neurological Surgeons	y
μl	Microlitre	y
χ^2	Chi-squared	y

Abstract

Introduction

Delayed ischaemic neurological deficit (DIND) following aneurysmal subarachnoid haemorrhage (aSAH) is a significant cause of morbidity and mortality. There is some evidence to suggest that its pathogenesis is related to inflammation and that clearing the subarachnoid space of potential pathogens via a lumbar drain may reduce the prevalence and severity of DIND.

Aims

The aims of the current study are two-fold:

1. To ascertain whether lumbar drainage of cerebrospinal fluid (CSF) following aSAH can reduce the prevalence and severity of DIND.

2. To investigate levels of inflammatory mediators in plasma and CSF and to look for an association with aSAH, DIND and outcome.

Material and methods

1. Prospective randomised controlled trial with randomisation into two arms: arm 1 control, no additional intervention; Arm 2 study, insertion of a lumbar drain in order to clear the visible blood load.

2. Prospective cohort study of patients with aSAH. Plasma and CSF samples were obtained on days 3, 5, 7 and 9 following haemorrhage and analysed for 11 mediators.

Results

1. Prevalence of DIND 35% (confidence interval (CI) 26.2-45.2%) in the control versus 21% (CI 13.6-30.0%) in the study group (p=0.021). A significant improvement in early clinical outcome in favour of lumbar drainage was noted (Modified Rankin Score 0-2 37.5% in the control versus 55.2% in the study group, p=0.009). There was no difference in outcome at six months.

2. Raised plasma and CSF levels of most mediators when compared with non-aSAH controls. Significantly higher levels of vascular endothelial growth factor within the CSF of patients with DIND on day 5 post ictus when compared to patients without DIND. Generally higher mediator levels were noted within the CSF when compared to plasma.

Conclusion

This provides some support for the routine use of lumbar drains following aSAH in good grade patients to reduce the prevalence of DIND and improve early clinical outcome. Both a central nervous system and systemic inflammatory response is initiated following aSAH. The former may be associated with DIND. Causality cannot be determined from this study.

Introduction

Introduction

The incidence of aneurysmal subarachnoid haemorrhage (aSAH) has been estimated at 6-8 per 100,000 annually, with a peak incidence in the fifth decade[1]. Delayed ischaemic neurological deficit (DIND) (also referred to as clinical/symptomatic vasospasm or delayed cerebral ischaemia) is a serious and poorly understood complication of aSAH, occurring in 20-40% of patients. This is characteristically defined as neurological deficit observed at least three days after aSAH, and features radiologically confirmed narrowing of the large cerebral blood vessels (radiological vasospasm). However, it has increasingly been suggested that a direct causative association between the two may be overly simplistic, despite their following a similar time course[2]. The variability in the reported incidence of DIND is partly due to the use of unstandardised diagnostic criteria. DIND does not necessarily imply the presence of angiographic vasospasm, although numerous studies have this (or a surrogate measure, such as flow velocities with transcranial doppler) as a prerequisite for diagnosis[3]. The observation that thick subarachnoid clot completely filling any cistern or fissure is an independent predictor of DIND suggests that causative compound(s) may be blood component(s), breakdown products, or be regulated/induced as a result of the presence of blood in or around the subarachnoid space[4].

The aim of this introduction is to: (i) summarise the main historical events resulting in the current understanding of DIND, (ii) discuss the evidence underlying established therapies for DIND, and (iii) broadly review the

translational and clinical research involved in elucidating the pathophysiology of DIND. This will subsequently lead to the aims of the current study.

Historical overview

Although ancient Egyptian writings indicated awareness of aneurysms, a causal association with subarachnoid haemorrhage was only first proposed in the late 1600s[5]. Almost a century later, Morgagni reported incidental posterior cerebral artery dilatations, suggesting that intracranial aneurysms may be a source of haemorrhage[6]. In parallel, Francesco Biumi presented the first documented report of an unruptured intracranial aneurysm[7], which was followed by the first verified account of aneurysmal rupture five decades later[8]. The earliest nineteenth century description of DIND came from William Gull, who described a young woman suffering a stroke and her subsequent neurological deterioration on the fifth day of her illness-she was shown to have a ruptured middle cerebral artery aneurysm at autopsy[9].

The first description of cerebral vasospasm was made in the 1920s, when rabbit cortical arteries were noted to contract following direct mechanical stimulus, but not in response to cervical sympathetic chain stimulation[10]. Current understanding of cerebrovascular abnormalities emerged following the work of Egas Moniz in developing clinical angiograms in the late 1920s[11], an imaging approach which underpinned Norman Dott's attempt at direct aneurysmal surgical repair and Walter Dandy's subsequent successful surgical clip repair[12]. Robertson's large series of ruptured intracranial aneurysms highlighted the presence of cerebral infarction despite patent blood vessels[13]. In the same period, Zucker described the presence of putative circulatory vasoconstrictive agents originating from lysed

erythrocytes[14], a notion supported by Jackson's observations that a meningeal reaction followed injection of blood products into the cisterna magna of dogs[15], with red blood cells eliciting the greatest reaction.

Cerebral vasospasm following aSAH was first described in Reid and Johnson's angiographic studies[16], although it was not until the following year that the images were published by Ecker and Riemenschneider[17]. This landmark study reported six patients who had suffered angiographically visible vasospasm defined as a change in vessel calibre between two identically performed angiograms at different time intervals. Angiographic vasospasm was noted to be self-limiting and greatest near the site of the aneurysm. Subsequent studies demonstrated a poorer operative prognosis, larger proportion of ischaemic lesions and worse outcome in patients with angiographic vasospasm[18], which was related to reduced total or regional cerebral blood flow (CBF)[19]. Techniques were developed to reduce the incidence of vasospasm during aneurysm surgery, including the timing of operations. Allcock and Drake advocated delayed surgical treatment because of a higher incidence of vasospasm in patients treated within ten days of ictus[20]. However, no consensus was reached regarding timing of surgery until 1976, following the publication of a large case series which indicated that mortality was decreased and the risk of vasospasm reduced in patients undergoing surgery within 48 hours[21]. In addition, an elegant study by Weir and colleagues based on serial angiograms in almost 300 patients demonstrated the onset, peak and subsidence of vasospasm at days 3, 6-8

and 12, respectively[22]. Mortality was confirmed to relate to the presence and severity of vasospasm.

Although the association between the size of the blood clot and the incidence of angiographic vasospasm is historically attributed to Fisher, it was Katada shortly followed by Takemae who were the first to report this association[23,24]. In 1980, the Fisher classification of aSAH was published[25], which was based on a series of 47 patients with aSAH. It was noted that severe angiographic vasospasm was almost invariably present in patients with subarachnoid clots larger than 5 x 3mm or layers of blood ≥1 mm in vertical cisterns and fissures. Conversely, vasospasm was extremely rare in patients without subarachnoid blood. This system was based on CT imaging performed in the 1970s with 16 mm slices. Two slices with blood within vertical cisterns implied at least a thickness of 8 mm. On three slices, this will imply at least a thickness of 32 mm. Although a widely used classification in modern practice, it's reliance on this out-dated method has led to further validation. The findings were confirmed in a study demonstrating that thick subarachnoid clot completely filling any cistern/fissure was an independent predictor of DIND[4]. Intraventricular haemorrhage had similar predictive value.

Management of aSAH

Although trauma is the most common cause of SAH, aSAH accounts for approximately 85% of all cases of spontaneous SAH[26]. The remainder of cases are often due to non-aneurysmal 'perimesencephalic' SAH or other rare causes. Clinical features consist of a spectrum ranging from a sudden onset headache to sudden death. Apart from altered consciousness, other associated features include vomiting, signs of meningism and seizures.

Investigation is based on clinical assessment followed by computerised tomography (CT) of the brain. In approximately 3% of patients presenting with clinical features of aSAH with a normal CT scan of the brain, examination of the cerebrospinal fluid (CSF) within 12 hours of the haemorrhage can reveal metabolites of haemoglobin such as bilirubin indicating a haemorrhagic event within this time frame[26]. All such patients should undergo further diagnostic imaging to confirm or exclude the presence of an aneurysm. The current gold standard remains four-vessel digital subtraction angiography although the sensitivity and availability of other imaging modalities such as CT-angiogram is improving[26,27].

Once diagnosis of aSAH is confirmed the aims of treatment are airway, ventilation and haemodynamic stabilisation followed by correction of reversible causes of neurological deterioration. This includes surgical evacuation of intracerebral haemorrhagic extensions of the SAH or correction of hydrocephalus. The former is seen in approximately one third of patients

although surgical evacuation is usually reserved for those haematomas that exert significant mass effect[26]. Patients with intraventricular and intracerebral haemorrhagic extensions are more likely to develop hydrocephalus. They may develop a gradual deterioration in conscious level following the haemorrhagic ictus or may initially present with an altered conscious level and ventriculomegaly. Approximately 20% of patients with aSAH will demonstrate ventriculomegaly on initial CT and approximately 80% of these patients will also have impaired consciousness[28]. Untreated however, within 24 hours around half of these patients will show spontaneous improvements in consciousness[26,28]. Treatment of hydrocephalus (symptomatic ventriculomegaly) usually involves continuous external ventricular drainage aimed at maintaining normal ventricular pressure.

Management of ruptured cerebral aneurysms

Subsequent management consists of early definitive aneurysm treatment followed by supportive measures to reduce the incidence and severity of complications. Within a few hours following haemorrhage, 15% of patients demonstrate a sudden deterioration in consciousness consistent with re-bleeding from the unprotected aneurysm[26]. Although pre-hospital re-bleeding has not been extensively studied, a recent prospective study has demonstrated that the greatest risk of re-bleeding is in the immediate few hours following haemorrhage[29]. For the continued course of patients surviving the first day following haemorrhage, the overall cumulative risk of re-bleeding at one month is 40% without treatment[26]. Re-bleeding is associated with a poorer clinical grade of haemorrhage and poorer outcome. An earlier study has demonstrated that a poor outcome was secondary to the initial haemorrhage in 32% of cases and secondary to a re-bleed in 35% of cases[30]. No specific risk factors for re-bleeding have been clearly demonstrated[31].

Traditional aneurysm treatment consists of craniotomy and surgical clipping. Endovascular occlusion of the aneurysm using detachable coils has emerged as a less invasive alternative treatment. Although there have been numerous observational studies, a large multi-centre randomised controlled trial comparing the two forms of treatment closed recruitment following an interim analysis which revealed a benefit of endovascular treatment[32]. The inclusion criteria for this trial included aSAH with the intention of aneurysmal treatment by surgical or endovascular means. Other criteria included a ruptured

aneurysm that was amenable to both endovascular and surgical treatment and an aneurysm that was not already clearly appropriate for one modality of treatment. There was recruitment of 2143 patients from 42 centres who were randomised into either the endovascular group (n=1073) or the surgical group (n=1070). Most patients were in a good clinical state with a small aneurysm of the anterior circulation. The relative and absolute risk reduction in the primary outcome measure of death or dependence at one year (Modified Rankin Score (MRS) of 3-6, see appendix 1.5) of endovascular versus surgical treatment was 22.6% and 6.9% respectively. A further analysis of these results has demonstrated that this early survival advantage was maintained for 7 years[33]. The analysis showed no good evidence that the benefit of endovascular surgery does not apply across all sub-groups (age, World Federation of Neurological Surgeons (WFNS) grade, Fisher grade and size and location of aneurysm). However for age, WFNS grade and aneurysm location, treatment effects were heterogeneous and difficult to interpret. The risk of late re-bleeding is low in both groups, although higher in the endovascular group.

The main criticisms of this trial include an expertise bias in favour of endovascular treatment. Most endovascular centres contributing to the trial employed dedicated interventional radiologists whereas contributions to the surgical arm were mainly from centres with general neurosurgeons without a specific neurovascular interest[34]. Secondly, a sample bias resulted in a propensity for smaller aneurysms to be included (92% less than 10 mm, 50% less than 5 mm) and an under-representation of middle cerebral and posterior

circulation aneurysms. This bias is in favour of endovascular treatment since large aneurysms and those of the middle cerebral aneurysm are anatomically more difficult to treat with this modality. However, the size and location of these aneurysms may represent the actual size and location of aneurysms seen in Europe[35]. Anterior circulation aneurysms do make up 90% of those encountered. In the UK, 85% of aneurysms are less than 1 cm and 66% are less than 5 mm[35]. A large number of patients eligible for the trial (9559 patients) were excluded by the treating physician. In the majority of these cases, the treating surgeon felt that surgery was the most appropriate form of treatment. As a result, patients in this trial are only included after they have satisfied this inclusion criteria and thus is not a true randomisation and does not represent an intention-to-treat randomised trial.

In summary, this trial is an important step in defining the roles of endovascular and microvascular surgery in the treatment of ruptured cerebral aneurysms although optimal management for groups of patients is still to be clearly defined.

Investigating DIND

The gold standard for the diagnosis of angiographic vasospasm has been accepted as arterial narrowing documented on digital subtraction angiography (DSA)[36]. Transcranial doppler (TCD) has been utilised as a surrogate measure of angiographic vasospasm with reasonable specificity and sensitivity but variable prediction of clinical deterioration/DIND[37,38]. The clinical entity of DIND better correlates with outcome and does not necessarily co-exist with angiographic vasospasm. The diagnostic criteria do vary slightly throughout the literature but it is in essence a diagnosis of exclusion and consists of two components. The first is often defined as a decrease in consciousness and/or new focal neurological deficit seen at least three days post ictus following exclusion of other causes of such a neurological deterioration (commonly re-bleed, seizure, hydrocephalus, hypoxia, sepsis and electrolyte disturbances). The second component is the presence of a new infarct not present on the initial and immediate post-operative imaging. Part of the variation in the diagnostic criteria seen in the literature is due to the requirement (or not) of angiographic vasospasm (or a surrogate measure of this from TCD) in diagnosing DIND. Computerised tomography angiogram (CT-A) and computerised tomography perfusion (CT-P) are increasingly being utilised to assess angiographic vasospasm (including distal microvascular vasospasm) and brain perfusion but are yet to be established as gold standards[39].

Management of DIND

At present, the mainstay of DIND treatment is neurocritical care management aimed at reducing secondary brain injury, oral nimodipine, haemodynamic therapy and endovascular techniques to improve angiographic vasospasm. What follows is an overview of the evidence underlying current therapy. Although statin/magnesium/nicardipine therapy and cerebrospinal fluid (CSF) drainage are not widely used, their inclusion herein is justified by recent studies highlighting their merit.

Haemodynamic therapy

Denny-Brown first noted that hypotension was associated with neurological deficits in patients with severe structural narrowing of cerebral blood vessels[40]. It was not until the 1960s and 70s that attempts at increasing systolic blood pressure with plasma expanders and vasopressors were noted to reverse ischaemia-related symptoms in cerebrovascular insufficiency[41]. In this regard, Kosnik and Hunt described the use of induced hypertension and hypervolaemia to reverse established DIND[42]. Larger series have shown that approximately two-thirds of patients with DIND improve with haemodynamic therapy, with the remaining patients either unchanged or worse despite maximal medical therapy[43,44]. Similar results were noted when induced hypertensive therapy was used alone[45]. This benefit may be seen following increases in cardiac index in addition to mean arterial pressure (MAP)[46].

The physiological explanation is that cerebral vasospasm results in a shift of cerebrovascular resistance to the large vessels of the Circle of Willis, thus impairing autoregulation in the distribution of the vasospastic vessel. Consequently, CBF varies directly with systemic blood pressure and according to the Hagen-Poiseuille law, increasing perfusion pressure or decreasing viscosity increases CBF. Although haemodynamic therapy has become widely accepted in the management of DIND, there are few published controlled trials investigating its efficacy and variable administration to patients, despite its association with significant morbidity: fluid overload (10-40%), pulmonary oedema (2-34%), dilutional hyponatraemia (3%), congestive heart failure (5-20%) and myocardial infarction (2%)[47]. Other complications include intracranial haemorrhage (less common with early aneurysm treatment), global cerebral oedema and death.

It is unclear which component of haemodynamic therapy (hypertension, hypervolaemia or haemodilution) confers benefit. The most contentious is haemodilution, following reports of reduced brain oxygen supply despite adequate CBF[48]. Isovolaemic haemodilution (haematocrit target of 0.28) has been shown to increase ischaemic brain volume despite a global increase in CBF[49]. Unfavourable outcome was associated with lower haemoglobin concentrations over the first two weeks following haemorrhage[50]. Conversely, higher haemoglobin levels have been associated with a better outcome following aSAH[51]. Similar questions have been raised regarding the additional benefit of hypervolaemic therapy[46,47,49], partly because of the increased risk of pulmonary oedema. There is an association between the

use of synthetic rescue colloids and impaired cerebrovascular parameters in poor WFNS grade patients, as well as poor outcome in all aSAH patients[52]. These findings are supported by other studies demonstrating that although induction of hypertension alone following aSAH increases regional CBF and brain oxygenation, additional hypervolaemic and haemodilutional therapy confers no additional benefit[53,54]. A systematic review of the literature concluded that there is no good evidence from controlled studies for a positive effect of triple-H or its components on CBF in aSAH patients[54]. Thus, there is a need for a carefully devised study utilising multimodal monitoring to investigate the effects of hypertensive therapy in hyper- and isovolaemic states without a concomitant decrease in haematocrit. The morbidity associated with such therapy needs further clarification.

Similar uncertainties are noted for the use of prophylactic haemodynamic therapy following aSAH. In a recent systematic review of 49 trials in which this therapy was administered prophylactically, only four were prospective, controlled and comparative studies[55]. Of these, three were not sufficiently/appropriately controlled or comparative to assess the efficacy of this therapy. However, two recent studies randomly assigned normovolaemic or hypervolaemic therapy to patients and reported no difference in the incidence of DIND between groups[56,57]. This may be explained by the observation that although hypervolaemic therapy results in higher daily fluid intake and cardiac filling pressures, it has no effect on net fluid balance, blood volume or CBF as assessed by ^{133}Xenon clearance[57]. Thus, earlier studies demonstrating improvements in CBF may have done so in initially

hypovolaemic patients given prophylactic hypervolaemic therapy soon after haemorrhage. This is almost certainly the case in studies prior to 1985, when fluid restriction was common practice[57].

Oral nimodipine

Numerous trials have investigated the use of oral nimodipine prospectively. The largest and most widely quoted of these (the British aneurysm nimodipine trial) recruited 554 patients to receive either placebo or 60 mg of nimodipine four hourly for 21 days[58]. There was a relative reduction in the incidence of cerebral infarction and poor outcome in the treatment group by 34 and 40%, respectively. Furthermore, a meta-analysis of seven nimodipine trials demonstrated that nimodipine almost halved the risk of neurological deficit and/or death secondary to vasospasm[59]. The incidence of infarction was also reduced by almost 60%, although mortality rate was largely unaffected. The suggestion that oral nimodipine is of clinical benefit in this setting was echoed by another meta-analysis and a recent Cochrane review of trials investigating several calcium antagonists[60,61].

Although oral nimodipine is accepted as standard care, its precise mechanism of action remains unclear. Despite being shown to reduce the incidence of DIND and infarction in clinical trials, it has negligible effects on angiographic vasospasm; indeed, nimodipine has been shown to improve behavioural outcomes in a murine SAH model without changing blood vessel diameter[62]. Treatment with nimodipine (which acts on the cerebral vasculature rather than at the neuronal level) resulted in worsening outcome despite increased blood

vessel luminal diameter. This suggests that nimodipine may be neuroprotective by blocking calcium influx at a neuronal level. A possible explanation for its limited effectiveness on angiographic vasospasm may relate to its predilection for small perforating arterioles rather than large proximal vessels: nimodipine results in greater vasodilatation of isolated rat intra-cerebral penetrating arterioles compared to pial arterioles. In a rat model of focal cerebral ischaemia, nimodipine reduced infarct size and both necrotic and apoptotic cell death[63]. It was also shown to antagonise spreading ischaemia induced by red blood cells in rats[64]. Its use in aSAH patients has been associated with an increase in fibrinolytic activity[65]. On balance, nimodipine's success in reducing the incidence of DIND in the clinical setting compared to its failure to improve outcome following ischaemic stroke may be reflective of the window of opportunity for preventing these cellular processes from taking place between aSAH ictus and the onset of DIND[62].

Endovascular therapy

Endovascular therapies for vasospasm have evolved since the first report of balloon catheter usage[66]. For patients with severe angiographic vasospasm and DIND that is refractory to haemodynamic therapy, the use of both endovascular balloon techniques and intra-arterial infusion of pharmacological vasodilators has become widespread. Research in this field consists largely of retrospective studies assessing the safety and efficacy of these techniques, with no randomised controlled trial to assess their impact on neurological outcome. For transluminal balloon angioplasty (TBA), several case series have reported angiographic improvements as high as 100%[67]. Overall

neurological improvement and incidence of infarction, however, has been modest[67-69]. Pharmacological agents such as papaverine were initially administered with fervour in an attempt to treat previously inaccessible distal vascular segments. However, criticism of its short active half-life, deleterious side effects (increasing intracranial pressure, which may depress capillary perfusion and venous outflow at the microcirculatory level[70]) and modest improvement in neurological outcome has kerbed this enthusiasm. A recent phase II randomised trial suggests that prophylactic TBA prior to the onset of DIND may reduce its incidence, although this study was inadequately powered to comment on the significance of this difference[71].

Intra-arterial nimodipine administration is gaining some acceptance in DIND management. Although the first descriptive studies gave mixed results about its effectiveness in treating both angiographic vasospasm and DIND, recent studies have demonstrated clinical improvements in approximately 75% of patients, although its effect on angiographic vasospasm (the primary endpoint) remained unclear[72-74]. A recent small, prospective study has contradicted these findings[75]. Although mean transit time and selective time to peak of the brain parenchyma were reduced one day after treatment, this effect was not sustained. Effects on CBF and volume were even less apparent. Half of assessable patients were clinically unchanged and 20% worsened following intervention. However, a third of patients failed to demonstrate angiographic improvements following the first treatment and were therefore excluded from further intervention despite the evidence that nimodipine may be beneficial without altering vessel diameter. In order to

counteract the problem of short duration of action, continuous intra-arterial infusion of nimodipine may represent a promising mode of delivery[76,77]. There is evidence to suggest that recurrent angiographic vasospasm and DIND is less frequently seen following intraarterial nimodipine when combined with TBA[78]. Future prospective studies to evaluate the effects of intra-arterial nimodipine should therefore be adequately powered to detect changes in clinical symptoms and outcome rather than angiographic vasospasm, and should also be carefully separated from other concurrent endovascular modalities.

Nicardipine

Nicardipine prolonged-release implants (NPRIs) have been subject to investigation in this setting[79,80]. Consecutive patients with aSAH implanted with NPRIs were shown to have a reduced incidence of angiographic vasospasm and DIND (11% in control group vs 6% in treated group)[79]. This was not a randomised study and was too small to comment on clinical differences. A small double-blinded randomised controlled trial which was not powered to detect changes in clinical outcome did demonstrate a significant reduction in delayed ischaemic lesions not attributable to operative morbidity in the study group given NPRIs[80]. Lower incidence of poor outcome and death were also noted in the treated group. This did not result in an improved quality of life at one year[81]. The use of nicardipine pellets or any drug infused via implants relies on placement during surgical aneurysm treatment. With the increasing trend towards endovascular treatment of ruptured aneurysms, future studies investigating such implants need to be devised with this in

consideration. The additional morbidity associated with application of these implants in those not undergoing surgical treatment of the aneurysm will have important implications on their potential role in the future.

Statin therapy

There has been much interest in 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors in the management of aSAH. These compounds are potent inhibitors of cholesterol production, although evidence suggests that statin therapy can also improve endothelial function and upregulate endothelial nitric oxide synthase (eNOS) without altering serum cholesterol levels. Other beneficial properties of statins include inhibition of platelet aggregration/adhesion, anti-inflammatory effects and decreased oxidative stress. Prospective randomised studies investigating the use of statins following aSAH have given promising results[82-84], with simvastatin and pravastatin reducing the incidence of DIND by 60-80%.

However, although these studies were blinded, they were inadequately powered to detect changes in clinical endpoints. The 60% incidence of DIND in the placebo group of the Lynch study is substantially higher than that documented in the literature. Less than 50% of patients completed the treatment in the Tseng study, although results were analysed as intention-to-treat. In a recent study comparing outcome before and after the introduction of statin therapy, no statistically significant difference in the incidence of DIND or poor outcome was found[85]. Although this study was retrospective in design and spanned a time period when endovascular aneurysm treatment was

being introduced, the study groups were similar and this represents the largest clinical investigation of statin use following aSAH to date. Other smaller studies have noted similar results[86,87]. Recent meta-analyses of this data have given mixed opinions regarding the benefits of statins in this setting, although there is a unified call for a prospective, randomised and controlled trial to investigate this further[88-90] as the detectable clinical differences may be far smaller than those noted in the current prospective studies. The results from such a trial are awaited (www.stashtrial.com).

There have been mixed reports of the effects of long-term statin use prior to haemorrhage. Evidence for its benefit comes from two retrospective reports demonstrating an association between statin therapy and a reduction in the incidence of DIND and improved outcome[91,92]. However, continuity of therapy following admission was not clearly discussed and the Parra study lacked complete three-month outcome data. Evidence against the beneficial effects of statins consists of two larger retrospective studies demonstrating no association with DIND and an increase in angiographic vasospasm risk in those patients receiving statin therapy[93,94]. Many statin users had their medication discontinued on admission in both studies, which may have impacted on the results given that the effect of pre-haemorrhage use of statins on the subsequent course of the haemorrhage and outcome remains unclear.

Magnesium

Magnesium sulphate acts as a non-competitive antagonist of voltage-dependant calcium channels and has been shown to have a neuroprotective effect by reducing vasospasm and infarct volume after experimental SAH. Hypomagnesaemia occurs in more than half of patients with aSAH and is associated with bleed severity[95] and, following ictus, is predictive of DIND. Treatment of aSAH patients with a continuous infusion of magnesium sulphate was associated with a 34% reduction in DIND risk and a better outcome[96]. A significant benefit was noted in delayed ischaemic infarction[97]. A recent Cochrane review of randomised trials investigating magnesium use in addition to oral nimodipine concluded that this represents a promising intervention that requires further investigation with a larger trial. Results from such a trial are awaited[98].

CSF drainage and thrombolysis

Since thick subarachnoid clot completely filling any cistern or fissure has been shown to be an independent predictor of DIND, it follows that clearance of blood from the subarachnoid space may reduce the prevalence and severity of DIND. The reported benefits of CSF drainage and thrombolysis in this setting will be discussed in the first part of this thesis.

Pathophysiology of delayed ischaemic neurological deficit

Although current therapies have improved the prognosis in patients with DIND, outcome remains poor. Interventions such as TBA and endothelin antagonists effectively target angiographic vasospasm but fail to improve outcome, lending notion to the theory that angiographic vasospasm may be a parallel end-point to the pathological process that results in ischaemic injury. Thus, the relationship between angiographic vasospasm and neurological outcome may be associative rather than causative, and neurological injury may not be entirely explained by ischaemia. However, it must be emphasised that this is a theory and that angiographic vasospasm will probably play some part in DIND. Some patients do respond to angioplasty and other patients with significant angiographic vasospasm do develop ischaemia and infarction in this distribution and their outcome is poor. New lines of research are therefore redefining our understanding of this process. What follows is a discussion of the current evidence for the pathophysiology of DIND.

Nitric oxide

Nitric oxide (NO) is a ubiquitous, inorganic and membrane-permeant gas produced by endothelial cells, neurons, glia and macrophages by three isoforms of nitric oxide synthase (NOS)[99]. Cerebrovascular tone requires continuous endothelial cell NO release[100] which, under physiological conditions, is produced by constitutive eNOS and neuronal NOS (nNOS). Paradoxically, NO has both neuroprotective and cytotoxic effects in the central nervous system (CNS). The relatively low levels of NO produced by eNOS are neuroprotective, whereas high levels of NO produced by macrophage nNOS and induced NOS (iNOS) during ischaemia may exacerbate/contribute to injury[99].

Much of the research investigating NO in aSAH has been based on animal models and has concentrated on association with angiographic vasospasm. It has been postulated that oxyhaemoglobin found in abundance in the perivascular space following aSAH, can scavenge NO and destroy nNOS-containing neurons[101]. Several clinical studies have reported conflicting findings of CSF nitrate (a stable NO metabolite) elevation or depression following aSAH[99,100,102]. Sustained overproduction of NO can lead to membrane peroxidative injury, change in blood vessel morphology and endothelial dysfunction. However, neuronal injury secondary to ischaemia can also produce NO, and so it is unclear as to whether NO is a cause or a consequence of DIND, or both[100]. Indeed, much of the research that has concentrated on endothelial cell dysfunction and associated mediators has yet to determine whether endothelial cell dysfunction is a true pathophysiological

factor in angiographic vasospasm and DIND or just an epiphenomenon. Although NO donors such as sodium nitroprusside have been successful in treating vasospasm in animal models, they have not consistently shown promise in clinical studies and are potentially limited by side-effects[103,104]. Newer modalities of NO donation should be subject to future investigation[105].

Endothelin

Endothelin-1 (ET-1) is a molecule produced principally by the endothelium which acts at a paracrine level to promote smooth muscle contraction, vasoconstriction, endothelial/smooth muscle cell proliferation and inflammation[106]. Several human studies have demonstrated elevated levels of ET-1 in plasma, CSF and microdialysate following aSAH[107-109]. However, there have been conflicting results as to which compartment this elevation is observed in, and its precise relationship with angiographic vasospasm. More importantly, the association between levels of ET-1 and DIND has been demonstrably weak. A randomised, double-blind, placebo-controlled trial investigated the use of clazosentan, a selective $ET\text{-}_A$ receptor antagonist, following aSAH demonstrated a significant dose-dependent reduction in the primary end-point of moderate to severe angiographic vasospasm in patients randomised to the treatment arm[110]. However, this did not translate into improved clinical outcome and was associated with an increased incidence of pulmonary oedema, adult respiratory distress syndrome and hypotension. Centrally assessed post-analysis of this data did demonstrate a trend towards improved outcome when DIND with mild angiographic vasospasm was excluded.

Lipid peroxidation

Accumulation of arachidonic acid may result in alterations in vasomotor regulation and disruption of blood brain barrier (BBB) integrity[111]. Haemoglobin breakdown products may activate lipooxygenase and cyclooxygenase, thus promoting conversion of arachidonic acid into products such as prostaglandins and leukotrienes. Levels of lipid peroxides were significantly higher in the CSF of aSAH patients with DIND than those without and were noted prior to the development of cerebral infarction. As such, these are more likely to have been produced by the blood clot rather than the infarction *per se*[112]. Efflux of free fatty acids is a marker of lipid peroxidation. CSF free fatty acid levels have been noted to undergo a biphasic increase after aSAH[111], with patients with DIND having higher CSF concentrations of arachidonic, palmitic and linoleic acid.

The arguments in favour of lipid peroxidation playing a role in the pathogenesis of DIND are attractive. Oxyhaemoglobin may catalyse free radical generation and lipid peroxidation, resulting in membrane perturbation and cellular dysfunction within the arterial wall. Lack of effective scavenger systems within CSF may account for the delayed nature of the deficits since systems originating in serum may suppress free radical reactions within three days of haemorrhage[112]. In addition, lipid peroxidation may directly stimulate smooth muscle contraction and generate an inflammatory response involving arachidonic acid metabolites[111]. Five multicentre randomised controlled trials have investigated the use of tirilazad, an inhibitor of lipid peroxidation, a

recent meta-analysis of which has shown that although tirilazad treatment was associated with a significant reduction in the incidence of DIND, overall outcome was unchanged[113,114].

Bilirubin oxidative products (BOXes)

In terms of oxidative stress, there has also been interest in free radical oxidation of bilirubin, biliverdin and haem to produce BOXes[115,116]. Interestingly, the formation of bilirubin and subsequent oxidation to BOXes results in a peak concentration of the latter which coincides with the peak period of angiographic vasospasm and DIND[116]. Cerebrospinal fluid from aSAH patients with angiographic vasospasm (with and without DIND) features higher levels of bilirubin, oxidative stress and BOXes than that of non-vasospasm patients[117]. Unfortunately, the emphasis of much of this elegant work has been on *in vitro* studies and angiographic vasospasm. BOXes applied to the brain surface of male Sprague Dawley rats led to a dose-dependent arteriolar vasospasm without any evidence of infarction[118]. This discrepancy was thought to be due to the single application of BOXes as opposed to prolonged exposure in the clinical setting or the possibility of other factors involved in the development of DIND and infarction[116]. Either way, further human studies are required to determine and compare the oxidative stress status and the level of BOXes in both DIND and non-DIND patients.

Microcirculation and autoregulation

It has been demonstrated that regional CBF is only affected once extraparenchymal vasospasm results in >50% luminal narrowing[119]. Although

large cerebral vessels contribute to vascular resistance, they do not significantly affect CBF[120]. A major determinant of microvascular pressure is the ratio of upstream to downstream resistance[121]. If large arteries constrict and arterioles dilate, there will be a decrease in microvascular pressure without a change in CBF, making microvascular pressure an independently regulated variable and contradicting the concept that large vessel vasospasm contributes significantly to downstream disturbances of CBF. Although severe extraparenchymal vasospasm is associated with reduced CBF, both reduced CBF and DIND are noted even with mild vasospasm or no vasospasm at all (i.e. severe vasospasm is not a prerequisite for DIND)[119,122,123]. Other sources of evidence against large vessel vasospasm as a significant cause of ischaemia and neurological deficit include several studies demonstrating a poor correlation between the site and severity of angiographic vasospasm and the clinical/measured ischaemia[119,120]. The location of cerebral infarction on CT could not be predicted by angiographic vasospasm in up to one-third of cases[124], although formal angiography was not performed in all patients. Post-mortem studies have demonstrated that most infarcts following aSAH do not have the typical appearance of large territorial infarcts and are cortical in >75% of aSAH patients[125]. Widespread and hypothalamic lesions consistent with those secondary to diffuse microangiopathy were noted in a subset of patients. Cortical lesions were associated with fluctuating blood pressure suggesting autoregulatory dysfunction. A direct effect of blood clot around the cortex has also been implicated with cortical infarcts[126].

These observations that there may be other factors contributing to ischaemia have prompted increasing interest in cerebral microvasculature and autoregulatory function. The evidence to suggest that vasospasm can occur at a microvascular level is based largely on animal models and has given conflicting views, although a clinical study has demonstrated a strong inverse correlation of peripheral cerebral circulation time (a measure of microvascular resistance) and regional CBF regardless of the severity of angiographic vasospasm by digital subtraction angiography[119].

Initial reports indicate that reduced autoregulatory responses to carbon dioxide are not due to autoregulatory capacity dysfunction, but may instead be due to maximal dilatation of peripheral arterioles in an attempt to maintain CBF when cerebral perfusion pressure (CPP) is reduced[48,119]. More recent positron emission tomographic (PET) studies have demonstrated a reduction in cerebral blood volume (CBV) implicating impairment of the vasodilatory capacity of distal vessels in the face of reductions in CPP[127]. It was concluded that since a reduction in CBV was noted in areas of angiographic vasospasm under hypoxic conditions that would normally increase CBV, then parenchymal vessels distal to arteries demonstrating vasospasm exhibited impaired autoregulatory dilation. This opinion has been echoed by other studies measuring autoregulation by different techniques[128-130]. For example, a recent study utilised brain tissue oxygen ($PtiO_2$) and pressure reactivity as a surrogate measure of autoregulation to demonstrate impairment of autoregulatory capacity in aSAH patients with DIND several days prior to the onset of ischaemic changes[128]. It is thought that perturbed cerebral

vasculature autoregulatory function fails to compensate for vasospasm-induced reductions in vessel diameter at the microvascular level, thereby increasing the risk of ischaemia[128]. This is probably an oversimplification of a complex process, highlighted by the wide variation in CBF patterns amongst patients with DIND, which range from reduced flow to hyperaemia on PET images[131].

Although current evidence points to autoregulatory dysfunction in DIND, more clarity is required regarding the extent of this dysfunction and the regions at risk. Whether this represents new autoregulatory dysfunction at the time of/immediately preceding DIND or a failure to re-establish autoregulation after the haemorrhage ictus is still unclear. New clinical modalities including real-time local CBF monitors used in conjunction with other local and global monitoring tools may help improve our understanding of microcirculation. These findings would clearly need to be considered in context with cerebral metabolism and oxygenation.

Microthrombosis

There has been increasing interest in the role of platelets and the coagulation system in DIND. Platelet aggregation is thought to impede circulation and aggravate brain injury in addition to causing scattered microinfarcts[132]. This is supported by human post-mortem studies following aSAH which have demonstrated the presence of infarcts with the appearance of those secondary to small thomboemboli[120]. Other possible mechanisms of injury resulting from platelet aggregation include release of vasoactive contents from

granules and alterations in microvascular structure following release of enzymes[133]. While an increase in platelet consumption in the days following aSAH was shown to be an independent predictor of DIND in an Asian population[134], these findings were not seen in a Caucasian population[135], although both studies lacked assessment of platelet function. A recent meta-analysis of seven trials investigating anti-platelet therapy following aSAH concluded that this treatment may reduce the risk of ischaemic complications and improve outcome, although these findings were not statistically significant[136].

Aneurysmal SAH has nevertheless been associated with a hypercoagulable state[134,137-139]. Markers of thrombin production and fibrinolytic activity were significantly raised and correlated with grade of aSAH and long term outcome[137-139], although this correlation was less apparent in a larger study [140] and no association has been found between these markers and DIND/cerebral infarction[137,139,141]. There have been conflicting reports as to whether D-dimer is an independent predictor of DIND[138,141]. Although uncertainty exists regarding CSF markers of fibrinolysis since most studies had been performed in conjunction with antifibrinolytic therapy, a recent study has demonstrated an association between CSF thrombin activation and infarction secondary to DIND[142]. Only the rate of blood clearance in the first three days following aSAH could predict DIND. Higher levels of thrombin activation were noted in patients with low blood clearance and in those with DIND. This raises the question as to whether the compartmentalised CNS coagulation cascade exerts its primary effect by slowing blood clearance and

allowing other factors to trigger DIND rather than by a direct thrombotic effect *per se*.

Inflammation and Complement

There is increasing evidence to suggest that a complex inflammatory response is initiated both systemically and within the CNS following aSAH. How this relates to ischaemic neurological injury and which exact mediators and mechanisms are involved is currently unclear. A detailed review of this subject is presented in part 2 of this thesis.

Apoptosis

Endothelial cell apoptosis has been implicated in breakdown of the BBB, oedema formation and secondary brain injury following aSAH[143]. A key event in this process is thought to involve apoptosis of neurons and cerebral endothelial cells. Neuronal cell death contributes to cytotoxic oedema while endothelial cell apoptosis is thought to result in a breakdown of the BBB, which subsequently contributes to vasogenic oedema.

Although experimental data is drawn principally from animal models (which operate on a different timescale to that seen in humans) following SAH, it has repeatedly been shown that the apoptotic apparatus is activated following SAH[143-147] (Table 1). While p53 is a transcription factor that has been implicated in the pathogenesis of neoplastic disease, there is evidence to suggest that it also plays a role in the apoptotic process following aSAH via caspase-dependent and independent pathways[144]. Both p53 and pan-

caspase inhibition attenuate apoptotic activation and, more importantly, reduce delayed (>24 h) BBB breakdown and increases in brain water content. Neurological outcome has also been shown to improve in most models. However, because of the broad mechanism of action of p53 and the complexity of the caspase-dependent and independent pathways, further information is required regarding the type and extent of inhibition required to induce these responses. Future clinical studies investigating apoptotic activation following aSAH may well underpin the development of new therapeutic strategies.

Spreading Depolarisation

Cortical spreading depolarisation (CSD) was first described in rabbit cerebral cortex[148] and refers to the wave of neuronal depolarisation which is ignited when passive cation influx across cell membranes exceeds adenosine tri-phosphate (ATP)-dependant sodium and calcium pump activity, which if prolonged can result in cell death[149]. Since under physiological conditions pump recruitment occurs, this is an energy consuming process. As a result, CSD induces vasodilation causing an increase in CBF. However, under ischaemic conditions, CSD can exacerbate ischaemia by causing severe microvascular vasoconstriction and reduced CBF[150]. This cortical spreading ischaemia (CSI) describes this CSD-induced perfusion deficit[149].

Table 1-Summary of experimental evidence investigating apoptotic pathways following SAH

Abbreviations: SAH-subarachnoid haemorrhage, TUNEL- Terminal deoxynucleotidyl transferase dUTP nick end labeling, BBB-blood brain barrier, TNF-tumour necrosis factor, IL-interleukin, BA-basilar artery, Bcl-B-cell lymphoma, bax-Bcl-2 associated X-protein.

Study Author	**Model Utilised**	**Apoptotic inhibition**	**Main findings (Effect of inhibition)**	**Effect on neurological scores/brain oedema/mortality**
Park et al., 2004	Sprague-Dawley rat SAH model.	Pan-caspase inhibition.	1. No change in caspase-3 and TUNEL staining in hippocampus and cortex but attenuation in endothelial cells. 2. Attenuation of vasospasm in blood vessels.	No significant difference in mortality. Improvement in BBB permeability, brain water content and neurological score after 24 hours in treated group.
Zhou et al., 2005	Mongrel dog double SAH model.	p53 inhibition.	1. Attenuation of angiographic and histological vasospasm. 2. Significantly reduced expression of all apoptotic proteins. 3. Reduced expression of TNF-α in endothelial and smooth muscle cells.	Delayed improvement in appetite and activity in the treated group.
Cahill et al., 2006	Sprague-Dawley rat SAH model.	p53 inhibition.	1. Attenuation of increased expression of all apoptotic mediators, particularly p53. 2. Attenuation of severe BA vasospasm at 24 and 72 hours.	Improved mortality and neurological scores at 72 hours. BBB function and brain water content improved at 24 hours only, effects had subsided by 72 hours.
Iseda et al., 2007	New Zealand white rabbit and Sprague-Dawley rat SAH models.	Pan-caspase inhibition.	1. Reduced angiographic/histological vasospasm. 2 Reduced CSF IL-1β. 3. Reduced caspase-1/IL-1β immunoreactivity in infiltrating macrophages in the subarachnoid space.	Not commented upon.
Gao et al., 2008	Sprague-Dawley rat SAH model.	Pan-caspase inhibition (tetramethylpyrazine)	1. Attenuation of p53 and TUNEL staining in cerebral cells. 2. Attenuation of histological vasospasm. 3. Reduced protein levels of Bcl-2, bax and caspase-3 in hippocampus and basal cortex.	Improvement in neurological function, brain water content and BBB permeability at 24 hours in treated group.

Animal studies have demonstrated that topical brain superfusion of artificial CSF with a high concentration of haemoglobin and potassium resulted in acute, spreading decreases in CBF[151], which transformed a normal spreading hyperaemic response into a CSI[152]. This pattern of ischaemia was similar to that of ischaemia-reperfusion seen in brain injury and was reversible by nimodipine. The similarity of these experimental conditions with aSAH has prompted the question of whether spreading depolarisation is associated with DIND. In a recent human study of aSAH, the evolution of ischaemic stroke was associated with clusters of CSD and increasingly prolonged periods of electrocorticographic depression, the latter being restricted to areas of radiologically new infarct evolution[149,153]. Only patients with DIND demonstrated a statistically significant delayed increase in the number of spreading depolarisations per day. Similarly, the presence and absence of a delayed cluster of CSD had high positive and negative predictive values for DIND. Although these are preliminary findings, spreading depolarisation in this context should be the subject of further investigation.

Summary

Although progress has been made in improving the management of aSAH and established DIND, morbidity and mortality associated with DIND remains significant. There is a paucity of good evidence to support even the most established therapies. Carefully devised studies are required to investigate the components of haemodynamic therapy and the continuously evolving endovascular technologies. In particular, emphasis must be placed on clinical endpoint with the aid of multimodal monitoring where appropriate. This has important implications for trial size and design since DIND is an endpoint occurring only in a third of patients.

The significant progress in our understanding of the pathophysiology of DIND has been marred by difficulties in differentiating between the effects of the initial haemorrhage and early brain injury with those of DIND and, similarly, between the causes and consequences of ischaemia. Distinguishing DIND from angiographic vasospasm is also paramount in improving our understanding of this pathological process since both are closely related spatially and chronologically and are likely to be caused by similar mechanisms. This bears on animal-based studies that have traditionally focused on angiographic vasospasm. Nevertheless, the development of new therapeutic interventions that currently lie at the translational level hold much promise for future patient prognosis.

Aims of the current study

There are two arms to the current study:

1. A prospective, randomised and controlled trial (RCT) investigating the use of lumbar drainage of CSF following aSAH in reducing the prevalence and severity of DIND.
2. Assessment of the hypothesised inflammatory response following aSAH and the effects of lumbar CSF drainage on this inflammatory response.

Part 1: Lumbar drainage of cerebrospinal fluid following aneurysmal subarachnoid haemorrhage: A prospective, randomised and controlled trial (LUMAS)

Introduction

The reported prevalence of DIND is 20-35% although in those with a higher blood load this may be as high as 40%[154-156]. It is thought to result in cerebral infarcts in approximately 20% of patients[157] and cause 13% of all death and disability following aSAH[155,158]. Although the underlying pathophysiological process is not known[159], the presence of blood or its breakdown products within the subarachnoid space and cisterns is clearly associated with DIND[4,25,160]. An association between the size of the blood clot following aSAH and the prevalence of angiographic vasospasm and DIND is historically attributed to Fisher[25]. This has subsequently been verified[161,162] and thick subarachnoid clot completely filling any cistern or fissure has been shown to be an independent predictor of DIND[4].

It follows from this that attempts at clearing the subarachnoid space of blood may potentially reduce the prevalence and severity of DIND. Numerous techniques have been described to reduce the blood load including external ventricular drainage, cisternal drainage, intrathecal thrombolysis, head-motion therapy, third ventriculostomy and incision of Lillequist's membrane[163-167]. Studies investigating cisternal drainage have reported mixed results[163,168-172]. They are small, retrospective and often combine results with other forms of CSF drainage (lumbar and external ventricular drainage) and intrathecal thrombolysis. Trials investigating recombinant tissue-plasminogen-activator following aneurysm clipping have also given mixed results although a meta-analysis of over 600 patients has demonstrated an absolute risk reduction of 14% for DIND, 10% for poor Glasgow Outcome Scale scores and 5% for

death[173]. Although the overall rate of complications secondary to thrombolysis was considered low, cisternal drains and intrathecal thrombolysis are not without morbidity. Given the trend towards endovascular treatment for ruptured aneurysms there is now restricted access to the subarachnoid cisterns in most patients who traditionally would have undergone a craniotomy and clipping of the ruptured aneurysm.

There has been some evidence to suggest that drainage of CSF via the lumbar cistern may be of some benefit in reducing the prevalence of DIND[156,174]. A lumbar drain is less invasive than other forms of CSF drainage and is a device that is regularly used for other clinical purposes. A recent study has retrospectively analysed the effects of lumbar drainage in aSAH by comparing the practice of two vascular neurosurgeons during the period of 1994 and 2003[156]. One surgeon routinely placed an intra-operative lumbar drain during clipping or endovascular coiling of an aneurysm (unless contraindicated), whilst the second surgeon did not. Data was collected on 266 patients, although following adherence to strict exclusion criteria, the study included a group of 167 aSAH patients.

All patients received standardised care including early definitive treatment for the aneurysm (surgical or endovascular coiling) and optimal medical therapy. Patients presenting with radiological evidence of raised intracranial pressure and/or hydrocephalus were treated with external ventricular drainage regardless of future insertion of lumbar drainage. Patients were divided into two groups, those that had lumbar drainage (number of patients 81) and those

that did not (number of patients 86). Outcome measures included the development of clinically evident vasospasm, the need for endovascular interventions (including angioplasty and/or intra-arterial papaverine) and the development of vasospasm-related infarction. Results were in favour of lumbar drainage. There was a statistically significant difference in the incidence of clinically symptomatic vasospasm (51% in the non-lumbar drain group, 17% in the lumbar drain group), need for endovascular therapy (45% in the non-lumbar drain group, 17% in the lumbar drain group) and vasospasm-related infarction (27% in the non-lumbar drain group, 7% in the lumbar drain group). There was also a statistically significant difference between the two groups in disposition at discharge, length of in-patient stay and Glasgow outcome scores (GOS) (better in the lumbar drain group).

Although a robust retrospective study, there were certain limitations. The study had a degree of selection bias with a tendency of higher Fisher grades (those in group 3-4 and group 4) not to have lumbar drainage. Logistic regression analysis and subgroup analyses showed that following correction for this bias, results were still in favour of lumbar drainage. Other limitations of the study include the long period in which patients presented to the department (9 years) and the inherent limitations to retrospectively analysing case notes. In addition, comparing the practice of two neurosurgeons introduces another bias. Other features which could be addressed in a future study include quantification of the amount of CSF drainage (including the amount of intraoperative drainage) and the use of lamina terminalis fenestration during the operation and the implications this would have in the

outcome measures[175]. These factors have not been adequately addressed in this study. A prospective, randomised and controlled trial will provide a solid scientific platform upon which to investigate the use of lumbar drainage following aSAH[156,175,176].

Aims of the study

The aim of this study is to conduct a prospective, randomised and controlled trial to investigate the use of lumbar drainage of CSF following aSAH.

The following questions are to be answered:

1. In aSAH patients, does lumbar drainage reduce the prevalence and severity of DIND?
2. Does lumbar drainage improve long-term clinical outcome following aSAH?
3. Does lumbar drainage affect the prevalence of chronic hydrocephalus and the need for CSF shunting following aSAH?
4. Can lumbar drainage be incorporated into the treatment plan for aSAH in a safe and effective manner?

Materials and Methods

Patients

This single-centre prospective randomised controlled trial commenced recruitment of patients with aSAH in October 2006 and closed recruitment in July 2010 as per protocol (appendix 1). Patients admitted to the neurosurgery department at Leeds General Infirmary with aSAH were assessed for suitability according to inclusion criteria (table 2). The protocol was approved by the Leeds West Research Ethics Committee (October 2006).

Procedure and interventions

Written consent (or assent obtained from relatives in those unable to give informed consent) (appendices 2 and 3 respectively) was obtained and the patient was randomised to either the study group of insertion of a lumbar drain in addition to standard therapy or the control group of standard therapy alone.

Standard therapy consisted of management in a neurosurgical high dependency unit by a multidisciplinary neurovascular team, fluid replacement to maintain euvolaemia and 60 mg of oral nimodipine administered every four hours. CT angiography ± DSA was performed to confirm the presence of a cerebral aneurysm and the aim was for early aneurysm treatment. Aneurysm treatment was performed using either endovascular or surgical techniques as assessed by the treating neurovascular surgeon and the interventional radiologist. In those patients demonstrated to have multiple aneurysms,

Table 2-Inclusion criteria for recruiting patients.

Age 18 years or over.
Written informed consent or relative assent obtained.
Proven aneurysmal subarachnoid haemorrhage.
World Federation of Neurological Surgeons grade 1-3 (appendix 1.2)[1].
Fisher grade 2, 3 or 4 on initial CT scan (appendix 1.3).
Recruitment prior to 96 hours post-haemorrhage[2].
No contraindication to lumbar drain[3].

1. Assessed following transfer to the recruiting centre and resuscitation (including treatment of acute symptomatic hydrocephalus thought to be contributing to a deteriorating Glasgow Coma Score (GCS) with an external ventricular drain where relevant).
2. If presentation was beyond four days, according to the trial hypothesis, pathways that may lead to DIND may already have been established.
3. This includes bleeding diathesis, large supratentorial intracerebral/subdural haematomas causing midline shift and any size of posterior fossa intracerebral haematoma. Small intraventricular haemorrhage per se was not deemed a contraindication although significant ventricular haemorrhage was excluded.

attempts at treatment were applied to all aneurysms where possible although treatment priority rested with the aneurysm responsible for the haemorrhage. If a patient was diagnosed with a DIND, hyperdynamic therapy was instituted to improve cerebral perfusion (crystalloid and colloid fluids ± inotropes to augment MAP). If deemed appropriate, further DSA and intra-arterial nimodipine ± TBA were performed regardless of the presence or absence of large vessel angiographic vasospasm.

If randomised to the study arm, the patient underwent insertion of a Medtronic^{R} lumbar intrathecal catheter into the lumbar cistern. This was performed using an aseptic technique with local anaesthesia (or under general anaesthesia if at the same time as aneurysm treatment). A 14-gauge Tuohy needle was inserted into the lumbar cistern and lumbar catheter was inserted through this needle to the length of 20 cm and secured. The catheter was connected to a Medtronic^{R} Becker drainage system adjusted hourly to drain 5-10 ml of CSF per hour. This remained in-situ until the CSF was visibly clear or for ten days (whichever was the sooner). Following screening and recruitment, patients were not subjected to further DIND prevention trials.

Outcome measure

The primary outcome measure was the prevalence of DIND. This was defined as a drop in consciousness (one motor score or two eye/verbal scores of the GCS) or a new focal neurological deficit at least 96 hours post haemorrhage, not present immediately after aneurysm treatment and following exclusion of other causes. These other causes included seizure,

hydrocephalus, re-bleed, hypoxia/respiratory failure, end-organ failure, sepsis and electrolyte disturbance. This diagnosis could be made within 21 days of ictus. TCD, CT-A and CT-P were utilised when required to aid management. Angiographic vasospasm was not a pre-requisite for diagnosing DIND in this cohort of non-comatosed aSAH patients with a good WFNS grade[177].

Secondary outcome measures were: MRS at day 10 and at six months following haemorrhage, persisting neurological deficit at discharge in those who have had a DIND, radiologically confirmed established infarct (as assessed by CT when performed as part of clinical care only) and the prevalence of ventriculoperitoneal CSF shunting.

Randomisation

Randomisation to treatment group was performed by randomly permuted blocks of 20 with an allocation ratio of 1:1 using an online package (http://www.randomization.com). Instructions on the next intervention were sealed in an envelope and numbered at the start of the study. The research team were blinded to any information about block size and the envelopes were prepared independently from the research team involved in recruiting patients and managing the trial. The research team opened the envelopes sequentially at the time of randomisation only.

Blinding

Although treatment allocation could not be blinded, clinicians/investigators were masked to outcomes when possible. MRS at six months was obtained

by the investigators (Y.A and D.B) blinded to the treatment allocation via a structured telephone interview directly with the patient (or their primary carer if the patient was unable to communicate on the phone) as previously described[178]. Blinding of treatment allocation was not always possible for MRS obtained at ten days post haemorrhage but was performed by a physiotherapist involved with the patient's clinical care and verified by the investigators (Y.A and D.B). All diagnoses of DIND were made by a multidisciplinary neurovascular team (neurosurgeons, neurointensive care anaesthetists and neuroradiologists) and were prospectively and retrospectively verified (from prospectively collected clinical parameters (appendices 4 and 5) and electronically recorded blood results/picture archiving and communication system) by the investigators blinded to the treatment allocation. With the exception of a few additional clinicians, the multidisciplinary team had remained largely unchanged over the four-year study period. No disagreements occurred between prospective and retrospective assessments due to the relative ease of assessing the neurology of good grade patients, the absolute diagnostic criteria for DIND and the objective investigations required to exclude other causes of neurological deterioration. Neuroradiologists blinded to the treatment allocation reported radiologically confirmed infarcts as part of routine clinical care.

Statistical Analysis

The power calculation was based on a prevalence of DIND of 40% as previously audited in the neurosurgical department at Leeds General Infirmary

and an estimated benefit of a 20% absolute reduction in DIND with lumbar drainage (based on the current evidence for lumbar drains[156]). For 85% power this required 105 patients in each arm. Planned interim analyses were performed following recruitment of 40 patients (to establish adverse effects only) and 100 patients. The data monitoring committee/statistical consultation advocated continuation of the trial to completion following these analyses.

Baseline data on all patients with aSAH was collected prospectively on confirmation of the aneurysmal cause of the haemorrhage. The primary analysis was performed on an intention-to-treat basis. A secondary analysis with some patients being excluded was also performed (see below). The analysis of the primary outcome was a categorical frequency comparison with the Chi-squared (χ^2) test. Comparison of secondary outcome data between groups was performed with several tests. All numerical data were assessed for normality using the Shapiro-Wilks test and Q-Q plots. Normal numerical data was tested with the independent t-test and data not normally distributed with the Mann-Whitney U test. Categorical frequencies were compared with the χ^2 test or Fisher's exact test when a cell size was less than 5. $p<0.05$ was considered statistically significant. All statistical tests were conducted with IBM Statistics for the Social Sciences (SPSS) version 18. This study was registered with ClinicalTrials.gov (NCT00842049).

Results

The trial profile is shown in figure 1. From a total of 426 patients with aSAH, 210 were recruited and randomised to the trial. The main reasons for exclusions were poor WFNS grade and delayed presentation following ictus. The primary analysis followed intention-to-treat principles and included all 210 patients. A secondary analysis was performed that excluded those patients in the study group that could not receive lumbar drain/CSF drainage therapy due to an inability to place a functioning drain and those patients in the control and study groups that were treated for hydrocephalus with early external ventricular drainage (within 14 days of ictus). Two randomised patients that were initially diagnosed with aSAH based on CT-A were subsequently found not to be aneurysmal on formal angiogram and were therefore excluded as part of this secondary analysis.

Table 3 shows baseline patient characteristics including age, gender, WFNS grade, Fisher grade, aneurysm type and treatment modality in the two groups. There is a greater prevalence of multiple aneurysms in the study group and hydrocephalus requiring external ventricular drainage in the control group.

Table 4 shows the primary intention-to-treat analysis of primary and secondary outcome measures. There was a significantly lower prevalence of DIND in the study group (prevalence of DIND in the control group 35% (confidence interval (CI) 26.2-45.2%) versus 21% (CI 13.6-30.0%) in the study group (p=0.021)). A better outcome at ten days post ictus was noted for

those in the study group. There were no significant differences noted in the other outcome measures including MRS at six months.

Figure 1- Trial profile. 1. Between 2006 and 2008 endovascular services at Leeds General Infirmary were occasionally unavailable and so following initial assessment and stabilisation; some patients were transferred to other neurosurgical units for their continued management.

2. Initially good World Federation of Neurological Surgeons (WFNS) grade with very early subsequent neurological and/or systemic deterioration prior to recruitment to the trial.

3. Radiologically or due to heparin therapy given during endovascular treatment, which continued beyond the four-day post ictus window allowable for recruitment.

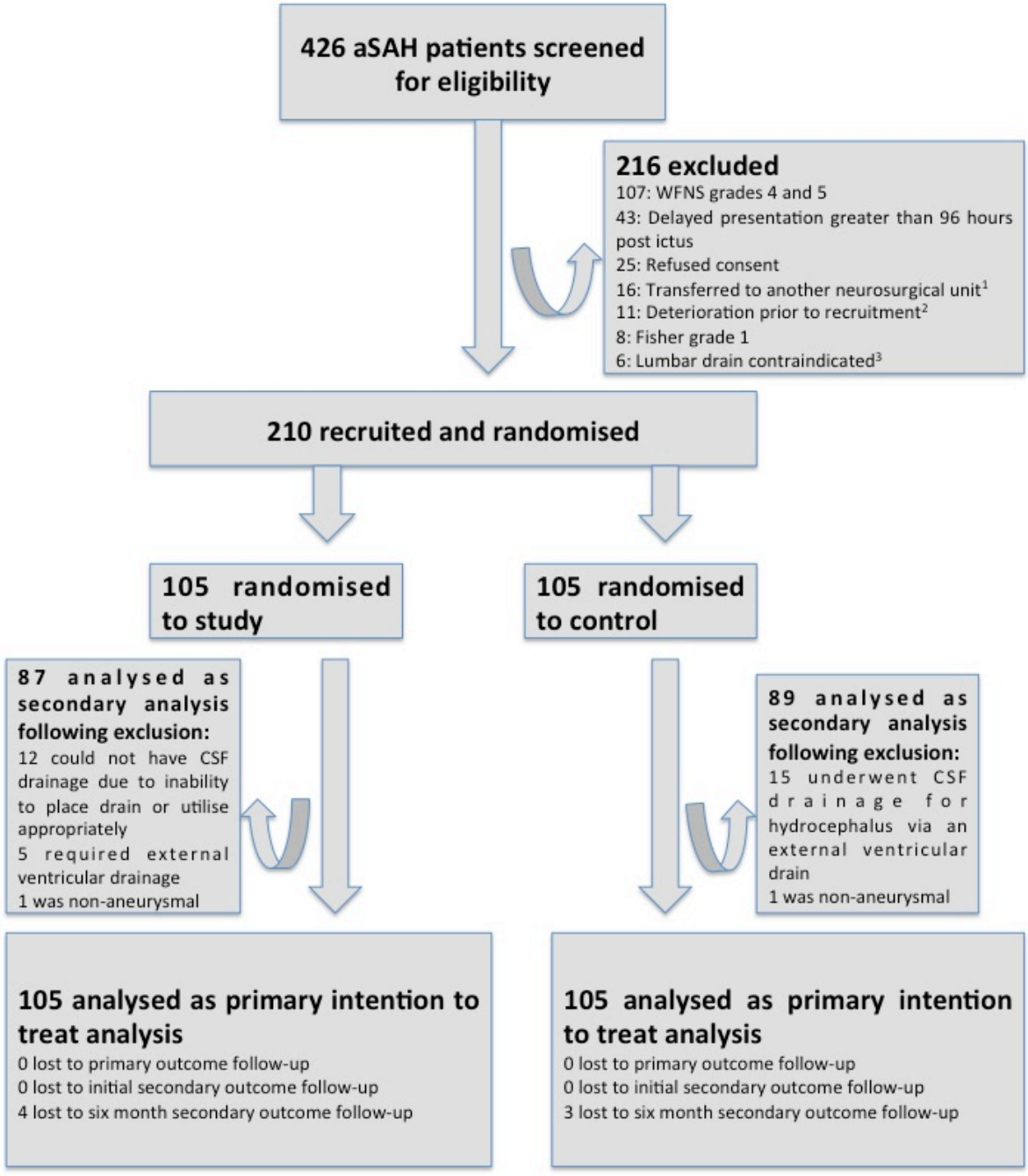


Table 3 (overleaf)-Baseline characteristics for patients in both arms of the trial

Abbreviations: M-male, F-female, WFNS-World Federation of Neurological Surgeons, ICA-internal cerebral artery, OA-opthalmic artery, PComA-posterior communicating artery, MCA-middle cerebral artery, ACA-anterior cerebral artery, PA-pericallosal artery, AComA-anterior communicating artery, VA-vertebral artery, BA-basilar artery, SCA-superior cerebellar artery, PICA-posterior inferior cerebellar artery, PCA-posterior cerebral artery.

Characteristic			**Control Group**	**Study Group**	**Significance**
Cohort size			105	105	N/A
Median age (interquartile range)/years			56.1 (45.0-63.6)	53.3 (44.9-62.2)	p=0.38
Sex (M:F)			1:3.6	1:4	p=0.74
WFNS grade (% of group)	**1**		63.8	68.6	p=0.47
	2		31.4	25.7	
	3		4.8	5.7	
Fisher grade (% of group)	**2**		43.8	37.1	p=0.33
	3		41.9	39.1	
	4		12.4	10.5	
	3+4		1.9	13.3	
Aneurysm type (number of patients)[1]	**Anterior circulation**	**ICA**	6	8	p=0.25
		OA	1	2	
		PComA	21	16	
		MCA	17	21	
		ACA	18	8	
		PA	2	5	
		AComA	26	25	
	Posterior circulation	**VA**	0	1	
		BA	3	10	
		SCA	1	1	
		PICA	6	4	
		PCA	3	3	
	None		1	1	
Multiple aneurysms/% of group			18.1	32.6	**p=0.017**
Aneurysm Treatment	**Coiling**		86	78	p=0.45
	Clipping		13	16	
	Both[2]		4	10	
	None[3]		2	1	
Procedural adverse events[4]	**Aneurysm rupture/coiling** **Aneurysm rupture/clipping** **Ischaemia post procedure[5]**		3 2 9	3 4 11	N/A
Hydrocephalus requiring early external ventricular drainage			15	5	**p=0.032**

Table 4-Primary intention-to-treat analysis

Abbreviations: DIND-delayed ischaemic neurological deficit, MRS-Modified Rankin Score, CSF-cerebrospinal fluid.

Outcome measure		Control Group (n=105)	Study Group (n=105)	Significance
Number of patients with DIND (% of group)		37 (35.2%)	22 (21.0%)	**p=0.021**
Relative risk of DIND for control versus study (95% confidence interval)		**1.7 (1.1-2.6)**		
Number of patients diagnosed with a DIND and a persisting neurological deficit at discharge (% of group)		13 (12.4%)	10 (9.5%)	p=0.51
Type of DIND (% of patients with DIND)	**Focal**	21 (56.8)	9 (40.9)	p=0.29
	Altered consciousness	9 (24.3)	8 (36.4)	
	Both	7 (18.9)	5 (22.7)	
Number of patients with radiologically confirmed infarct/number of patients imaged (% of total group)		22/66 (21%)	15/60 (14.3%)	p=0.21
Number of patients with MRS of 0-2 at day ten (% of group)		39 (37.5%)	58 (55.2%)	**p=0.009**
Number of patients with MRS of 0-2 at six months (% of group)		83 (81.4%)	81 (80.2%)	p=0.83
Number patients dead at six months (% of group)		5 (4.8%)	4 (3.8%)	P=1.00
Number of patients requiring permanent CSF shunting (% of group)		8 (7.6%)	6 (5.7%)	p=0.58

Table 5-Secondary analysis with exclusions

Abbreviations: DIND-delayed ischaemic neurological deficit, MRS-Modified Rankin Score, CSF-cerebrospinal fluid

Outcome measure		Control Group (n=89)	Study Group (n=87)	Significance
Number of patients with DIND (% of group)		32 (36.0%)	14 (16.1%)	**p=0.003**
Relative risk of DIND for control versus study (95% confidence interval)		**2.2 (1.3-3.9)**		
Number of patients diagnosed with a DIND and a persisting neurological deficit at discharge (% of group)		10 (12.2%)	7 (8.0%)	p=0.47
Type of DIND	**Focal**	20	4	**p=0.034**
	Altered consciousness	6	4	
	Both	6	6	
Number of patients with radiologically confirmed infarct/number of patients imaged (% of group)		18/51 (20.2%)	10/46 (11.5%)	p=0.11
Number of patients with MRS of 0-2 at day ten (% of group)		38 (42.7%)	52 (59.8%)	**p=0.02**
Number of patients with MRS of 0-2 at six months (% of group)		71 (82.6%)	72 (86.7%)	p=0.45
Number of patients dead at six months (% of group)		3 (3.4%)	3 (3.3%)	p=1.00
Number of patients requiring permanent CSF shunting (% of group)		3 (3.4%)	3 (3.4%)	p=0.98

Two patients with a lumbar drain developed meningitis and one patient developed a lumbar drain exit site infection. All cases were treated with antibiotics with no permanent morbidity. There were thirteen cases of organism growth in CSF or the lumbar drain tip that had no clinical significance. Lumbar drains could not be inserted/did not function on insertion in 9 patients. Concerns about a post-operative intracranial haematoma resulted in cessation of lumbar drainage in 3 patients. There was one case of symptomatic hydrocephalus requiring external ventricular drainage despite a patent and functioning lumbar drain in-situ. There was one case of continued low-pressure headaches several weeks after removal of the lumbar drain. This required a dural blood patch. There were no cases of neurological deterioration/re-bleed of aneurysm secondary to lumbar drain insertion.

Mean CSF drainage was 138 ml/24 hours (confidence interval 124-152 ml/24 hours) (data missing in 16 patients). The mean duration of drainage was 5.0 days (CI 4.5-5.6 days, range 1-12 days). Drain insertion occurred on day 1 post ictus in 31%, day 2 in 32%, day 3 in 26% and day 4 in 11% of patients. Drain insertion occurred prior to aneurysm treatment in 31%, on the day of aneurysm treatment in 40% (approximately half immediately prior to treatment and half several hours after treatment) and after aneurysm treatment in 29% of patients. For the first 53 patients in the lumbar drain group, the opening pressure was recorded. Mean opening pressure (95% confidence intervals) was 27.3 cmH_2O (24.9-29.7 cmH_2O).

Table 6 shows the prevalence of DIND in the control and study groups stratified by Fisher grade. There was a trend for a lower prevalence of DIND in Fisher grades 2, 3 and 4 although this was statistically significant in Fisher 3 patients only. Table 7 shows the prevalence of DIND in the study group stratified by the day of drain insertion relative to the haemorrhagic ictus. The relative risk of DIND for control versus study group was greater in those with drain insertion on days 3 and 4 post ictus. Approximately 94% of all patients had presented to the neurosurgery department within 48 hours of ictus.

Table 6-Prevalence of DIND stratified by Fisher grade

Abbreviations: DIND-delayed ischaemic neurological deficit

Fisher Grade	Control Group: Number of patients with DIND/Total number in subset (% of subset)	Study Group: Number of patients with DIND/Total number in subset (% of subset)	Significance	Relative Risk of DIND for control versus study (95% confidence intervals)
2	8/46 (17.3)	3/39 (7.7)	p=0.21	2.53 (0.62-10.3)
3	22/44 (50)	9/41 (22.0)	**p=0.013**	**3.56 (1.38-9.16)**
4	6/13 (46.2)	3/11 (27.3)	p=0.42	2.29 (0.41-12.7)
3+4	1/2 (50)	7/14 (50)	p=1.00	0.50 (0.036-6.86)

Table 7-Prevalence of DIND in the study group stratified by day of lumbar drain insertion following ictus

Abbreviations: DIND-delayed ischaemic neurological deficit

Day of insertion post ictus	Number of patients in each sub-group	Prevalence of DIND (% of sub-group)	Relative Risk of DIND for control versus study (95% confidence intervals)
1	34	26.7	1.5 (0.9-2.6)
2	31	20.0	
3	28	15.4	2.2 (1.0-4.7)
4	12	18.2	

Discussion

Summary of findings

This study has demonstrated that the use of lumbar drainage of CSF following aSAH significantly reduces the prevalence of DIND and improves early clinical outcome. There is no significant difference noted in the clinical outcome at six months or in the need for permanent CSF shunting although there was a non-significant decrease in radiologically confirmed infarct. The incidence of CSF infection in those with lumbar drains is less than 2%, with no permanent morbidity associated with their use. A secondary analysis designed to exclude patients that may have introduced bias into the intention-to-treat model mirrored the primary analysis in findings. Patients with DIND in the study group tended to have a higher rate of altered consciousness (either with or without a focal deficit) than the equivalent patient in the control group.

CSF blood clearance

Lumbar drains have been described as a less invasive form of CSF drainage[156,174,179]. In a retrospective comparison of two patient cohorts with and without lumbar drains following aSAH, the difference in the prevalence of DIND was noted to be 51% in those patients without lumbar drains and 17% with lumbar drains[156]. This difference is larger than that demonstrated in the current study (35% vs 21%) and may be explained by the retrospective study methodology and a selection bias between the two cohorts in the non-randomised study. Their prevalence of DIND of 51% is higher than that reported in the literature. The reported prevalence is significantly influenced

by diagnostic criteria and is generally reported to be 20-35% with a slightly higher prevalence in those with high blood loads[154-156].

The hypothesis that lumbar drains confer benefit by reducing blood load has support from the current study. The trial protocol consisted of volume-driven CSF drainage with the aim of 5-10 ml CSF per hour until the CSF was visibly clear. This objective was achieved in most patients. Supporting this hypothesis is the finding that lumbar drains conferred the most benefit to patients with Fisher 3 grade aSAH. On the other hand however, there was no additional benefit to placing drains very early (i.e. within 48 hours of haemorrhage). This is not explained by the late presentation of patients to the neurosurgical centre since 94% of all patients in the study had presented within 48 hours of ictus (i.e. there is no selection bias of late lumbar drain insertion being associated with late patient presentation to the hospital which may have been a result of milder symptoms and thus a milder haemorrhage). This subgroup analysis must be interpreted with caution due to the small number of patients involved.

Intracranial pressure control

An alternative hypothesis regarding the mechanism of benefit conferred by lumbar drainage is intracranial pressure (ICP) control. In the current study, opening lumbar cistern pressure was invariably raised in all of the patients administered with lumbar drains (when tested) and drainage resulted in immediate improvements in the severity of headaches in most patients. Although drainage of CSF was volume driven, intracranial pressure was likely to have been lowered by the volumes of CSF drained via the lumbar cistern.

This is supported by the observation that most patients with a lumbar drain in situ for more than 72 hours exhibited clinical symptoms of low intracranial pressure very distinct from the initial presenting features of the aSAH. These symptoms improved following removal of the drain. Previous studies have demonstrated elevated ICP (as measured in the ventricle) several days after aSAH regardless of the volume of blood load[180-182]. Although associated with poor clinical grade, elevated ICP has been seen in 50-90% of patients with good clinical grade[180,181,183] and is associated with a poor clinical outcome[180,184]. Severe angiographic vasospasm has been shown to be more common with high ICP although association with DIND is weak[185]. A previously reported comparison of simultaneous lumbar and ventricular pressure in this setting demonstrated that both pressure readings reflect each other closely[186]. Drainage of 5-20 ml of CSF via a lumbar drain has been shown to approximately halve ICP in aSAH and brain injury patients[187]. Additional benefits observed included an improvement in regional CBF and $PtiO_2$. Hydrocephalus requiring treatment with external ventricular drainage was more frequently seen in the control group rather than the study group. Some patients with lumbar drainage may have had this clinical effect masked by the presence of a lumbar drain. This adds further support to the hypothesis that patients with a lumbar drain in the current study would have been likely to benefit from a reduction in ICP. It is plausible that this may have improved ischaemic thresholds by improving oxygenation and CBF and thus reducing the prevalence of DIND.

Complications

Since the current study cohort consisted of good WFNS grade patients with aSAH (grades 1-3), the benefits of lumbar drainage have been demonstrated in this population only. Although results cannot be extrapolated outside of the study cohort, there is no reason to suggest that aSAH patients of poor grade (grades 4-5) would not benefit from lumbar drainage. Patients of poor grade are more likely to have higher blood loads and a higher ICP. They are however also more likely to have an intracerebral and extensive intraventricular haemorrhage, which raises the issue of safety. Neurological deterioration that has been reported following lumbar drain insertion[156,188] is more difficult to assess in this cohort of ventilated and comatose patients. Although re-bleed of the ruptured aneurysm has been reported following insertion of an external ventricular drain or lumbar puncture/drain insertion, this risk has not been proven[179,189,190]. This problem was not encountered in the current study despite the fact that approximately half the patients underwent insertion of a lumbar drain with an unprotected aneurysm. One patient reported continued low-pressure headaches requiring a blood patch. This is consistent with the reported incidence of this complication with lumbar drain use following trans-sphenoidal surgery (1.3%)[191]. Meningitis secondary to lumbar drainage has been shown to be less than 2% in the current study, which is half that previously described[192]. No permanent morbidity was associated with infection.

Permanent CSF diversion

Although lumbar drainage has previously been shown to reduce the need for permanent CSF diversion (24% with lumbar drain vs 36% without lumbar drain)[156], this reduction has not been consistently demonstrated[174]. In the current study, there was no difference in the need for permanent CSF diversion between the two groups. In contrast, cisternal CSF drainage and irrigation has been associated with a higher rate of permanent CSF diversion, with the volume of drainage proportional to this rate[168,172,193]. It is unclear why lumbar drainage did not increase the occurrence of long-term hydrocephalus. A possible explanation is that the patient's natural CSF channels are encouraged rather than bypassed (which may occur with cisternal and external ventricular drains) so allowing normal pathways to re-establish following blood clearance. Acute and chronic hydrocephalus is thought to result from tentorial/ventricular obstruction and blockage of arachnoid granulations with blood respectively[194,195]. Either mechanism is likely to be resisted and minimised by the negative downward pressure drawing CSF into the lumbar cistern, an advantage of lumbar drainage over supratentorial CSF drainage.

Clinical outcome

Although patients in the study group demonstrated improved clinical outcome ten days following ictus, this improvement was not maintained at six months (there was a trend for patients with lumbar drains to have improved six-month outcome but this was only seen in the secondary analysis and was not statistically significant). It is well recognised that clinical trials powered to detect changes in DIND occurrence often report difficulties in detecting

patient-centred 'down-stream' clinical outcome[196]. The effect size of a treatment on DIND does not translate into the same effect size on the clinical outcome. In addition, the MRS has been noted to lack specificity in detecting subtle yet meaningful changes in cognition and functioning.

Limitations of the trial

There are subtle differences in the baseline cohort characteristics between the two groups of the trial. There is no explanation as to why there are more patients with multiple aneurysms in the study group. More patients in the control arm had required external ventricular drainage for hydrocephalus. This is likely to be due to the masking of hydrocephalus in those with a lumbar drain and so obviating the need for additional supratentorial. Limitations of the study include a reliance on DIND as the primary endpoint, which can be a subjective endpoint[177]. This subjectivity has been minimised by using a homogenous cohort of good WFNS grade aSAH patients with very strict diagnostic criteria for DIND, objective investigations to exclude other causes of neurological deterioration and multiple verification of the diagnosis of DIND. A second limitation to the study is the reliance on imaging performed as part of routine clinical care only; although there was a trend for fewer radiologically confirmed infarcts in the lumbar drain group, this is based on the incorrect assumption that those without clinical need for imaging will not have infarcts. A third limitation to this study is the difficulties associated with blinding treatment allocation and obtaining unbiased outcome measures. Being a single-centre trial has the advantage that only few clinicians were involved during the study period and so quality and consistency of diagnoses and treatment should be high. The disadvantage is the small size of the trial not

powered to detect changes in clinical outcome and the lack of information regarding reproducibility in other neurosurgical centres, particularly those in other countries where management of patients is likely to be markedly different.

Part 2: The role of cytokines in aneurysmal subarachnoid haemorrhage and delayed ischaemic neurological deficit

Introduction

The physiology and immunology of inflammation

The immune system is designed to defend against foreign antigens. It constitutes both cellular components such as B and T lymphocytes, monocytes, macrophages and neutrophils; and molecular components such as complement, acute-phase proteins and cytokines[197]. When the immune system is activated, a delicate balance between pro-inflammatory and anti-inflammatory states is maintained by the innate and adaptive immune systems[198]. T-cell lymphocytes, B-cell lymphocytes and natural killer (NK) cells all constitute part of the adaptive immune system. T-cell lymphocytes include helper $CD4^+$ T-cells and cytotoxic $CD8^+$ T-cells. Following antigen activation, the former can differentiate into Th_1 (associated with pro-inflammatory cytokines and cell mediated immunity), Th_2 (associated with anti-inflammatory cytokines and humoral immunity) and regulatory T-cells[198].

An immune response involves both cellular and humoral components and can be subdivided into four distinct stages: 1. recognition and activation, 2. proliferation, 3. effector and 4. memory[199]. The first stage relies on presentation of peptide antigens on the surface of antigen-presenting cells (such as microglia, dendritic cells and monocytes/macrophages) to T-cells[197]. Cytokines are also actively involved, both for activation and suppression of this process. The second proliferation phase ensures that activated lymphocytes can produce expanded populations of T- and B-lymphocytes. Effector killer T-cells and B-cells can induce apoptosis and antibodies respectively, resulting in elimination of foreign antigens. The final phase of

memory constitutes the persistence of a small number of these immune cells that can respond quickly following further exposure to the same antigens[197].

CNS inflammation

Our understanding of inflammation in the CNS is largely based on animal studies and the demonstration of various inflammatory mediators and cells in plasma, serum, CSF and pathological brain tissue[200]. These compartments may not reflect the injured brain itself. Intracerebral microdialysis catheters have been developed with increasingly larger membrane cut-offs that provide some insight into the extracellular fluid (ECF). Inflammation is a cardinal host defence response to injury, tissue ischaemia, autoimmune responses or infection[201]. This process often involves recruitment and invasion of immune cells and activation of mediators such as kinins, cyclooxygenase products and cytokines. Inflammation may manifest as a local response or a more generalised systemic 'acute phase' response. Historically, the brain has been considered to be an immune privileged organ. Early pioneering work had demonstrated that allogeneic tissue grafts were not rejected when implanted into the brains of experimental animals[202]. This was subsequently thought to be due to the lack of connection between the lymphatic system and the CNS. The CNS was thought not to have native antigen-presenting cells[197].

More recently, it has been demonstrated that rejection does occur following graft implantation into the CNS, there is a connection between CSF compartments and cervical lymphatics and resident antigen-presenting cells do exist[197]. In healthy CNS tissue, inflammatory mediators are expressed at very low levels and cytokine receptors are expressed constitutively in the

CNS[201]. In response to tissue injury or infection, early and rapid glial activation and release of inflammatory mediators is followed by slower leukocyte recruitment[201]. In a disease state, the presence of damaged cells and debris causes resting microglia to transform into migrating macrophages. These in turn can produce cytokines and trophic factors that can affect neighbouring cells and recruit peripheral inflammatory cells.

Cytokines are well recognised as low molecular weight pleiotropic glycoproteins that act autocrinally and paracrinally on target cells to promote and control inflammatory processes[203]. Much of our understanding of cytokines is derived from studies of their immunoregulatory properties that depict cytokines as operating within tightly regulated complex networks where individual elements operate to potentiate and/or modulate each other's effects. Furthermore, functional redundancy within these networks can enable different cytokines to induce similar responses through their use of a common receptor. Among their numerous roles, cytokines regulate the expression of cell adhesion molecules in microvascular endothelial cells. Cell adhesion molecules consist of three families responsible for interactions between leukocytes and endothelial cells: endothelial cells express the immunoglobulin supergene family members, intercellular adhesion molecules (ICAMs) (including ICAM-1) and P- and E-selectin, while leukocytes express L-selectin and integrins[204].

In addition to the inflammatory components produced within the CNS, the BBB may also be compromised in disease states and thus allow transport

(active or passive) of inflammatory mediators into the CNS[201]. Inflammatory mediators may be themselves involved in the breakdown of the BBB in certain pathologies[205]. In ischaemic brain damage, a local inflammatory response in addition to peripheral inflammatory processes and immune alterations occur with interleukin (IL)-6 considered a key mediator in this acute phase systemic response[206]. To support this, peripheral white cell count has been shown to be elevated soon after ischaemic stroke and leukocyte infiltration has been detected within the ischaemic lesions and the CSF of patients with ischaemic stroke[207-209]. In the acute phase of ischaemia, cell death in the core is likely to be secondary to necrosis, whereas following reperfusion, penumbral neurons may undergo apoptosis[210]. An increasing body of evidence implicates CNS inflammation in this process[210].

What follows is a brief overview of the key inflammatory mediators under investigation in the current study. The evidence for their involvement in traumatic brain injury (TBI) and ischaemic stroke will be included. Only experimental evidence for their involvement in SAH will be discussed at this stage (for clinical studies in aSAH, see next section).

Pro-inflammatory Mediators

Interleukins-1α and β (IL-1α and 1β)

The IL-1 family is a group of cytokines considered key mediators of neural injury in the CNS following ischaemic stroke and TBI[211]. IL-1α and β are constitutively expressed in the brain at low concentrations but their expression can be stimulated by bacterial and viral products, other cytokines (such as tumour necrosis factor (TNF)-α), cellular injury and hypoxia. They are involved in the host defence against disease and many of their actions are in the CNS resulting in systemic effects (including fever and the acute-phase response). They are considered to be largely pro-inflammatory, whilst their effects are antagonised by the endogenous IL-1 receptor antagonist (IL-1ra)[200].

Although the mechanism by which IL-1 exerts CNS injury is unknown, there is some experimental data to suggest that increases in body temperature may exacerbate neuronal injury and is associated with a poor clinical outcome in stroke or head injury[211,212]. Experimental data would suggest that IL-1 has positive effects to reduce cell death (such as inhibition of glutamate release, inhibition of calcium influx and inhibition of γ-aminobutyric acid (GABA) transmission)[213]. This inhibition of excitatory and inhibitory pathways may result in excitation, inhibition and cell death. Calcium influx may also be stimulated via N-methyl-D-aspartate (NMDA)-receptor ion channels. Effects on glial cells including astrocytes are also variable. The effect of IL-1 on cultured astrocytes can change the expression of numerous genes encoding

chemokines, growth factors, adhesion molecules, matrix metallopeptidase (MMPs) and cytokines (including IL-6) which may contribute to neurotoxicity and paradoxically also promote neuronal survival[211]. IL-1 has numerous effects on the endothelium that result in expression of molecules involved in leukocyte recruitment and adhesion (including ICAM-1). This has implications for the integrity of the BBB. The overall effect depends on the temporal expression profile of these molecules. This makes the interpretation of these findings difficult in the context of IL-1-mediated effects *in-vivo*. Figure 2 is a summary of the proposed cellular actions of IL-1β in neuronal injury.

In experimental SAH models, IL-1β was activated early after SAH and a selective caspase-1 inhibitor inhibited its activation and attenuated the mortality, neurological impairment, BBB disruption and brain oedema[214]. S-100B protein concentration in the serum (a marker of BBB permeability) was elevated following experimental SAH[215]. This effect was attenuated by the neutralization of IL-1β activity. This protective effect was also associated with reduced MMP-9 induction. This is a proteolytic enzyme that has been implicated in the breakdown of the BBB in ischaemia and in haemorrhagic transformations in ischaemic stroke[216,217].

Similarly, simvastatin treatment resulted in reduced IL-1β expression and ischaemic injury in a rat model of middle cerebral artery occlusion[218]. The transcription factor nuclear factor-kB (NF-kB) (responsible for expression of proinflammatory genes) was upregulated in both transient and permanent

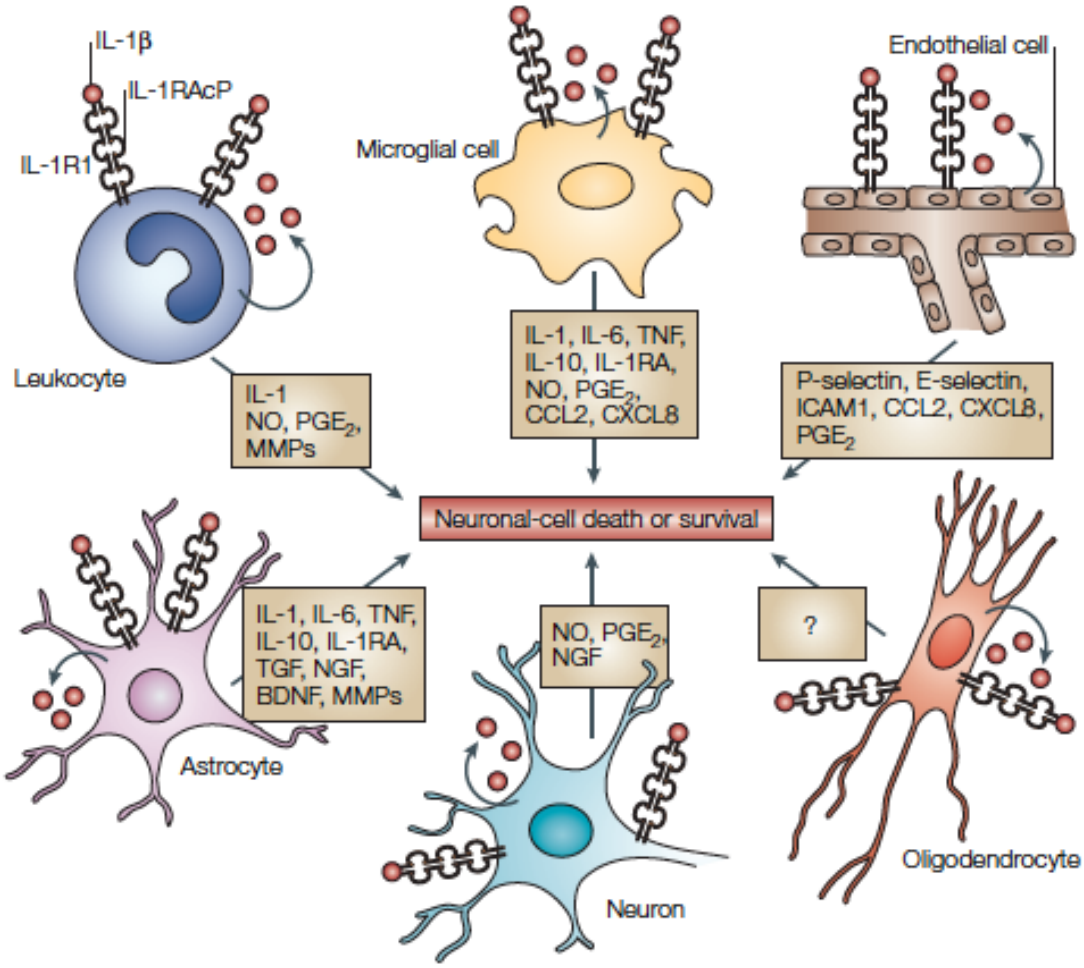
artery occlusion. Its activation was attenuated during ischaemia with simvastatin treatment.

Interleukin-6 (IL-6)

IL-6 is produced by mononuclear phagocytes, T cells and endothelial cells[197]. IL-6 stimulates the growth of mature B cells and promotes the synthesis of acute phase proteins by the liver including C-reactive protein and fibrogen[219]. IL-6 can stimulate expression of MMPs including MMP-9. It is also involved in liberating adhesins in the process of leucocyte aggregation and adhesion to the vascular wall. It is itself synthesised as an acute phase reactant. Although considered pro-inflammatory, IL-6 has been shown to exhibit some anti-inflammatory effects that are supported by its ability to inhibit TNF-α synthesis, induce the nerve growth factor and IL-1ra, promote neuronal differentiation and survival and counteract NMDA-mediated toxicity[220]. Its constitutive expression in the healthy brain would support its regulatory role during normal physiology.

Plasma IL-6 was induced following experimental stroke in mice, peaking at four hours[206]. In a clinical study, plasma IL-6 levels correlated with blood volume, mass effect and GCS following intracerebral haemorrhage[221]. In patients with ischaemic stroke, early plasma IL-6 levels were higher than in healthy controls and correlated with stroke volume and poorer outcome[222-224]. This is supported by similar findings in the CSF[225]. In a nested case-control study, very high levels of plasma IL-6 were found to be a significant predictor of recurrent ischaemic stroke[226]. It is increased following TBI in

Figure 2-Summary of proposed cellular actions of interleukin-1β in neuronal injury. All cell types can express and release IL-1β physiologically and following neuronal injury. IL-1 receptor (IL-1R1) is expressed at the surface of these cells, enabling them to respond to IL-1β in an autocrine and paracrine fashion. After receptor binding, cells can produce a wide range of pro-inflammatory and immunoregulatory mediators that are implicated in neuronal-cell death or survival depending on the relative contribution of synergistic/antagonistic cytokines. BDNF: brain-derived neurotrophic factor; CCL2: CC-chemokine ligand 2; CXCL8: CXC-chemokine ligand 8; E-selectin: endothelial-cell selectin; ICAM1: intercellular adhesion molecule 1; IL-1RA: IL-1-receptor antagonist; IL-1RAcP: IL-1-receptor accessory protein; MMP: matrix metallopeptidase; NGF: nerve growth factor; NO: nitric oxide; PGE_2: prostaglandin E_2; P-selectin: platelet selectin; TGF: transforming growth factor; TNF: tumour-necrosis factor. Source: Allan et al 2005, Nature Reviews: Immunology[211].



plasma, CSF, cerebral ECF and brain tissue proper[200,227-229]. In paediatric TBI patients, both plasma and CSF IL-6 levels were shown to correlate with the severity of the injury and outcome[227]. Contrary to this, following adult TBI, higher ECF IL-6 seemed to exhibit a protective role and correlated with good outcome and survival[230]. This supports a possible dual role for IL-6 in this setting.

Interleukin-8 (IL-8)

IL-8 is a chemokine and thus belongs to a family of cytokines that share the ability to stimulate leukocyte motility and directed movement. It is produced by mononuclear phagocytes, T cells, platelets, endothelial cells and fibroblasts[197]. As with IL-6, it has an important role in mediating the acute phase response and in stimulating adhesion molecules. Activated neutrophils may contribute to brain injury by causing microvascular occlusion and the production of further cytokines, reactive oxygen and nitrogen metabolites and lipid mediators[231]. Anti-neutrophil antibodies and neutrophil depletion have been shown to reduce neutrophil infiltration and infarct size in the ischaemic rat brain[232,233]. IL-8 is considered an important chemokine in post-ischaemic leukocyte accumulation and activation[234]. In an experimental ischaemia model, an IL-8 receptor antagonist reduced neutrophil infiltration but had no effect on tissue damage during permanent ischaemia[234]. During transient ischaemia however, tissue damage was reduced and neurological function was improved[234,235]. This suggests that these benefits are conferred during reperfusion. IL-8 levels were not changed, indicating blockade of downstream factors.

Increased CSF concentration of IL-8 has been reported in humans following TBI and correlates with a fatal outcome[228]. In patients with ischaemic stroke, intracellular leukocyte IL-8 concentrations, plasma IL-8 levels and IL-8 mRNA expressing blood mononuclear cells were noted to be higher than in controls[236-238]. Data from a rabbit basilar SAH model suggests that IL-8 expression within cerebral vessels may also be associated with angiographic vasospasm[239].

Interleukin-15

IL-15 is a pleiotropic cytokine which is constitutively expressed by a large variety of cell types and tissues including monocytes/macrophages, dendritic cells, keratinocytes and epidermal skin cells[240]. It enhances the phagocytic activity of monocytes and macrophages and induces the production of pro-inflammatory factors such as IL-8 and monocyte chemoattractant protein-1 (MCP-1)[240]. It is implicated in various inflammatory conditions such as coeliac disease, inflammatory bowel disease, systemic sclerosis and rheumatoid arthritis. The pathogenic role of IL-15 in multiple sclerosis has been extensively investigated. IL-15 mRNA is up-regulated in blood mononuclear cells from patients in the secondary progressive phase of the disease and in active plaques[241,242]. Serum and CSF levels of the mediator have been shown to be elevated in patients with multiple sclerosis, with a suggestion that systemic production was the source of CSF IL-15[243]. There is limited evidence for its involvement in cerebral ischaemia. In the ischaemia/hypoxia model of the immature rat brain, IL-15 was initially produced by blood cells

penetrating the injured brain but was later also synthesised by reactive astrocytes forming dense astrogliosis around necrotic centres[244].

Interleukin-17

IL-17 is a homodimer activated by CD4+ and CD8+ activated memory T cells[245]. It plays an important role in the maturation of haematopoietic progenitor cells and induces production of proinflammatory mediators. It can induce stromal cells such as fibroblasts, endothelial cells and epithelia, to produce IL-6, IL-8, granulocyte colony-stimulating factor and ICAM-1[246]. In both *in-vitro* and *in-vivo* models, IL-17 has also been implicated in the disruption of BBB tight junctions[247].

Its putative role in ischaemia is unclear. Following neuronal injury, activated microglia accumulate and secrete pro-inflammatory cytokines such as IL-23 via Toll-like receptors (TLRs)[248]. TLRs are a family of transmembrane receptors able to recognise pathogen-associated molecular patterns and are essential in the microglial innate immune response. This is evidence suggesting that a TLR2, IL-23 and IL-17 axis does exist which leads to neuronal apoptosis[248]. This pathway is activated in animal models of ischaemia/reperfusion, with IL-23 functioning in the immediate stage of ischaemia/reperfusion injury and IL-17 (being a downstream factor) more important in the delayed phase[248,249]. Both exogenous IL-17 addition and microglia release of IL-17 lead to apoptosis in an *in-vitro* oxygen-glucose-deprivation reperfusion system[248]. Both animal and human studies suggest that IL-17 expression is elevated in the ischaemic brain. Neuronal injury

during oxygen-glucose deprivation may be mediated by IL-17 via the IL-17 receptor[250,251].

Interleukin-18

IL-18 is a pro-inflammatory cytokine, formerly known as interferon-γ inducing factor and is a member of the IL-1 family. IL-18 and IL-1 act through related receptor complexes to trigger common signalling pathways[252]. This cytokine is produced constitutively in many different cell types including macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells and Kupffer cells. Increased expression of IL-18 mRNA and protein has been reported during Wallerian degeneration of the rat nervous system following a nerve crush injury[253]. The role of IL-18 following brain injury and ischaemia is unclear, although it could contribute to exacerbating hypoxic brain injury. Up-regulation of the intracerebral IL-18 gene occurs following TBI in mice[254,255]. IL-18 has been shown to induce intracellular expression of IL-1α, a precursor of IL-1β and IL-6 from mixed glia in the mouse. There is some evidence to suggest that IL-18 is modulated and can be inhibited by TNF-α, alluding to a possible dual function for the latter[254].

In ischaemia, there are conflicting reports as to whether regulation is actually induced in animal models, with some evidence for its late induction[256,257]. Mice lacking IL-18 were not protected in the early phase after ischaemia[256]. In neonatal hypoxic ischaemia, IL-18 showed an early increase, peaking at 14 days with attenuation of brain injury seen in IL-18 deficient neonatal mice and rats[252,258]. This protective effect was not noted in IL-1α and IL-1β deficient

mice, suggesting the importance of IL-18 in neonatal brain. In a nested case-control study, plasma IL-18 levels were not a predictor of recurrent ischaemic stroke[226]. Contrary to this, plasma IL-18 was noted to be an independent predictor of 90-day major adverse clinical outcome in patients with ischaemic stroke[259]. Furthermore, IL-18 functional promoter polymorphisms are associated with an increased risk of ischaemic stroke[260].

MCP-1

MCP-1 is a member of the cysteine-cysteine chemokine gene family characterised by its chemoattractant properties for monocytes, memory T-cells and natural killer cells *in-vitro*[261]. Monocytes, smooth muscle cells, fibroblasts and vascular endothelial cells produce it in response to stimuli such as IL-1, TNF-α, interferon-γ and lipopolysaccaride[262].

MCP-1 is expressed in experimental models of ischaemia, with gene knockout animals lacking MCP-1 sustaining smaller infarct volumes and reduced monocyte and polymorphonuclear neutrophil infiltration compared to wild-type[263-268]. The relatively late change to cellular infiltration is unlikely to explain this protective effect. An early reduction in IL-1β within ischaemic tissue was noted and may explain this protective effect[265]. Similarly, overexpression of MCP-1 caused larger infarcts and greater chemoattraction of monocytes and macrophages into the ischaemic area[269]. MCP-1 seemed to be necessary in recruiting blood-borne cells to the site of injury but did not seem to be involved in the microglia activation and migration that occurs during ischaemic injury[266].

In clinical studies, MCP-1 was elevated 24 hours following ischaemic stroke in the CSF but the evidence for plasma elevation is conflicting[224,270]. Plasma MCP-1 was an independent predictor of poor outcome as several early time points following ischaemic symptoms[224]. Neuroblasts derived from the neural progenitors in the subventricular zone have been shown to migrate physiologically and during focal ischaemia[268]. There is evidence to suggest that MCP-1 may be involved in attracting neuroblasts from the subventricular zone to the infarct[268].

In canine and rat models of SAH, MCP-1 was upregulated in vasospastic cerebral arteries and cerebral cortex[271-273]. Sphingolipid sphingosylphosphorylcholine (SPC) is released by platelets and engages in a wide range of intracellular signalling pathways in different cell types[274]. There has been a suggestion that SPC has a vasoconstrictor effect and pro-inflammatory function in the cerebral vasculature. SPC increased the release of MCP-1 in cultured vascular smooth muscle cells, implicating the latter in SPC-mediated pro-inflammatory effects[274].

Tumour necrosis factor-α

The role of TNF-α in immunoactivation elicited in the brain has been subject to extensive investigation but is still unclear. Following TBI, TNF-α has been detected in human CSF and plasma and is up-regulated in cerebral tissue in experimental models[275]. TNF-α has been associated with activation of ICAM-1, BBB dysfunction and intrathecal infiltration of activated leukocytes following

TBI[275]. There is evidence largely from experimental knockout models that in addition to being a mediator of injury, TNF-α may confer protection[275]. Although there was no difference in the post-traumatic pathophysiology of knockout versus wild type animals (including intracerebral neutrophil infiltration and BBB permeability), increased mortality in certain knockout models would suggest a protective role for TNF-α following TBI[275].

TNF-α up-regulation was detected in brain autopsies after acute stroke[276]. CSF and plasma demonstrated significantly higher TNF-α levels in patients with ischaemic stroke within 24 hours of onset when compared with control[277]. In a nested case-control study, very high levels of plasma TNF-α were found to be a significant predictor of recurrent ischaemic stroke[226]. TNF-α is also thought to release MMP-9[217]. TNF-α appears to have different effects based on its intrinsic concentration and the relative contribution of synergistic/antagonistic cytokines.

Vascular endothelial growth factor (VEGF)

VEGF is an endothelial mitogen expressed in neuronal and vascular tissues in the brain. VEGF may induce angiogenesis and increased vascular permeability. It binds to a receptor tyrosine kinase to activate Sarcoma (Src) tyrosine kinase and mitogen-activated protein kinase (MAPK) pathways. It has also been shown to stimulate neurogenesis[278]. It is upregulated after experimental TBI and hypoxia, resulting in an increase in angiogenesis and vessel homeostasis[278,279]. This has implications for the integrity of the BBB.

Conversely, blockade of its receptor has been shown to aggravate experimental brain trauma, suggesting a potentially protective role[280].

It has been implicated in the pathogenesis of aSAH. MAPK pathway can be activated by various stimulants including VEGF[281]. MAPK and VEGF are activated in the cerebral arteries and, to a lesser extent, in the cerebral cortex following experimental SAH[282]. The resulting alterations in the BBB, enhanced brain oedema and death were attenuated by inhibition of the Src-family tyrosine kinase, in addition to reducing VEGF expression. This would implicate Src as both a downstream factor to VEGF receptor activation leading to MAPK, and an upstream factor involved in the signalling pathways that lead to VEGF activation.

Anti-inflammatory mediators

Interleukin-4

IL-4 is a Th_2-derived anti-inflammatory cytokine[283,284]. IL-4 downregulates the release of pro-inflammatory cytokines from activated monocytes. How IL-4 is involved in the regulation of immune responses in acute cerebrovascular disease is not known. The number of blood mononuclear cells producing IL-4 and plasma levels of IL-4 were not significantly different in acute stroke and neurological deterioration[283,284]. In a mixed cohort of patients with TBI, aSAH and intracerebral haemorrhage, plasma IL-4 levels taken from the internal jugular vein were higher in patients that did not survive compared with those that survived the injury[285]. Levels were inversely proportional to arterio-jugular venous differences in oxygen content. Local CNS evaluation of this

mediator and its evaluation specifically in the context of aSAH have not been previously documented.

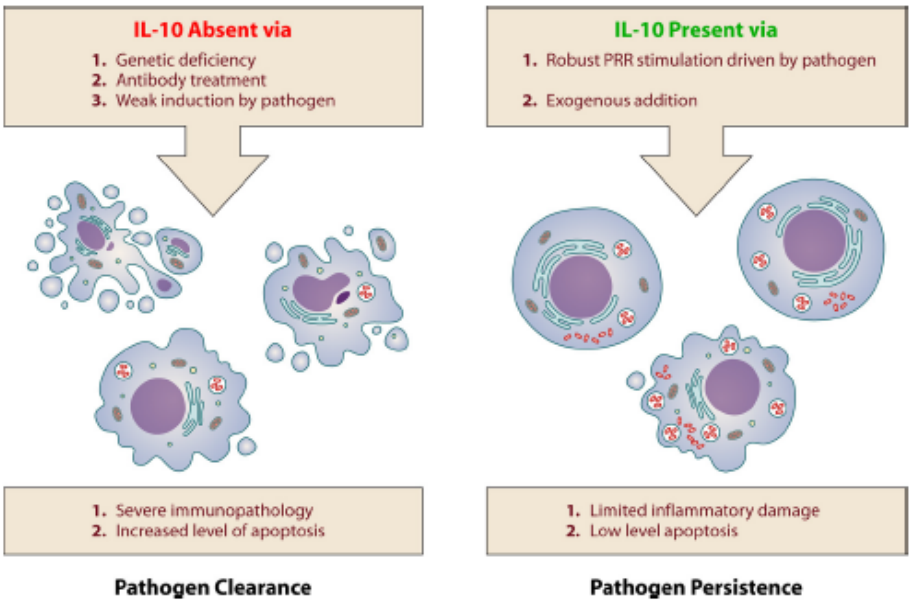
Interleukin-10

In-vitro post-ischaemic microglial cells are thought to produce reactive oxygen species and cytotoxic cytokines such as TNF-α and IL-1β[286]. In addition to this, they are thought to produce anti-inflammatory cytokines such as IL-10. IL-10 is also produced by T helper cells, macrophages, monocytes and B cells. Its main target cell is the macrophage. It is anti-inflammatory because it inhibits the production of cytokines from Th_1 cells, the expression of cyclo-oxygenase-2 and the production of TNF-α[287]. There is some evidence to suggest that its neuroprotective properties are conferred by blocking caspase-3 activity and reducing pro-inflammatory cytokine production[288,289]. IL-6 and IL-10 negatively correlate with each other in normal subjects, indicating a balance between the pro-inflammatory and anti-inflammatory molecules[287].

In-vivo, over-expression of IL-10 markedly protected cortical tissue from ischaemia in transgenic mice[290]. A similar neuroprotective effect was noted following spirochetal brain infection in mice[291]. IL-10 deficiency resulted in early death from subarachnoid/intraparenchymal haemorrhage following infection. This was also associated with apoptosis of brain microvascular endothelial cells and increased systemic production of TNF-α. Conversely, post-ischaemic gene transfer of IL-10 into the rat focal and global ischaemia model attenuated brain infarction and hippocampal damage[292].

It is unclear what happens to IL-10 levels following brain trauma and ischaemia in humans. Intracerebral haemorrhage has been shown to be associated with elevated plasma IL-10 levels (taken within 48 hours of haemorrhage)[221]. There are conflicting reports in stroke patients of both increased and decreased plasma IL-10 levels and number of IL-10 secreting cells[222,223,284,287,293]. Similarly, there have been mixed reports at to whether IL-10 levels are associated with poor outcome following ischaemic stroke[223,287,293,294]. Elderly patients with a history of stroke were shown to have a significantly lower plasma IL-10[295]. The risk of fatal stroke during the prospective study period was higher in those with low or intermediate baseline IL-10 production levels. IL-10-1082 G/A, promoter polymorphism is associated with lower IL-10 production and ischaemic stroke in a south Indian population[296]. This would suggest that following ischaemic stroke, there may be an anti-inflammatory cascade that combats the disease process and a genetic predisposition resulting in a weaker response maybe detrimental to outcome. This is supported by the finding that in acute ischaemic brain injury, IL-10 was an independent predictor of stroke-associated infection[297]. This suggests that a blood-borne anti-inflammatory response is initiated following ischaemic stroke that decreases microbial resistance in the immune system. This has been noted in other disease states[298]. IL-10 dampens TNF-α-mediated inflammation during bacterial infection. The presence or absence of IL-10 in infected tissue dictates the strength of the immune response but also the amount of apoptosis incurred due to inflammation (figure 3).

Figure 3-The balance between apoptosis and pathogen persistence. A heavily inflamed apoptotic environment is not conducive to pathogen survival. Some pathogens induce interleukin (IL-10) via pattern recognition receptors to subvert the development of acute inflammation. An abundance of IL-10 decreases activation-induced inflammation and apoptosis allowing pathogen persistence intracellularly. Conversely, if IL-10 is not induced, robust inflammation develops leading to high levels of apoptosis and parenchymal damage. Source: Cyktor et al., 2011, Infection and Immunity[298].



Inflammation following aSAH

Cytokines in aSAH

There is increasing evidence to suggest that a complex inflammatory response is initiated both systemically and within the CNS following aSAH. How this relates to ischaemic neurological injury and which exact mediators and mechanisms are involved is currently unclear. Numerous clinical studies have investigated the association between aSAH, DIND and cytokine production[219,299-312]. The most widely investigated mediators include interleukin (IL)-1α, IL-1β, TNF-α, IL-6 and IL-8 (Table 8). Although these mediators are generally detected in higher than normal concentrations in CSF following aSAH, there is some evidence to suggest that increases in CSF IL-6 may be specifically associated with radiological vasospasm and DIND[219,306,311,313]. CSF IL-6 levels were significantly higher on days 4 and 5 following haemorrhage. These changes often preceded clinical signs of DIND, thus giving IL-6 predictive power for the onset of DIND, with a significant correlation between IL-6 levels and DIND on day 7[219,306]. Serum and CSF concentrations of MCP-1 were significantly higher following aSAH than in controls undergoing surgery for an unruptured aneurysm[262]. Serum and CSF concentrations of MCP-1 were associated with poor outcome and angiographic vasospasm respectively.

In recent years, microdialysis has become an established method for biochemical surveillance of patients in the neurointensive care setting. Catheters with a high molecular weight cut-off have been developed to allow

Table 8-Summary of clinical studies investigating inflammatory mediators following aSAH. * indicates significance at p=0.05

Abbreviations: aSAH-aneurysmal subarachnoid haemorrhage, IL-interleukin, CD-cluster of differentiation, CSF-cerebrospinal fluid, DIND-delayed ischaemic neurological deficit, TNF-tumour necrosis factor, IL-1ra-interleukin-1 receptor agonist.

Study	No of patients	Mediators investigated	Compartment (plasma/CSF)	Key findings (* statistical significance)	Association with DIND (*statistical significance)
Mathiesen et al., 1993	8 aSAH	IL-6, IL-2, CD-8	Plasma & CSF	↑CSF IL-6*	Levels peaked on day 6. Higher in DIND. Not paralleled by plasma levels.
Kikuchi et al., 1995	7 aSAH	IL-1α&β, IL-6, IL-8, TNF-α	Plasma & CSF	CSF and plasma IL-6 & IL-8 detected. CSF level > plasma level	None
Mathiesen et al., 1997	22 aSAH 10 Controls	IL-1ra, TNF-α	CSF	Both were higher than normal controls. Increased in those with eventual poor outcome.	IL-1ra was increased during DIND
Osuka et al., 1998	9 aSAH 9 Controls	IL-6, IL-8, IL-1β	Plasma & CSF	↑CSF IL-6/IL-8*, > plasma level. IL-1β no change.	↑CSF IL-6 & IL-8 in those with DIND*
Gaetani et al., 1998	31 aSAH 10 Controls	IL-6, IL-8	CSF	↑IL-6/IL-8* for those operated within 72 hours.	↑IL-6 in those with DIND*
Kwon et al., 2001	19 aSAH 12 Controls	IL-1β, TNF-α, IL-6	CSF	Only levels within 24 hours investigated. IL-1β & TNF-α higher compared to controls*.	↑ IL-1β & TNF-α in those with DIND ↑IL-6 in those with DIND*
Fassbender et al., 2001	35 aSAH 20 Controls	IL-1β, TNF-α, IL-6	Plasma & CSF	↑CSF IL-1β, TNF-α & IL-6*. Peaking at days 5 to 9. Paralleled by plasma levels in TNF-α only.	Not commented upon.
Schoch et al., 2007	64 aSAH	IL-6	CSF	↑ CSF IL-6 overall*, individual variability evident.	IL-6 on days 4/5 predicted DIND.

measurement of cytokines and other macromolecules from the extracellular space of the brain. Serial measurements of IL-6 in plasma, CSF and the extracellular fluid (ECF) using microdialysis following aSAH demonstrated that the highest concentration was found in the CSF followed by the ECF and plasma respectively[313]. All compartments demonstrated significantly higher levels than normal controls[312,313]. Both ECF and CSF IL-6 were predictive of DIND[312]. For those patients with elevated ICP, IL-6 increased in the ECF and decreased in the CSF after four days post-ictus[313]. During this time, plasma IL-6 and other systemic markers of inflammation were elevated. This state was also associated with a poor outcome. Although the results are conflicting, overall evidence favours intrathecal cytokine production, such that increases in CSF/ECF are not paralleled in plasma, highlighting the compartmentalisation of this process. How the CSF and ECF levels are related is unclear. Although high cut-off membranes have the potential to expand microdialysis to the study of protein chemistry, there are numerous difficulties with their use and in the interpretation of the results obtained[314].

Adhesion molecules and aSAH

Several studies have demonstrated an association between soluble adhesion molecule levels and markers of endothelial cell activation following aSAH and DIND[315-320]. Von Willebrand Factor (vWF) is a blood glycoprotein involved in haemostasis and is one such marker of endothelial cell activation. Levels of vWF measured within 72 hours of haemorrhage were slightly raised and weakly associated with subsequent DIND[315,316], which was thought to be an

acute response reflecting haemorrhage severity and thus subsequent delayed complications. Serial measurements also demonstrated that soluble P-selectin increased significantly in patients with DIND (in contrast to those without, where levels decreased)[315,316,320]. Although an association between ICAM-1 and poor neurological outcome has been made[317,319], this has not been consistently associated with DIND[315,316,320].

Genetic predisposition

Genetic predisposition to inflammation following aSAH has been subject to some investigation. Although polymorphisms of IL-1β (-511) genotype were not associated with aSAH per se, carrying a T/T genotype was associated with a poor aSAH grade and poor 6-month outcome[321]. There are mixed reports as to whether IL-6 gene polymorphisms are associated with both ruptured and unruptured intracranial aneurysms[322,323]. Patients with aSAH exhibiting the TNF-α non-wild type allele resulting in a lower serum TNF-α concentration had a higher risk of poor outcome[324].

To test the hypothesis that DIND occurs in some people and not in others because of the variation in the inflammatory response that occurs between individuals, the relationship between immune activation and DIND following aSAH was investigated[325]. Northern hybridization was utilised to evaluate the expression of IL-1β and TNF-α in cultured monocytes from patients with aSAH. Activation indices for TNF-α were high in all cases and showed individual variation for IL-1β. This variation correlated significantly with

angiographic vasospasm of the anterior cerebral artery only. Those with DIND showed significantly higher activation indices than those without.

Cellular infiltration and systemic inflammation in aSAH

Based on both animal and clinical studies, a three-step inflammatory process has been suggested to occur following aSAH, which may be associated with DIND. The three-step model begins with an unidentified signal within the subarachnoid space that initiates a cascade of molecular events leading to an upregulation of ICAM-1[204]. Leukocytes then interact with endothelial selectins via their sialylated carbohydrates, resulting in their tethering and rolling along the endothelium. Leukocyte arrest is initiated when an integrin interacts with endothelial ICAM-1. Subsequently, leukocytes migrate through the vessel wall and are thought to mediate ischaemic brain injury, although endothelial cell activation has also been implicated in this process[326].

Early studies correlated DIND with a raised systemic temperature following aSAH[327,328]. A fever curve beginning on the fifth day following haemorrhage was noted in 88% of patients with DIND versus 18% without[327]. Patients with DIND have also been noted to have a higher peripheral white cell count[329-332], with a peak leukocyte count greater than 15 X 10^9/L increasing the odds of DIND three-fold[333]. There have been several studies demonstrating the presence of circulating immune complexes and complement activation in patients with angiographic vasospasm and DIND[334,335]. Immune complex deposits and cellular infiltration have been demonstrated in the arterial media of cerebral blood vessels following aSAH[325]. Following aSAH, an increase in

lymphocyte sub-populations within the CSF occurs in those with DIND, particularly intrathecal suppressor/cytotoxic/NK-cells[336].

There is evidence to suggest that early activation of the systemic inflammatory response syndrome (SIRS) occurs following aSAH in most patients[337,338]. SIRS represents a collection of clinical findings originally described in association with sepsis that is thought to arise as a result of cytokine or circulating catecholamine release, which promotes immune activation[339]. SIRS has been associated with poor clinical grade, thick cisternal blood, large aneurysm size, surgical aneurysm treatment and hypertension[337], and the magnitude of this response is an independent predictor of DIND and poor outcome but not of angiographic vasospasm. However, the interpretation of this study is limited by its retrospective analysis of prospectively collected data, its investigation of a complex, multifactorial physiological response and lack of standardisation of corticosteroid regimens.

The complement cascade is a principal effector of erythrocyte haemolysis and activator of inflammatory mediators. Early studies demonstrated an association between circulating/CSF immune complexes and complement components, and the development of angiographic vasospasm and DIND[340-342]. Plasma C3a and C5a were shown to be elevated following aSAH when compared with normal controls, while early C3a levels were independently associated with outcome[343].

Summary

Both systemic and compartmentalised inflammatory/immune responses following aSAH have been associated with DIND, although causality is less clear. Investigating the systemic component is complicated by numerous confounding causative factors and endpoints that are difficult to quantify. Does this systemic inflammatory response truly contribute to DIND or is this an epiphenomenon related only to the severity of the aSAH as measured by clinical grade and blood volume load? A large prospective study is required to determine this and account for confounding factors during regression modelling. Similarly, investigating the CNS inflammatory response is complicated by that observed following ischaemia in ischaemia/reperfusion models[133]. Is CNS inflammation a cause or a consequence of DIND? Measurement of inflammatory mediators in blood, CSF and brain ECF is technically difficult and is subject to variability between patients and their courses following aSAH, regardless of the development of DIND. Clinical studies utilising various anti-inflammatory agents such as cyclosporin and methylprednisolone have unsurprisingly been disappointing[344], highlighting that inflammation following aSAH is pleiotropic and multifaceted. New studies utilising specific interleukin antagonists and antibodies to block leukocyte-endothelial interactions are awaited.

Aims of the study

The aim of the current study is to answer the following questions:

1. Do plasma and CSF cytokine levels change following aSAH?
2. If so, do plasma and CSF levels correlate with each other and to what extent is this response compartmentalised within the central nervous system?
3. Are there any changes associated with DIND?
4. If so, what is the time course for these changes in relation to ictus of haemorrhage and do these markers hold predictive characteristics?

Materials and methods

Patients

Patients with aSAH recruited into the lumbar drain trial were eligible for this secondary study. Ethical approval was obtained by the Leeds (West) Research Ethics Committee. Control non-aSAH patients were recruited after obtaining written informed consent as part of a separate ethical approval obtained by the Bradford Research Ethics Committee.

Procedure and Interventions

Patients that present with aSAH were assessed for eligibility for inclusion into the lumbar drain trial (see above). As part of the consent/assent process, permission was obtained to collect and store plasma and CSF. These were collected at certain time-points following the presumed time of ictus (taken as time=0). Once aneurysmal pathology had been confirmed and consent/assent was obtained, the first blood and CSF samples were collected. Samples of plasma and CSF were collected simultaneously and at 9am where possible. In the event of randomisation into the control group, only plasma was obtained (due to lack of accessible CSF).

Venous blood was taken from the antecubital fossa and drawn into EDTA containers. CSF was removed from the lumbar drain using an aseptic technique. The first 2 ml of CSF drawn from the drain was discarded, as this would represent the dead space volume of the lumbar drain. Subsequently

drawn CSF was utilised for analysis. Both blood and CSF samples were immediately placed on ice and centrifuged at 3000 rpm for 15 minutes (at room temperature). The supernatant was removed and stored at -80^{o}C until further analysis.

Fixed time points for sampling were at days 5, 7 and 9 following ictus where possible. Due to the variability associated with when patients present to our unit in relation to time of ictus, the first sample was taken at a variable time point defined as within 96 hours of ictus and within 12 hours following intervention (endovascular or surgical). Additional time points that were collected include at the time of onset of DIND. Occasionally patients were discharged from the neurosurgery department before day 9 or lumbar drainage was stopped prior to retrieval of all samples. As a minimal requirement, at least the initial sample and days 5 and 7 were required to be included in this study.

There were four cohorts of aSAH patients. For each arm of the trial, some patients developed DIND and others did not. Therefore the cohorts of aSAH patients are:

Group 1: Lumbar drainage with DIND

Group 2: Lumbar drainage without DIND

Group 3: Control with DIND

Group 4: Control without DIND

Both CSF and plasma were available for groups 1 and 2, whereas only plasma was available for groups 3 and 4.

A control group of normal subjects (non-aSAH) were also sampled (group 5). This group consisted of patients undergoing elective surgery requiring spinal anaesthesia (hip and knee replacements).

Exclusions

Patients with aSAH that developed clinical and biochemical signs of sepsis/infection were excluded from the analysis. Patients in group 5 that were undergoing elective joint replacement due to rheumatoid arthritis were also excluded from the analysis.

Molecular analysis

Assay format

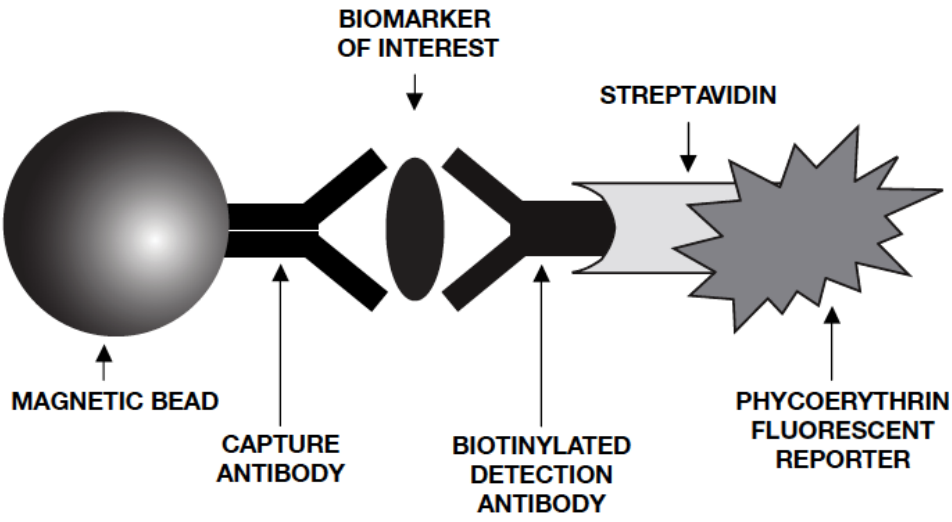
Analysis was performed using the Bio-Plex® suspension array system manufactured by Biorad® as previously described [345]. This system is built around the three core elements:

1. Fluorescently dyed microspheres (also called beads), each with a distinct spectral address. This allows simultaneous detection of more than 100 different types of molecules in a single well of a 96-well microplate.
2. A dedicated flow cytometer with two lasers and associated optics to measure the different molecules bound to the surface of the beads.

3. A high-speed digital signal processor that efficiently manages the fluorescence data.

The assays are immunoassays formatted on magnetic beads similar to a sandwich enzyme-linked immunosorbent assay (ELISA) (Figure 4). Capture antibodies directed against the desired biomarker are covalently couples to the beads, which react with

Figure 4-Bio-Plex sandwich immunoassay



the sample containing the biomarker of interest. A series of washes removes the unbound protein and a biotinylated detection antibody is added to create a sandwhich complex. Streptavidin-phycoerythrin (PE) conjugate is added to form the final detection complex (the latter serving as a fluorescent indicator).

Data acquisition and analysis

The data was acquired with a Luminex-100 cytometer. The assay suspension was drawn into the reader and a red (635 nm) laser illuminates the fluorescent dyes within each bead to provide a bead classification. This identifies the target analyte. Simultaneously, a green (532 nm) laser excites the PE part of the conjugate to generate a reporter signal that is detected by a photomultiplier tube (PMT). Bio-Plex Manager ™ software presents data as Median Fluorescence Intensity (MFI) as well as concentration (picograms (pg)/ml). The concentration of analyte bound to each bead is proportional to the MFI. A calibration curve was used to obtain the concentration in pg/ml.

Plate preparation

The plate layout was planned and labelled. Assay reagents were equilibrated to room temperature. The samples were removed from the freezer and allowed to thaw. The wash station was primed and the system was calibrated. The 96-well filter plate was washed via vacuum filtration using a vacuum manifold.

Figure 5 shows a typical sample plate layout. In the 96-well filter, columns 1 and 2 are assigned to the calibration curve standards. Two wells were assigned as blanks, which consisted of standard diluent (serum-based matrix that mimics the matrix in 1:4 diluted samples). The Bio-Plex Manager™ software automatically subtracted the assay blank FI value from the standard and sample FI values.

Standards preparation

A single vial of standards was reconstituted in 500 µl of the standard diluent. This was vortexed and incubated on ice for 30 minutes. An eight point standard dilution series was prepared and blanked. The lyophilized standard was gently tapped to ensure the pellet is at the bottom of the vial. 500 µl of the standard diluent were used to reconstitute the standard. This was gently vortexed for 1-3 seconds and incubated on ice for 30 minutes.

The following dilution series produced an eight-point standard curve with a four-fold dilution between each point. New pipette tips were used for every volume transfer. Nine 1.5 ml tubes were labelled S1 through to S8 and Blank. Diluent was added to tube S1 (72 µl) and tubes S2-8 (150 µl). Figure 6 demonstrates the steps in the serial dilution. The reconstituted standards were gently vortexed for 1-3 seconds prior to removal of any volume. 128 µl of the standard were added to S1. 50 µl were transferred from S1 to S2. This was continued for the remaining tubes as illustrated in Figure 6. Samples were vortexed throughout each step of the serial dilutions.

Figure 5-Plate formatting

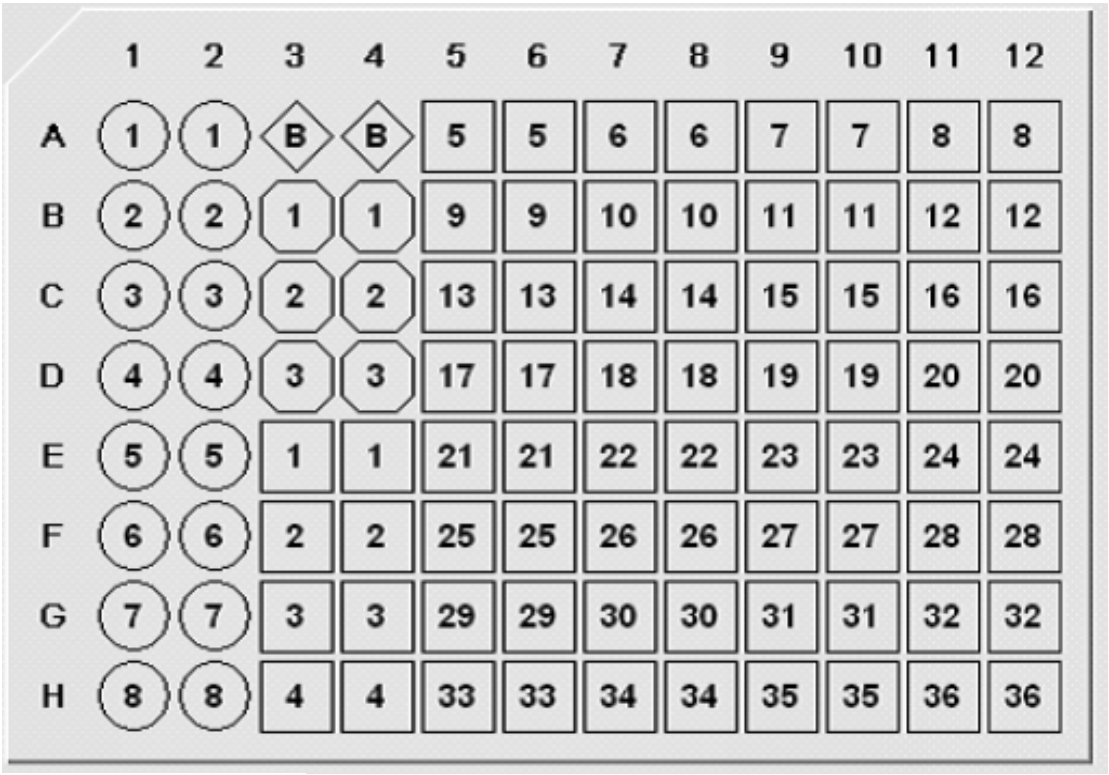
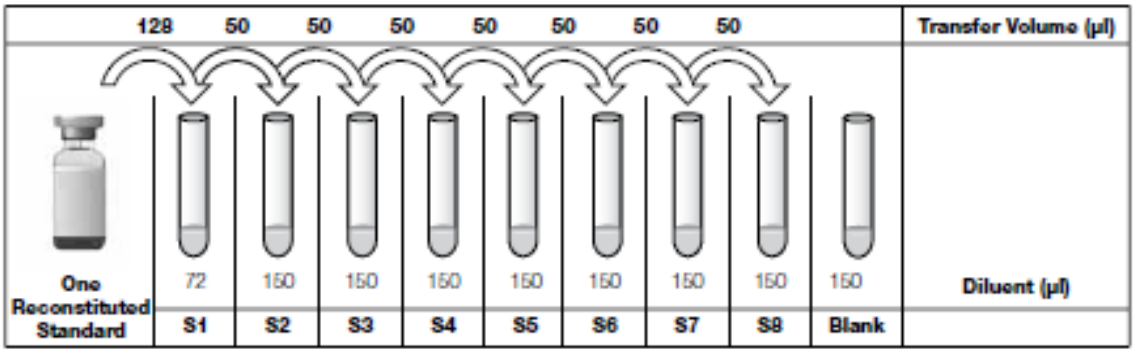


Figure 6-Preparing a fourfold dilution series with a single reconstituted standard



Sample preparation

Once the samples were thawed, they were centrifuged at 1000 x g for 15 minutes and the plasma and CSF transferred to a clean polypropylene tube. Dilution with Bio-plex sample diluent by the order of 1:4 was performed by adding 1 volume of sample to 3 volumes of sample diluent. The first analysis consisted of five wells dedicated to CSF samples in order to confirm that this dilution was appropriate for this biological fluid.

Preparation of coupled beads

The coupled beads were prepared in assay buffer whilst being protected from light. The volume of coupled beads was calculated as follows:

1. Each well required 50 μl of 1x coupled beads.
2. For 96 wells, this required 4800 μl total volume.
3. As 20% excess was included to ensure enough volume: 960 μl
4. Total volume of 1x coupled beads: 5760 μl
5. Volume of 20x coupled beads stock: 5760/20 = 288 μl
6. Volume of assay buffer required: 5760 – 288 = 5472 μl

The assay buffer was placed into a 15 ml universal tube. The coupled beads were vortexed at mid speed for 30 seconds. The required volume of 20x stocked coupled beads was pipetted into the polypropylene tube with the assay buffer. The beads were protected from light with aluminium foil while

they were allowed to equilibrate at room temperature for 20 minutes prior to use to prevent their photobleaching.

Wash and incubation

All buffers, diluted standards, diluted coupled beads and samples were brought to room temperature prior to the assay run. Repeated washes and incubations on shaker procedures were required. This consisted of:

1. The filter assay plate was placed on a calibrated vacuum apparatus and the buffer was removed by vacuum filtration. 100 μl of wash buffer was added to each well and the contents aspirated by vacuum filtration. The bottom of the filter plate was thoroughly blotted with a clean paper towel between each vacuum step to prevent cross contamination and plate leakage.
2. The plate was covered with a new sheet of sealing tape and placed on a microplate shaker and covered with aluminium foil to prevent photobleaching. It was shaken at room temperature at 1100 rpm for 30 seconds and then at 300 rpm for the incubation time.

Addition of coupled beads, standards and samples

The filter plate was prewetted with 100 μl assay buffer and the liquid was removed by vacuum filtration. The bottom of the filter plate was thoroughly blotted on a clean paper towel. The diluted coupled beads were vortexed for 30 seconds at medium speed and the beads poured into a reagent reservoir. 50 μl were added to each well using a multichannel pipette. The wells were subsequently washed twice as described above.

The diluted standards, blanks, samples and controls were gently vortexed for 1-3 seconds and 50 µl of diluted standard, control and sample were added to each well. The plate is subsequently incubated on shaker at room temperature for 30 minutes.

Preparation and addition of detection antibodies

The volume of detection antibodies was determined as follows:

1. Each well required 25 µl detection antibodies (1x). For 96 wells this was a total volume of 2400 µl.
2. A 25% excess to ensure enough volume was also included: 600 µl leaving a total volume of 1x detection antibodies: 3000 µl.
3. Volume of 20x detection antibodies stock: 150 µl.
4. Volume of detection antibody diluent required: 2850 µl.

This volume of detection antibody diluent was added to a 15 ml tube. The detection antibodies were vortexed for 15-20 seconds at medium speed followed by a 30 second spin to collect the entire volume at the bottom of the vial. For each detection antibody, the required volume (150 µl) was pipetted into a 15 ml polypropylene tube. Each well of the assay required 1.25 µl of 20x detection antibody adjusted to a final volume of 25 µl.

After incubating the samples, the sealing tape was slowly removed and discarded. The plate was washed three times as described above. The diluted detection antibodies were vortexed for 1-3 seconds and poured into a reagent reservoir. 25 µl were added to each well using a multichannel pipette.

The plate was covered with a new sheet of sealing tape and the wells sealed. The plate was incubated on shaker at room temperature for 30 minutes.

Preparation and addition of Streptavidin-PE

The volume of streptavidin-PE and assay buffer were calculated as follows:

1. Each well required 50 μl of 1x streptavidin-PE: 4800 μl
2. 25% excess to ensure enough volume: 1200 μl
3. Total volume of 1x streptavidin-PE required: 6000 μl
4. Volume of 100x streptavidin-PE required: 60 μl
5. Volume of assay buffer required: 5940 μl

This assay buffer was added to a 15 ml tube. The streptavidin-PE tube was vortexed for 15-20 seconds at medium speed followed by a 30 second spin to collect the entire volume at the bottom of the vial. The required volume of stock streptavidin-PE was pipetted into a 15 ml polypropylene tube containing the assay buffer in order to dilute the streptavidin-PE to a 1x concentration.

After detection antibody incubation, this was slowly removed and the sealing tape discarded. The plate was washed three times and the diluted streptavidin-PE was vortexed at medium speed for 3-5 seconds. This was poured into a reagent reservoir and 50 μl were added to each well using a multichannel pipette. Incubation on shaker was performed at room temperature for 10 minutes.

After the streptavidin-PE incubation step, the wells were washed three times. 125μl assay buffer was added to each well and the plate covered with a new sheet of sealing tape. The plate was shaken at room temperature at 1100 rpm for 30 seconds.

Plate reading and data acquisition

A protocol file specifying the analytes used in the reading, the plate wells to be read, the sample information, the values of the standards and controls, bead region number and instrument settings was prepared in advance using the Bio-Plex Manager 6.0 software as shown in table 10. The wells were defined as shown in figure 5.

To enter the standards, the highest concentration of each analyte was entered into the top row (labelled S1) of the table. A dilution factor of 4 was entered and the concentration for each standard point was populated in the table by the software. The assay plate was shaken at 1100 rpm for 30 seconds and the sealing tape is removed prior to placing the plate in the plate reader. The protocol was subsequently run.

Specifications of assay

The manufacturer's specification list is as follows:

Sensitivity ≈ 10 pg/ml (based on detectable signal >2 SD above background).

Precision: Inter-assay CV: <30%; intra-assay CV: <20%

Cross-reactivity: Negligible

Table 10-Cytokine assay bead regions

Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor.

Cytokine	Bead Region	Group
IL-1β	39	1
IL-4	52	1
IL-6	19	1
IL-8	54	1
IL-10	56	1
IL-15	73	1
IL-17	76	1
MCP-1	53	1
TNF-α	36	1
VEGF	45	1
IL-1α	63	2
IL-18	42	2

Statistical analysis

All analyses were performed in duplicate with mean values analysed. The spread of data for aSAH patients was investigated in order to highlight and exclude extreme values greater than 2 standard deviations from the mean. All day 9 CSF samples were removed from the analysis due to a very small sample size ($n<5$). All mediator data was tested for normality with the Shapiro-Wilks test and Q-Q plots. Time serial analyses were performed using one-way analysis of variance (ANOVA) with Games-Howell *post hoc* analysis at a significance level of 0.05. Comparison of DIND and non-DIND data was performed with the independent t-test and Mann-Whitney-*U* test for parametric and non-parametric data respectively. A further sub-group analysis of the distribution of when DIND was diagnosed was not possible due to small sample sizes. Independent t-tests were conducted with Levene's test for equality of variances. Binary logistic regression analysis was performed with MRS at day 10 and 6 months post ictus as the outcome variable (MRS 3-

6 considered as poor outcome). All mediators were forced into the model. Any mediators identified as significant predictors were placed into a second model as a second step following age, WFNS and Fisher grade placement. $p < 0.05$ was considered statistically significant. All statistical tests were performed with IBM SPSS version 18 (and StataCorp STATA for the Games-Howell analysis).

Results

Cohort demographics

Table 11 demonstrates the clinical characteristics of this cohort of aSAH patients.

Table 11-Cohort characteristics

Abbreviations: aSAH-aneurysmal subarachnoid haemorrhage, WFNS-World Federation of Neurological Surgeons.

		Patients with aSAH	Control patients
Number of patients		43	12
Median age/years (interquartile range)		58 (48-63)	65 (52-68)
Male : female		1 : 2.9	1 : 2
WFNS grade	**1**	32	N/A
	2	10	N/A
	3	1	N/A
Fisher grade	**2**	10	N/A
	3	26	N/A
	4	1	N/A
	3 + 4	6	N/A
Aneurysm treatment	**Coiling**	27	N/A
	Clipping	11	N/A
	Both	5	N/A

Plasma

Time serial analysis and control comparison

Figure 7 demonstrates box plots of the plasma mediator levels at each time-point following aSAH. Compared to controls, significantly higher levels were noted in the plasma for IL-6, IL-8 and IL-15 at days 3, 5 and 7. No other significant differences were noted.

Comparison of DIND and non-DIND

Figure 8 demonstrates mediator levels at each time-point for DIND and non-DIND patients. IL-10 was significantly lower in patients with DIND at day 3 (p=0.031). There was a trend for lower mediator levels in DIND patients in IL-1β, IL-10, IL-15 and TNF-α at all time-points although this did not reach statistical significance.

Comparison of DIND samples on the day of clinical symptoms with non-DIND patients

Figure 9 demonstrates a comparison of plasma mediator levels of DIND patients (on the day of commencement of DIND) with mediators taken from non-DIND patients at other time points. The analysis did not show any significant differences between DIND levels and non-DIND patients at all time-points. There was a tendency for day 9 plasma samples to show elevated mediator levels for all patients with a larger spread of data. Day 9 sample numbers were small for this analysis (n=4).

Logistic regression analysis

With ten-day MRS placed as the outcome variable, the regression analysis for mediators at day 3 revealed IL-18 to be a significant predictor of poor outcome (odds ratio (OR) 0.91, CI 0.83-0.99, p=0.031). This significance was lost in the second model when age, WFNS grade and Fisher grade were accounted for (OR 0.96, CI 0.92-1.004, p=0.074). At day 5, IL-1α was a significant predictor of poor outcome (OR 113, CI 1.1-11888, p=0.047). This

significance was lost in the second model (OR 5.9, CI 0.34-101, p=0.22). At day 7, IL-1α (OR 65.9, CI 1.1-4103, p=0.047), IL-18 (OR 0.93, CI 0.88-0.996, p=0.037), IL-4 (OR 0.03, CI 0.001-0.80, p=0.036) and IL-6 (OR 1.24, CI 1.03-1.49, p=0.021) were significant predictors of poor outcome. This significance was lost in the second model.

Figure 7: Box plots demonstrating median plasma mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9-post haemorrhage. Control values are also shown. Comparisons are between each time-point and control values; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor.

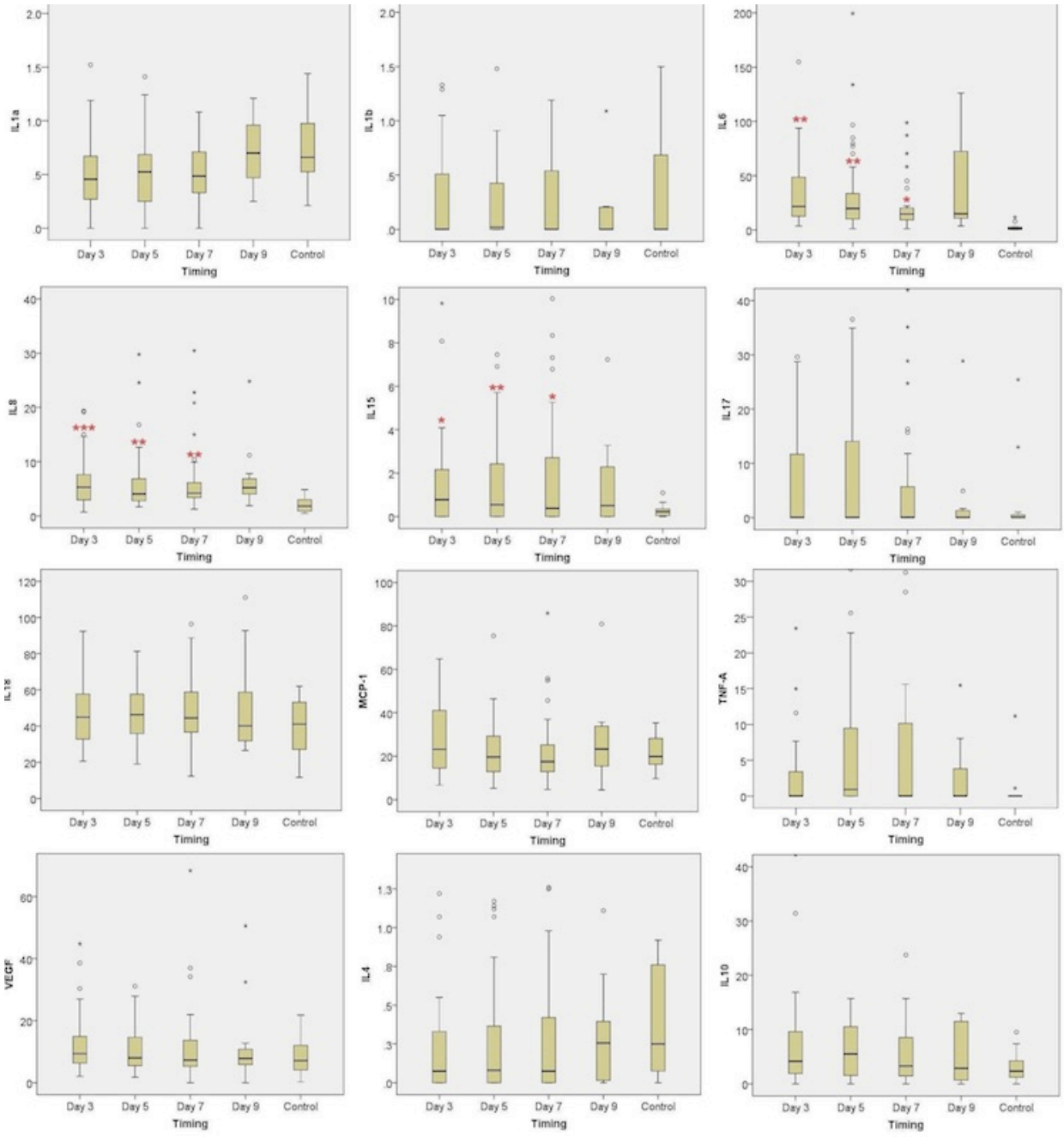


Figure 8-Box plots demonstrating median plasma mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9-post haemorrhage for both DIND and non-DIND patients. Control values are also shown. Comparisons are between DIND and non-DIND; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor, DIND-delayed ischaemic neurological deficit.

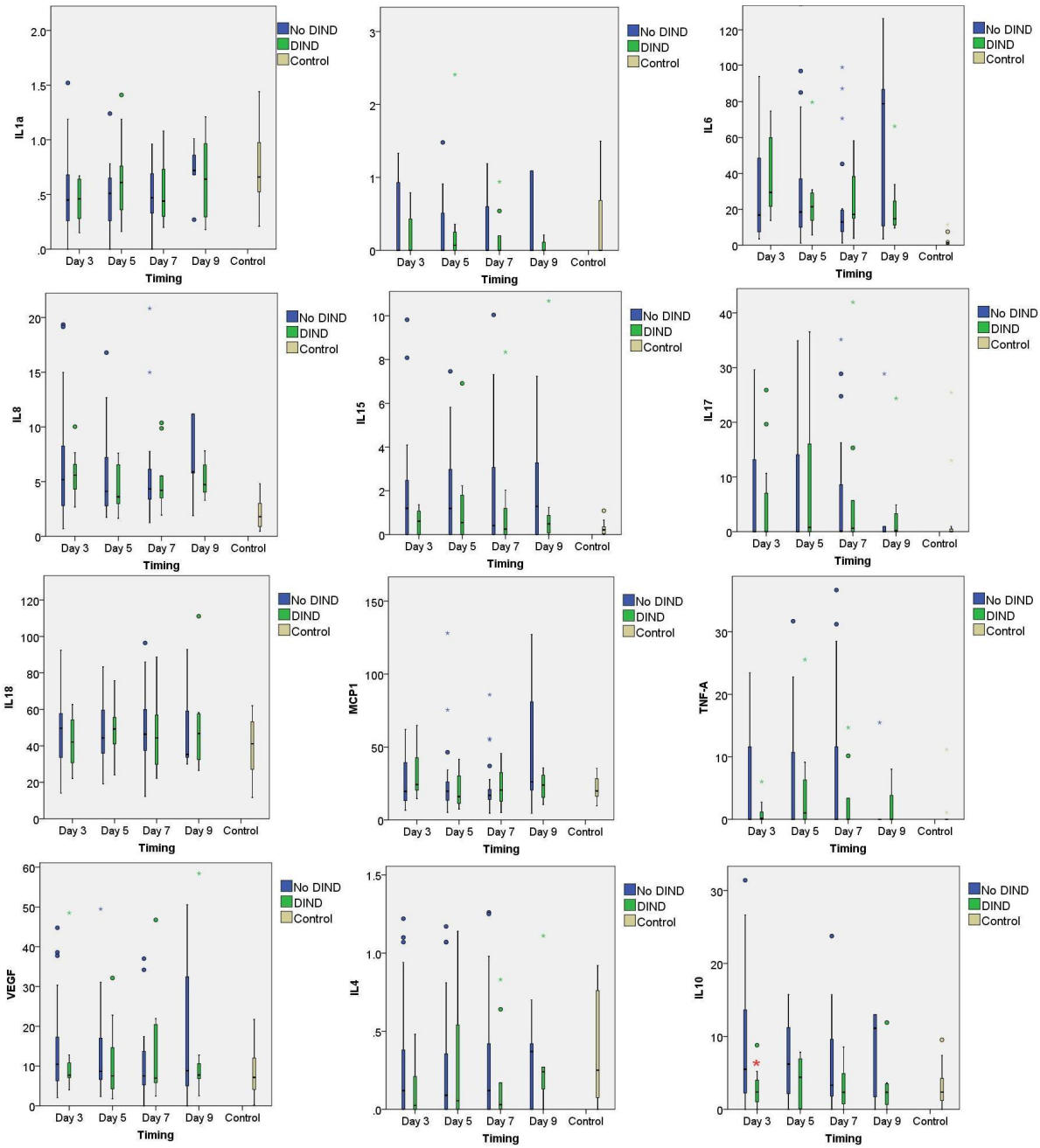
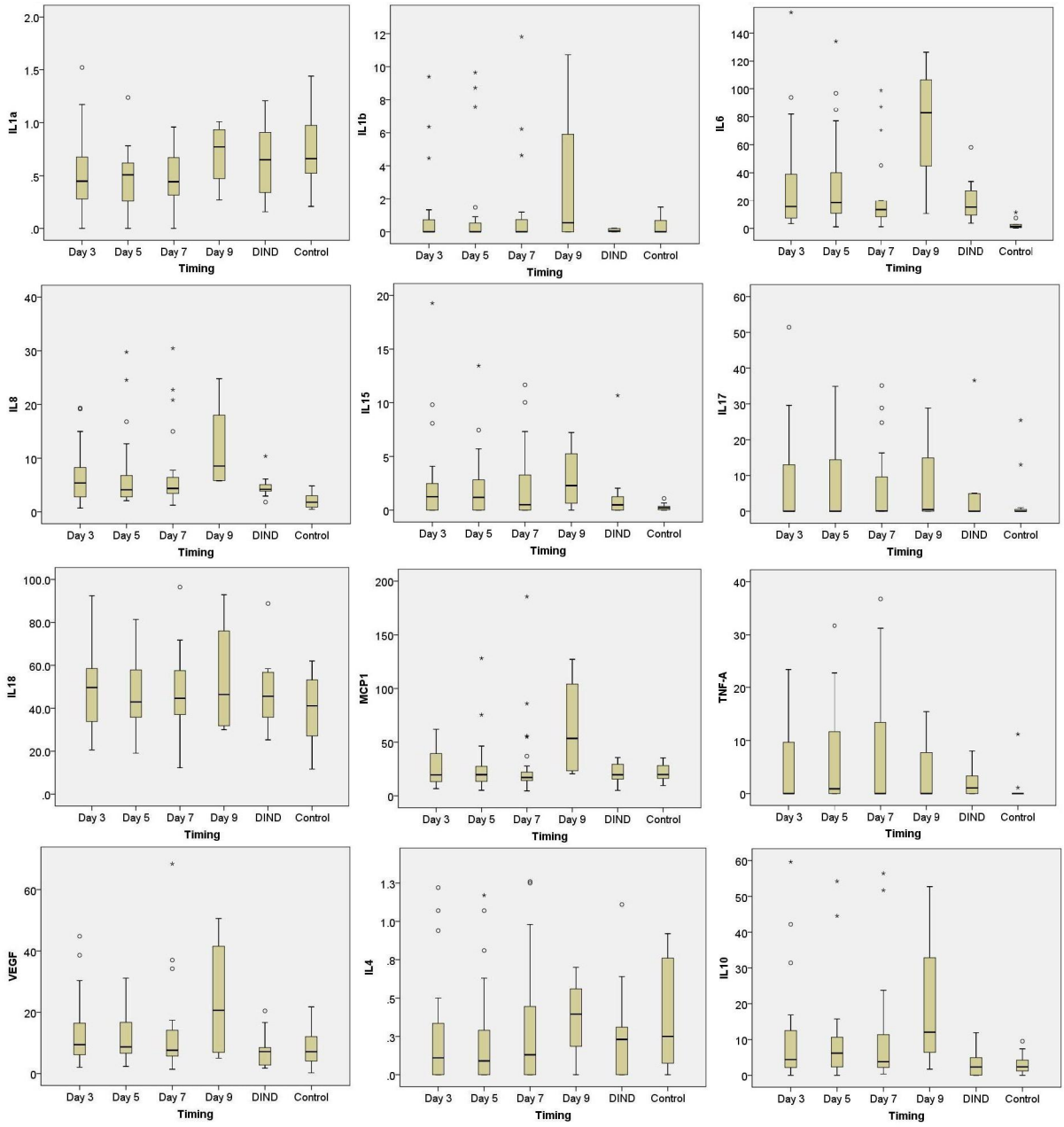


Figure 9-Box plots demonstrating median plasma mediator concentrations. **I**nterquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9-post haemorrhage for non-DIND patients and values on the day of commencement of symptoms in those with DIND. Control values are also shown. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor, DIND-delayed ischaemic neurological deficit.



With six-month MRS placed as the outcome variable, no significant predictors of outcome could be found from the mediators.

CSF

Time serial analysis and control comparison

Figure 10 demonstrates box plots of the CSF mediator levels at each time-point following aSAH in addition to control values. There is a significant increase in CSF IL-1α, IL-1β, IL-4, IL-6, IL-8 and TNF-α levels between day 3 and day 5. Levels of IL-18, IL-1β, IL-4, IL-6, IL-8, IL-15, IL-17, MCP-1, TNF-α and VEGF were significantly higher than controls on days 3, 5 and 7. IL-10 was significantly higher than controls on days 5 and 7 only.

Comparison of DIND and non-DIND

Figure 11 demonstrates mediator levels at each time-point for DIND and non-DIND patients. Although there was a trend of higher CSF levels in DIND patients at all time-points for IL-1β, IL-4, IL-17, TNF-α and VEGF, this was only statistically significant for VEGF on day 5 ($p=0.033$).

Comparison of DIND samples on the day of clinical symptoms with non-DIND patients

Figure 12 demonstrates a comparison of CSF mediator levels of DIND patients (on the day of commencement of DIND) with mediators taken from non-DIND patients at other time points. DIND values were significantly higher

Figure 10-Box plots demonstrating median CSF mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9 post-haemorrhage. Control values are also shown. Comparisons are between each time-point; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Comparisons between each time-point and control values are shown in blue; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor.

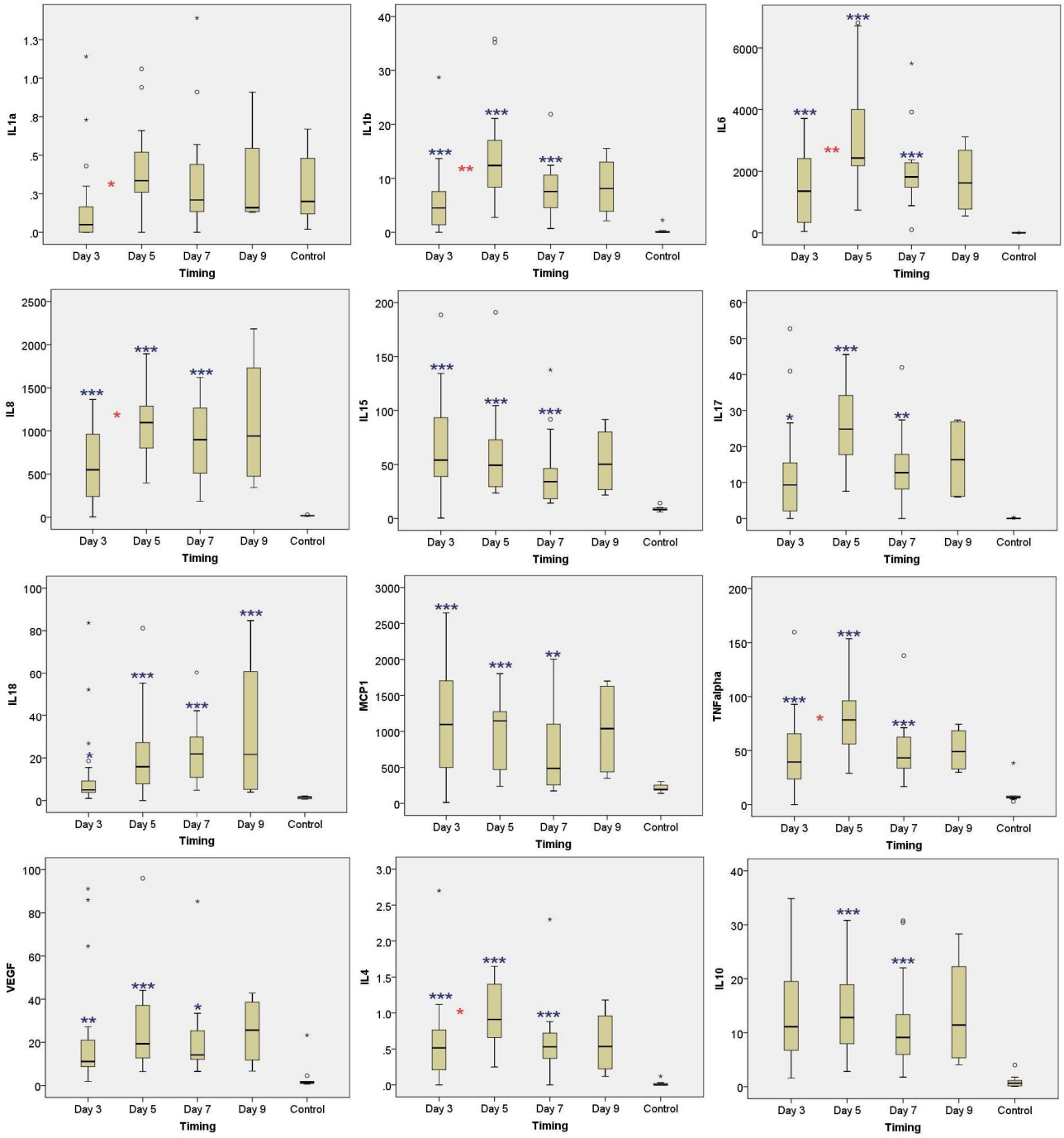


Figure 11-Box plots demonstrating median CSF mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9-post haemorrhage for both DIND and non-DIND patients. Control values are also shown. Comparisons are between DIND and non-DIND; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor, DIND-delayed ischaemic neurological deficit.

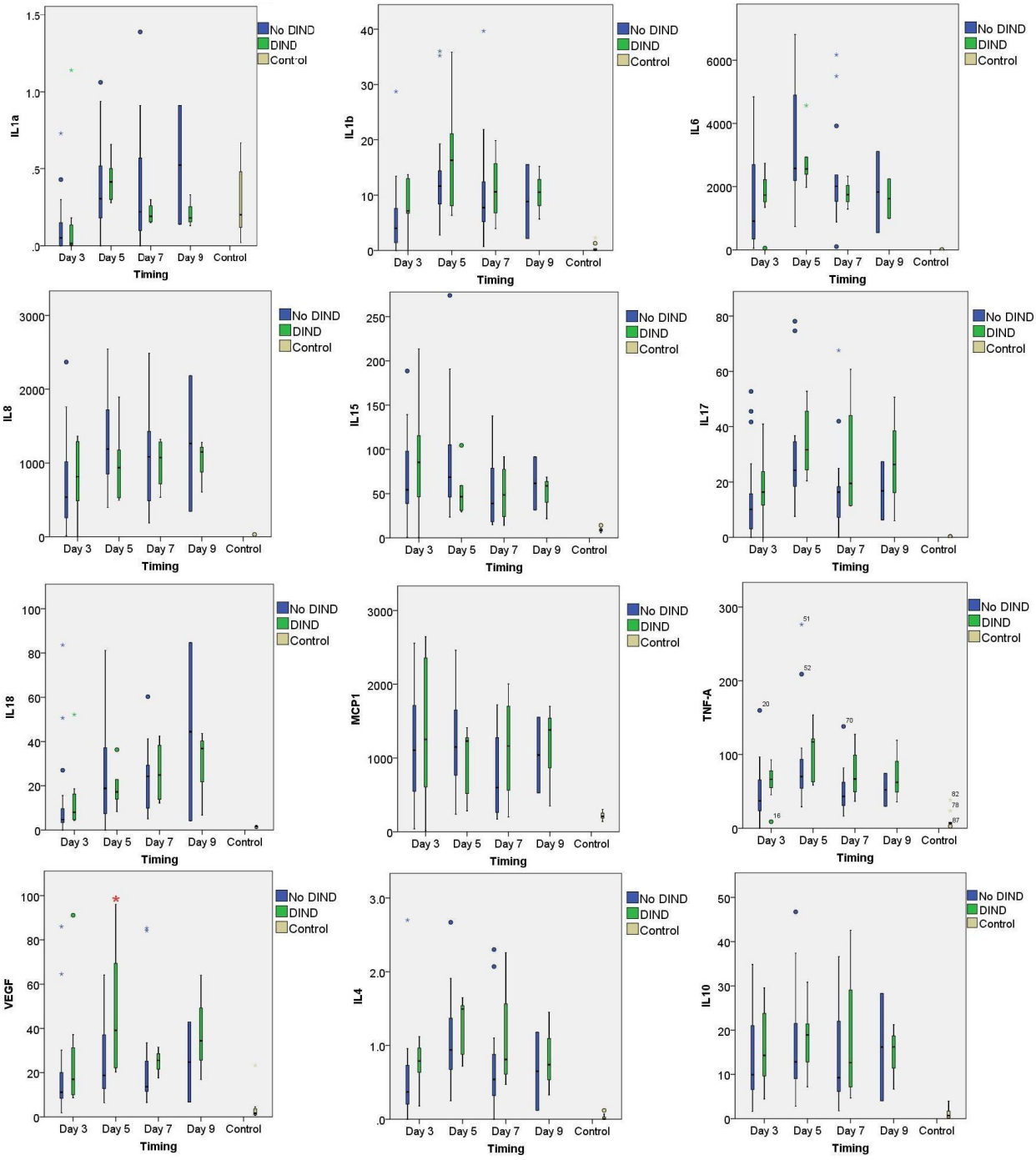
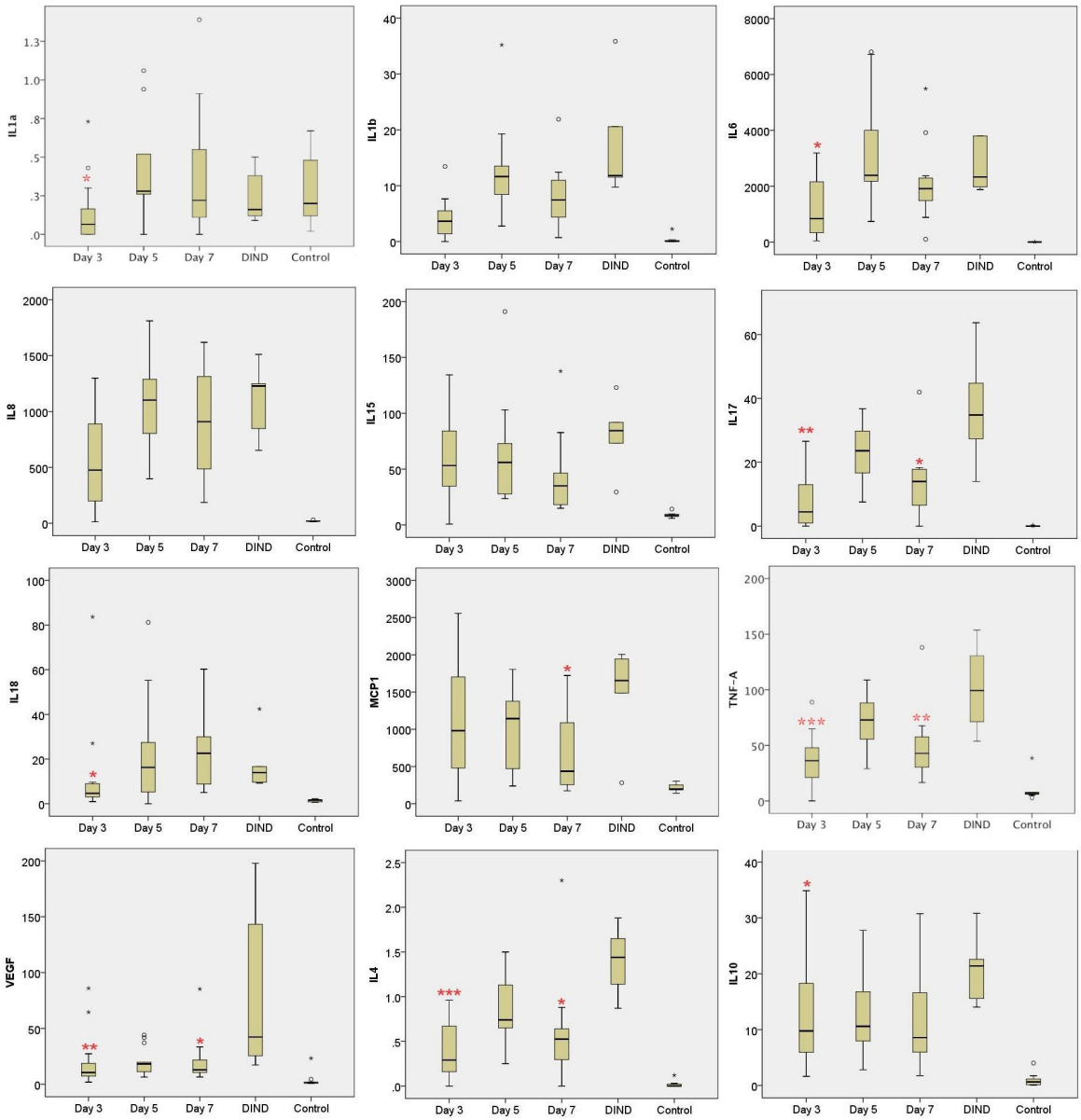


Figure 12-Box plots demonstrating median CSF mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9 post-haemorrhage for non-DIND patients and values on the day of commencement of symptoms in those with DIND. Control values are also shown. Comparisons are between DIND values and non-DIND values at all time-points; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor, DIND-delayed ischaemic neurological deficit.



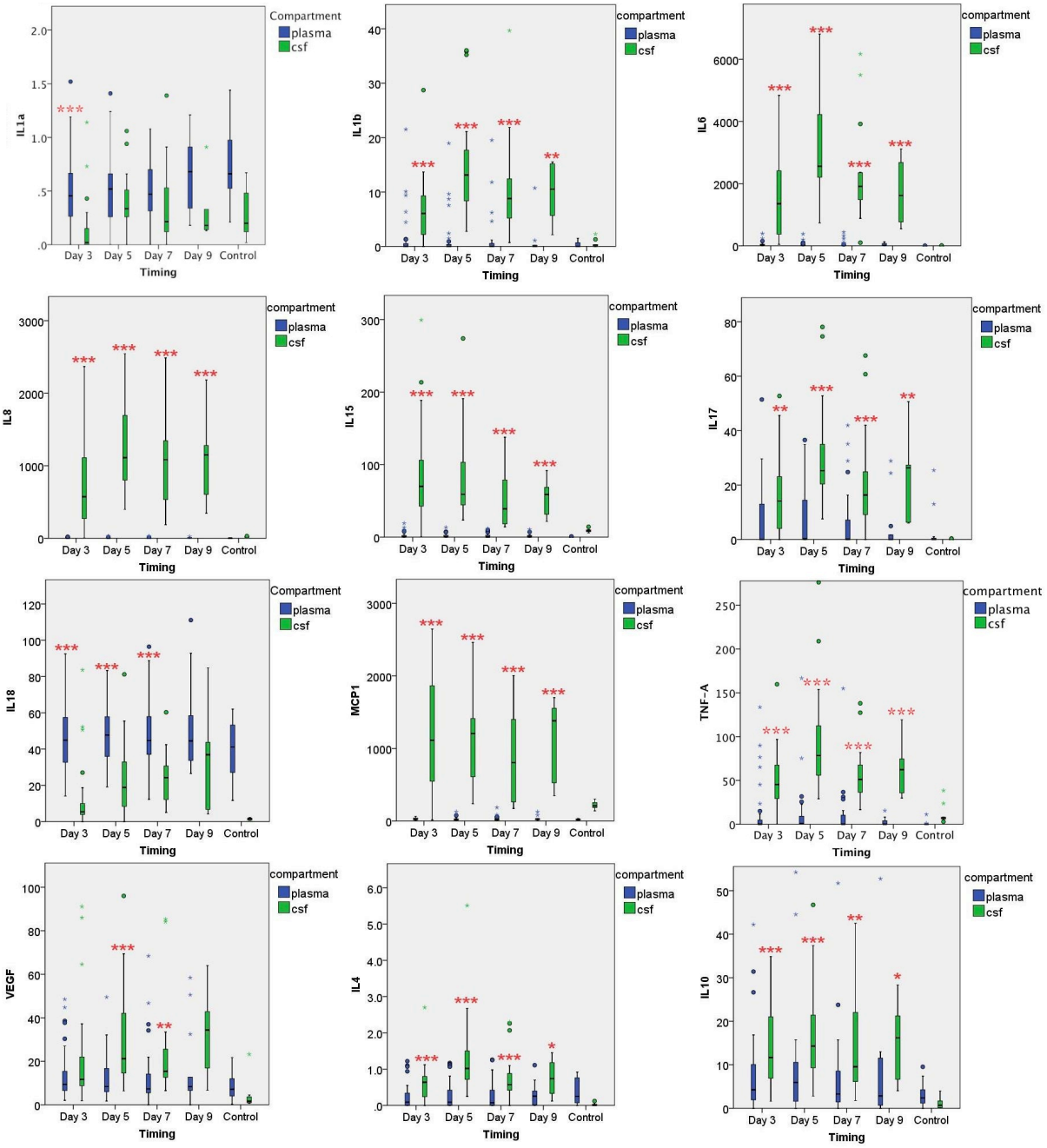
Logistic regression analysis

No significant predictors of 10-day or 6-month MRS were found amongst the mediators in CSF.

Comparison of plasma and CSF levels

Figure 13 demonstrates box plots comparing plasma and CSF levels of each mediator at all time-points and in controls. All levels were significantly higher in the CSF apart from IL-1α and IL-18, which were higher in the plasma. No markers demonstrated changes in the plasma that were mirrored by CSF levels.

Figure 13-Box plots demonstrating median plasma and CSF mediator concentrations. Interquartile range and extreme values are highlighted (o and * denote greater than two and three lengths of the box respectively, values greater than 3 standard deviations have been excluded). Units are pg/ml. Values are given at days 3, 5, 7 and 9 post-haemorrhage. Control values are also shown. Comparisons are between plasma and CSF at all time-points; * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001. Abbreviations: IL-interleukin, MCP-monocyte chemotactic protein, TNF-tumour necrosis factor, VEGF-vascular endothelial growth factor, CSF-cerebrospinal fluid.



Discussion

Key Findings

Plasma levels of IL-6, IL-8 and IL-15 were greater in aSAH when compared to controls within the first seven days of ictus. CSF levels of all mediators except for IL-1α were higher than controls throughout the course of aSAH. For aSAH patients, no specific trends could be identified in the plasma during the course of aSAH. For CSF, however, IL-1α, IL-1β, IL-4, IL-6, IL-8 and TNF-α increased in the early stages following aSAH (between days 3 and 5). Although patients with DIND did show a trend for lower plasma mediator levels of IL-1β, IL-10, IL-15 and TNF-α, this reached statistical significance at day 3 for IL-10 only. Conversely, CSF levels of IL-1β, IL-4, IL-17, TNF-α and VEGF tended to be higher in patients with DIND at all time points although this reached significant values on day 5 for VEGF only. Although no specific trends in plasma could be identified on the day of commencement of DIND, CSF levels were significantly higher than non-DIND patients for most mediators.

Systemic and CNS inflammatory response following aSAH

Plasma levels of IL-6 and IL-8 were higher in aSAH patients than in controls. This supports the role of both mediators being produced systemically and promoting an acute phase response[219]. A more substantial finding however is that CSF levels of IL-6 and IL-8 have been shown to be raised and compared to non-aSAH controls, shown to be anywhere between 1.5-10000-fold higher[219,299-302,305,307]. This is in agreement with the current study that demonstrated 680-fold and 50-fold higher CSF IL-6 and IL-8 levels

respectively in aSAH patients than when compared with non-aSAH controls. Absolute concentrations and the large variation and spread of the data are also similar[219,299,305]. As noted in the current study, CSF IL-6 and IL-8 levels have been shown to increase and peak around day 5 post ictus, with a subsequent decline to lower, albeit pathological levels by day 14[219,303,305]. The relatively slower peak at day 5, supports the known mechanisms of action for IL-6. In the CNS following ischaemia, early release of IL-1β and TNF-α results in a second and more persistent inflammatory response mediated by IL-6 and IL-8. β-adrenergic stimulation may be involved in the production of IL-6 in this setting[346]. Both IL-6 and IL-8 are important in leukocyte aggregation and adhesion which is thought to play a role in the periadventitial space around the microvasculature surrounded by the spilled blood in the subarachnoid space[204].

As has been seen in the present study, the concentration of IL-1β in the CSF soon after aSAH was shown to be greater than in non-aSAH controls [302]. It is unclear from the literature what the absolute values are following aSAH, with a range of 2-80 pg/ml being reported[347]. The time-course demonstrates a peak at day 5 post ictus followed by a steady decline to lower levels by day 10 post ictus, although this time-course has not been consistently demonstrated[299,305]. In the current study, there is a similar time-course observed with absolute values lying within those reported in the literature. IL-1α increased in the CSF early after aSAH but this difference was not significant. Levels were never significantly different from controls, with a trend for lower plasma values in aSAH patients. IL-1α was one of only two

investigated mediators that was found more abundantly in the plasma than in CSF. This lack of association with aSAH and DIND has some support from another study that could not detect IL-1α in the CSF following aSAH[301].

Levels of CSF TNF-α in control subjects in the current study are 40-fold higher than that described in the literature[299]. This may be explained by the different types of controls utilised. In the literature, control subjects often constitute patients undergoing lumbar puncture for suspected aSAH or meningitis without a subsequent positive diagnosis. In the current study, control subjects are patients undergoing an elective joint replacement (knee or hip) requiring spinal anaesthesia. These patients did not present with any acute pathology and were screened for any systemic inflammatory response. Patients with inflammatory arthropathy were not included. TNF-α has been shown to be 5-30 fold higher in the CSF of aSAH when compared to control, peaking at around day 7[299,302,304]. This increase may be related to those with an unfavourable outcome[304]. In the current study, TNF-α peaked slightly earlier on day 5, with absolute values considerably higher than those previously documented[299]. The global increase in TNF-α in both controls and CSF may suggest a fundamental assay difference for this mediator even though multiplex analysis were used for both studies.

MCP-1 has been shown to be elevated in the plasma and CSF following aSAH in comparison with non-aSAH controls and this difference noted to be more marked in the CSF[262,300,307]. In the current study, this effect was noted in the CSF only. As is seen in the present study, control values were higher in

plasma than in CSF[262]. The level of MCP-1 was shown to be proportional to the grade of the patient and the weaker response in plasma was thought to represent a weaker systemic inflammatory response in good grade patients. The lack of difference between plasma levels in aSAH patients and controls in the current study may be a result of the good grade cohort being investigated.

Systemic and CNS inflammatory response associated with DIND

CSF IL-6 and IL-8 levels have been shown to be higher in patients with DIND[219,299,300,302,305,306,311] and associated with a poor outcome at three months following haemorrhage[310]. This increase was as early as day 1 post ictus and sustained throughout the course of aSAH. CSF IL-6 levels were also higher in patients with increased flow velocities on TCD examination[299]. This cohort exhibited a greater peak of IL-6 concentration. CSF IL-6 levels were elevated 0-3 days post-haemorrhage in those of poor clinical grade with subsequent DIND[306]. According to binary logistic regression, there was a statistically significant effect on days 4 and 5 for the prediction of DIND and day 7 for cerebral ischaemia related to DIND[219]. A cut-off point of 2000 pg/ml on day 4 came with an eleven-fold relative risk of DIND. An alternative cut-off point has been described as 400 pg/ml on day 3[311]. This has not been demonstrated in the current study. Although median levels were higher in DIND patients at day 3, this was not a significant or sustained effect. This was also noted for CSF IL-6 levels taken at the time of commencement of DIND. Half of patients with DIND and half without DIND had a CSF IL-6 level of >2000 pg/ml in the current study. A possible explanation for this is the

smaller number in the current study unable to confirm the significance of this change at day 3. It is interesting to note that in the study by Schoch and colleagues, no association was found between DIND/delayed cerebral ischaemia and WFNS grade, Fisher grade and length of stay in the critical care unit. This may highlight fundamental differences between this study and the current study in relation to the aSAH cohort and diagnostic criteria for DIND. It was noted in their study that there was a disproportionate weighting towards poor grade patients; the opposite effect was noted in the current study, which was limited to good grade aSAH patients only.

Blockade of IL-8 effects has been shown to be beneficial following transient ischaemia but not permanent ischaemia[235,348]. This would suggest that IL-8 (or a downstream factor) confers damage during the re-perfusion phase of ischaemia. Leukocyte accumulation may have an important role in this. This is especially relevant for patients with new onset DIND who have not yet established an infarct and are likely to exhibit focal compromises to cerebral blood flow that are initially dynamic and subject to fluctuation at the microvascular level.

CSF IL-1β levels have been shown to be higher in patients with increased flow velocities on TCD examination, with a greater peak concentration at day 5 post ictus[299]. Although a very similar trend was seen in the current study, the difference was not statistically significant for patients with DIND. This is likely to be due to the smaller numbers in the current study and the inherent differences between angiographic vasospasm and DIND. A higher trend in

DIND was noted at all time-points, particularly at day 3. This is supported by the observation that CSF IL-1β taken at admission was three-fold higher in those with subsequent DIND and subsequent samples taken between days 8 and 15 were higher in those with DIND[302,306]. Given the diverse set of circumstances that can result in IL-1α and β release and the diversity of their subsequent response, it is difficult to predict exactly what role they have in aSAH and the subsequent clinical course. There is a lack of consensus in the literature as to the correlation of IL-1β levels and the occurrence of DIND (table 8). This is likely to be due to the temporal expression profile of these molecules and the potentially conflicting pro- and anti-inflammatory effects they may have depending on the relative concentration and contribution of other existing cytokines. This of course needs to be interpreted in the context of anatomical location; between the systemic compartment and the CNS and also temporal differences within the CNS.

CSF TNF-α was higher in patients with increased flow velocities on TCD examination, peaking at day 7 and subsiding at a slower rate in this subset of patients[299]. CSF TNF-α taken at admission was double in those with subsequent DIND[302]. These findings are reflected in the current study that has demonstrated a higher trend in DIND patients at all time-points and a peak at day 5 that is sustained until day 9 in those with DIND.

VEGF was the only mediator in the current study to be significantly higher in patients with DIND (only on day 5). Although it has been implicated in BBB disorders and is upregulated following experimental TBI and hypoxia[278,279],

this is the first demonstration in humans of an association with DIND. It is difficult to interpret this finding given that it was elevated in the CSF in all aSAH patients when compared with controls and tended to be even higher in those with DIND at all measured time-points. It is not possible to determine whether this response is a cause or consequence of ischaemia or simply epiphenomena. A possible mechanism of how VEGF can cause or contribute to DIND lies in its ability to disrupt the BBB. Cerebral oedema (even in a focal/microvascular capacity) may be the starting point which compromises cerebral blood flow and triggers the secondary insults resulting in DIND. Alternatively, DIND is triggered by some another unknown mechanism and the subsequent rise of VEGF triggers/worsens cerebral oedema and starts the cycle of secondary brain injury. Src-family tyrosine kinase and MAPK are important pathways associated with VEGF[281,282]

CSF and not plasma levels of MCP-1 have been shown to correlate with angiographic vasospasm in aSAH patients[262]. In a subgroup of good grade patients with and without vasospasm, this correlation was no longer present. Although DIND and not angiographic vasospasm was investigated in the current study, this association was not seen. This may in part be explained by the good grade cohort in this study and by the different pathological processes that are likely to be taking place in angiographic vasospasm and DIND.

Regression analysis in the current study did not highlight any mediators that were independent predictors of early and long-term outcome in this cohort.

Compartmentalisation of inflammation following aSAH

The overall consensus from the literature is that the mediators found abundantly in the CSF following aSAH are not as prominent in the plasma, suggesting intra-thecal production[299,305,308]. There has been some evidence implicating raised IL-6 values in the peripheral and central venous system with DIND[349]. In agreement with previous studies IL-1β, IL-6 and IL-8 were lower in the plasma in the current study compared to simultaneous CSF samples although in the case of IL-6 and IL-8, plasma levels were still significantly higher than non-aSAH controls[299,303,305,308]. It is not possible to determine whether this represents two separate sites of mediator production in a combined systemic/CNS inflammatory cascade, or production at one of these sites with subsequent migration. The existence of SIRS in aSAH would support the former whereas the overwhelmingly larger mediator concentrations seen in the CSF would support the latter. In the case of IL-1β, another study has shown this to be completely undetected in the plasma[305]. TNF-α was significantly higher in the CSF in the current study and although this does have some support[308], others have shown no difference between the two concentrations[299].

Mediator concentrations within the ECF are also uncertain following aSAH. Although studied in a mixed cohort of aSAH, ischaemic stroke and TBI patients, microdialysis catheters placed during the first 72 hours following haemorrhage demonstrated very high IL-1β concentrations (approximately 20 pg/ml) in the first 6 hours, followed by a steady decline to less than 4 pg/ml[309]. These findings closely precede the CSF findings in the current study (where

the first measurement is at 72 hours) and other CSF findings[299]. ECF IL-6 levels did also rise but in a slightly delayed fashion when compared with IL-1β (6-12 hours)[309]. Absolute values are also comparable with CSF[299]. This would suggest that IL-6 levels do fluctuate as early as 12 hours post-ictus: activity that would have been missed in the current study due to the first sample being taken at 72 hours. In a study of aSAH exclusively, IL-6 was elevated in all three compartments in all patients[312]. This was followed by a systemic inflammatory response (as measured by CRP) between days 7-9 only in those with DIND. IL-6 was higher in those with symptomatic DIND particularly in the CSF and ECF (no associated with plasma levels).

ECF IL-10 values were not subject to any significant fluctuations within the first 72 hours following haemorrhage, with a median concentration of 10-12 pg/ml[309]. This is supported by the current study demonstrating median concentrations in the CSF between 9-12 pg/ml betweens days 3-9. Plasma levels of IL-10 were also not subject to fluctuation and were found to be approximately one-third of CSF levels in magnitude.

The chemokine IL-8 demonstrated a peak value (approximately 4000 pg/ml) on initial assessment in the ECF at 6 hours post haemorrhage and underwent a steady decline to 500 pg/ml at 72 hours[350]. The 72 hour levels reflect those found in the current study within the CSF at that time. Plasma levels were found to be approximately 10-fold less than CSF levels and not subject to these fluctuations. This would support the early rise and fall of IL-8 within the CNS.

Whereas the median concentration of VEGF in the ECF following aSAH and other intracranial pathologies was relatively constant for the first 36 hours, our results would suggest that absolute values were approximately four-fold greater in the CSF and plasma[350].

Limitations of the study

Single samples taken every 48 hours only capture a limited picture of the true inflammatory profile. The changes taking place in the 7-10 days following aSAH are likely to impact heavily on mediator levels regardless of the presence or absence of DIND or the initial severity of the haemorrhage. In order to assess this more thoroughly, more frequent samples need to be taken (every 4-6 hours) in order to construct a better impression of molecular events.

Although cytokines are measured together using Multiplex technology, the analysis in the current study has kept those mediators separate. Cytokine profiling is a complex process and cytokines are likely to modulate and potentiate each other. Each cytokine is likely to behave in a completely different way depending on its concentration, site of action and the temporal expression of other mediators. Improved data modelling is required to aid interpretation of this complex dataset (particularly if more frequent samples are taken). Using the Games-Howell test for post-hoc analysis helps adjust p-values for multiple comparisons and is particularly useful for unequal group sizes with unequal variances and for small sample sizes per cell. It is based

on Welch's correction to degree of freedom with the t-test and uses the studentized range statistic.

The current study relied upon CSF obtained from a lumbar drain. This device is likely to stimulate its own local inflammatory reaction with time. An ideal control for this (not available in the current study) would be CSF obtained from a single lumbar puncture at days 3, 5 , 7 and 9 in a patient randomised to the control arm of the lumbar drain trial (and so without a lumbar drain).

Summary

Although definite conclusions cannot be drawn from the current study regarding the role of inflammation following aSAH, it is clear that an inflammatory response is initiated following the haemorrhage, likely to peak at day 5-7 post ictus and localised within the CNS relative to the systemic compartment. VEGF may have a particular association with incipient DIND but any further conclusions about the chronology of events are not possible from the current study. This mediator should be subject to further investigation in a larger study extracting it from the ECF, CSF and plasma simultaneously.

Final conclusions and future direction

The prevalence of DIND following aSAH has been reduced with the use of lumbar drainage of CSF post ictus. This is likely to be due to a combination of blood clearance and control of ICP although the former is a more likely explanation for this. Patient recovery was quicker with lumbar drains but long-term outcome was no different. Despite this, there is reasonable support from this trial for the routine use of lumbar drains in all good grade aSAH patients. Similar results from a second trial (ideally incorporating poor grade aSAH patients) would give strong support for the routine use of lumbar drains. Larger patient numbers and better outcome measures would need to be incorporated in order to detect any possible subtle differences in long-term outcome.

Cytokines appear to be important mediators involved in DIND. Whether their effects are the cause or consequence of ischaemia or a response that is seen concurrently in patients with DIND but not directly related to it, is unclear. In fact, all the inflammatory mediators that did show non-significant trends following aSAH and associations with DIND were not conclusively shown to be causative mediators. VEGF is highlighted from the current study as a new target for a future investigation. This should extend to include sampling the ECF with microdialysis technology and involve multiple sampling throughout a 24-hour period in order to ascertain if there are frequent fluctuations in mediator levels. Snap-shots every 24-48 hours will not suffice. Advanced modelling techniques will need to be applied in order to interpret such large amounts of data correctly and accurate correlation to clinical parameters needs to be made. Single mediator analyses are unlikely to yield meaningful

results since cytokines are likely to function as dynamic signalling networks. The same mediators may simultaneously be able to stimulate both a pro-inflammatory and anti-inflammatory state depending on its concentration and interaction with other mediators. In the CNS, these conflicting responses may be occurring simultaneously in different parts of the brain anatomically and physiologically in different compartments. In fact, the terms pro-inflammatory and anti-inflammatory are likely to be over-simplifications. As opposed to focusing on single mediators, multiplex technology allows multiple mediators to be measure in duplicate or triplicate. This could potentially allow mediator results to be combined in order to help elucidate what the overall inflammatory status was at the time and place of sampling. This may be a more constructive way of interpreting this complex data.

References

1. Linn FH, Rinkel GJ, Algra A, van Gijn J. Incidence of subarachnoid hemorrhage: role of region, year, and rate of computed tomography: a meta-analysis. Stroke 1996;27:625-9.

2. Macdonald RL, Pluta RM, Zhang JH. Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution. Nature clinical practice 2007;3:256-63.

3. Nolan CP, Macdonald RL. Can angiographic vasospasm be used as a surrogate marker in evaluating therapeutic interventions for cerebral vasospasm? Neurosurg Focus 2006;21:E1.

4. Claassen J, Bernardini GL, Kreiter K, et al. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: the Fisher scale revisited. Stroke 2001;32:2012-20.

5. Bonet T. Sepulcretum Anatomicum. Geneva 1679.

6. Morgagni J. De Sedibus et Causis Morborum per Anatomen Indagatis. Book 1, Letters 3 and 4 1769.

7. Biumi F. Observationes anatomicae, scholiis illustrati. Observatio V, in Sandifort E (ed). Thesaurus Diessertationem Milan, S & J Luchtmans 1765:373.

8. Blackall J. Observations on the Nature and Cure of Dropsies. London, Longman, Hurst, Rees, Orne, and Brown 1814.

9. Gull W. Cases of aneurism of the cerebral vessels. Guys Hosp Rep 1859;5:281-304.

10. Florey H. Brain 1925;48:43.

11. Moniz E. L'angiographie cérébrale, ses applications et résultats en anatomic, physiologie te clinique (Cerebral angiography, its applications and results in anatomy, physiology, and clinic). 1934.

12. Dandy W. Intracranial aneurysms of the internal carotid artery. Cured by operation. Ann Surg 1938;107:654-9.

13. Robertson EG. Cerebral lesions due to intracranial aneurysms. Brain 1949;72:150-85.

14. Zucker M. A study of substances in blood serum and platelets which stimulate smooth muscle. Am J Physiol 1944;142:12-26.

15. Jackson IJ. Aseptic hemogenic meningitis; an experimental study of aseptic meningeal reactions due to blood and its breakdown products. Archives of neurology and psychiatry 1949;62:572-89.

16. Reid BG, Johnson RT. Proceedings of the Sixth International Congress of Radiology in London 1950.

17. Ecker A, Riemenschneider PA. Arteriographic demonstration of spasm of the intracranial arteries, with special reference to saccular arterial aneurysms. J Neurosurg 1951;8:660-7.

18. Stornelli SA, French JD. Subarachnoid Hemorrhage--Factors in Prognosis and Management. J Neurosurg 1964;21:769-80.

19. Kak VK, Taylor AR. Cerebral blood flow in subarachnoid hemorrhage. Lancet 1967;1:875-7.

20. Allcock JM, Drake CG. Ruptured Intracranial Aneurysms--the Role of Arterial Spasm. J Neurosurg 1965;22:21-9.

21. Suzuki J, Yoshimoto T. [Early operation for the ruptured intracranial aneurysms--especially the cases operated within 48 hours after the last subarachnoid hemorrhage (author's transl)]. No shinkei geka 1976;4:135-41.

22. Weir B, Grace M, Hansen J, Rothberg C. Time course of vasospasm in man. J Neurosurg 1978;48:173-8.

23. Katada K, Kanno T, Sano H, Shibata T, Shah MY. [Computed tomography of ruptured intracranial aneurysm in acute stage (author's transl)]. No shinkei geka 1977;5:955-63.

24. Takemae T, Mizukami M, Kin H, Kawase T, Araki G. [Computed tomography of ruptured intracranial aneurysms in acute stage--relationship between vasospasm and high density on CT scan (author's transl)]. No to shinkei = Brain and nerve 1978;30:861-6.

25. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1-9.

26. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. Lancet 2007;369:306-18.

27. Chappell ET, Moure FC, Good MC. Comparison of computed tomographic angiography with digital subtraction angiography in the diagnosis of cerebral aneurysms: a meta-analysis. Neurosurgery 2003;52:624-31; discussion 30-1.

28. Hasan D, Vermeulen M, Wijdicks EF, Hijdra A, van Gijn J. Management problems in acute hydrocephalus after subarachnoid hemorrhage. Stroke 1989;20:747-53.

29. Ohkuma H, Tsurutani H, Suzuki S. Incidence and significance of early aneurysmal rebleeding before neurosurgical or neurological management. Stroke 2001;32:1176-80.

30. Roos YB, Beenen LF, Groen RJ, Albrecht KW, Vermeulen M. Timing of surgery in patients with aneurysmal subarachnoid haemorrhage: rebleeding is still the major cause of poor outcome in neurosurgical units that aim at early surgery. J Neurol Neurosurg Psychiatry 1997;63:490-3.

31. Brilstra EH, Algra A, Rinkel GJ, Tulleken CA, van Gijn J. Effectiveness of neurosurgical clip application in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;97:1036-41.

32. Molyneux A, Kerr R, Stratton I, et al. International Subarachnoid Aneurysm Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised trial. Lancet 2002;360:1267-74.

33. Molyneux AJ, Kerr RS, Yu LM, et al. International subarachnoid aneurysm trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised comparison of effects on survival, dependency, seizures, rebleeding, subgroups, and aneurysm occlusion. Lancet 2005;366:809-17.

34. Britz GW, Newell DW, West GA, Lam A. The ISAT trial. Lancet 2003;361:431-2; author reply 2.

35. Sellar R, Whittle I. The ISAT trial. Lancet 2003;361:432-3; author reply 3.

36. Killeen RP, Mushlin AI, Johnson CE, et al. Comparison of CT perfusion and digital subtraction angiography in the evaluation of delayed cerebral ischemia. Academic radiology 2011;18:1094-100.

37. Carrera E, Schmidt JM, Oddo M, et al. Transcranial Doppler for predicting delayed cerebral ischemia after subarachnoid hemorrhage. Neurosurgery 2009;65:316-23; discussion 23-4.

38. Carrera E, Schmidt JM, Oddo M, et al. Transcranial Doppler ultrasound in the acute phase of aneurysmal subarachnoid hemorrhage. Cerebrovascular diseases 2009;27:579-84.

39. Hickmann AK, Langner S, Kirsch M, et al. The value of perfusion computed tomography in predicting clinically relevant vasospasm in patients with aneurysmal subarachnoid hemorrhage. Neurosurg Rev 2012.

40. Denny-Brown D. The treatment of recurrent cerebrovascular symptoms and the question of "vasospasm". The Medical clinics of North America 1951;35:1457-74.

41. Farhat SM, Schneider RC. Observations on the effect of systemic blood pressure on intracranial circulation in patients with cerebrovascular insufficiency. J Neurosurg 1967;27:441-5.

42. Kosnik EJ, Hunt WE. Postoperative hypertension in the management of patients with intracranial arterial aneurysms. J Neurosurg 1976;45:148-54.

43. Awad IA, Carter LP, Spetzler RF, Medina M, Williams FC, Jr. Clinical vasospasm after subarachnoid hemorrhage: response to hypervolemic hemodilution and arterial hypertension. Stroke 1987;18:365-72.

44. Mori K, Arai H, Nakajima K, Tajima A, Maeda M. Hemorheological and hemodynamic analysis of hypervolemic hemodilution therapy for cerebral

vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 1995;26:1620-6.

45. Darby JM, Yonas H, Marks EC, Durham S, Snyder RW, Nemoto EM. Acute cerebral blood flow response to dopamine-induced hypertension after subarachnoid hemorrhage. J Neurosurg 1994;80:857-64.

46. Joseph M, Ziadi S, Nates J, Dannenbaum M, Malkoff M. Increases in cardiac output can reverse flow deficits from vasospasm independent of blood pressure: a study using xenon computed tomographic measurement of cerebral blood flow. Neurosurgery 2003;53:1044-51; discussion 51-2.

47. Raabe A, Beck J, Keller M, Vatter H, Zimmermann M, Seifert V. Relative importance of hypertension compared with hypervolemia for increasing cerebral oxygenation in patients with cerebral vasospasm after subarachnoid hemorrhage. J Neurosurg 2005;103:974-81.

48. Hino A, Mizukawa N, Tenjin H, et al. Postoperative hemodynamic and metabolic changes in patients with subarachnoid hemorrhage. Stroke 1989;20:1504-10.

49. Ekelund A, Reinstrup P, Ryding E, et al. Effects of iso- and hypervolemic hemodilution on regional cerebral blood flow and oxygen delivery for patients with vasospasm after aneurysmal subarachnoid hemorrhage. Acta neurochirurgica 2002;144:703-12; discussion 12-3.

50. Kramer AH, Zygun DA, Bleck TP, Dumont AS, Kassell NF, Nathan B. Relationship between hemoglobin concentrations and outcomes across subgroups of patients with aneurysmal subarachnoid hemorrhage. Neurocrit Care 2009;10:157-65.

51. Naidech AM, Drescher J, Ault ML, Shaibani A, Batjer HH, Alberts MJ. Higher hemoglobin is associated with less cerebral infarction, poor outcome, and death after subarachnoid hemorrhage. Neurosurgery 2006;59:775-9; discussion 9-80.

52. Tseng MY, Hutchinson PJ, Kirkpatrick PJ. Effects of fluid therapy following aneurysmal subarachnoid haemorrhage: a prospective clinical study. British journal of neurosurgery 2008;22:257-68.

53. Muench E, Horn P, Bauhuf C, et al. Effects of hypervolemia and hypertension on regional cerebral blood flow, intracranial pressure, and brain tissue oxygenation after subarachnoid hemorrhage. Critical care medicine 2007;35:1844-51; quiz 52.

54. Dankbaar JW, Slooter AJ, Rinkel GJ, Schaaf IC. Effect of different components of triple-H therapy on cerebral perfusion in patients with aneurysmal subarachnoid haemorrhage: a systematic review. Crit Care 2010;14:R23.

55. Treggiari MM, Walder B, Suter PM, Romand JA. Systematic review of the prevention of delayed ischemic neurological deficits with hypertension, hypervolemia, and hemodilution therapy following subarachnoid hemorrhage. J Neurosurg 2003;98:978-84.

56. Egge A, Waterloo K, Sjoholm H, Solberg T, Ingebrigtsen T, Romner B. Prophylactic hyperdynamic postoperative fluid therapy after aneurysmal subarachnoid hemorrhage: a clinical, prospective, randomized, controlled study. Neurosurgery 2001;49:593-605; discussion -6.

57. Lennihan L, Mayer SA, Fink ME, et al. Effect of hypervolemic therapy on cerebral blood flow after subarachnoid hemorrhage : a randomized controlled trial. Stroke 2000;31:383-91.

58. Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. BMJ (Clinical research ed 1989;298:636-42.

59. Barker FG, 2nd, Ogilvy CS. Efficacy of prophylactic nimodipine for delayed ischemic deficit after subarachnoid hemorrhage: a metaanalysis. J Neurosurg 1996;84:405-14.

60. Feigin VL, Rinkel GJ, Algra A, Vermeulen M, van Gijn J. Calcium antagonists in patients with aneurysmal subarachnoid hemorrhage: a systematic review. Neurology 1998;50:876-83.

61. Rinkel GJ, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, van Gijn J. Calcium antagonists for aneurysmal subarachnoid haemorrhage. Cochrane database of systematic reviews (Online) 2005:CD000277.

62. Mesis RG, Wang H, Lombard FW, et al. Dissociation between vasospasm and functional improvement in a murine model of subarachnoid hemorrhage. Neurosurg Focus 2006;21:E4.

63. Korenkov AI, Pahnke J, Frei K, et al. Treatment with nimodipine or mannitol reduces programmed cell death and infarct size following focal cerebral ischemia. Neurosurg Rev 2000;23:145-50.

64. Dreier JP, Windmuller O, Petzold G, Lindauer U, Einhaupl KM, Dirnagl U. Ischemia triggered by red blood cell products in the subarachnoid space is inhibited by nimodipine administration or moderate volume

expansion/hemodilution in rats. Neurosurgery 2002;51:1457-65; discussion 65-7.

65. Roos YB, Levi M, Carroll TA, Beenen LF, Vermeulen M. Nimodipine increases fibrinolytic activity in patients with aneurysmal subarachnoid hemorrhage. Stroke; a journal of cerebral circulation 2001;32:1860-2.

66. Zubkov YN, Nikiforov BM, Shustin VA. Balloon catheter technique for dilatation of constricted cerebral arteries after aneurysmal SAH. Acta neurochirurgica 1984;70:65-79.

67. Zwienenberg-Lee M, Hartman J, Rudisill N, Muizelaar JP. Endovascular management of cerebral vasospasm. Neurosurgery 2006;59:S139-47; discussion S3-13.

68. Jestaedt L, Pham M, Bartsch AJ, et al. The impact of balloon angioplasty on the evolution of vasospasm-related infarction after aneurysmal subarachnoid hemorrhage. Neurosurgery 2008;62:610-7; discussion -7.

69. Jun P, Ko NU, English JD, et al. Endovascular treatment of medically refractory cerebral vasospasm following aneurysmal subarachnoid hemorrhage. AJNR Am J Neuroradiol 2010;31:1911-6.

70. Uhl E, Lehmberg J, Steiger HJ, Messmer K. Intraoperative detection of early microvasospasm in patients with subarachnoid hemorrhage by using orthogonal polarization spectral imaging. Neurosurgery 2003;52:1307-15; disacussion 15-7.

71. Zwienenberg-Lee M, Hartman J, Rudisill N, et al. Effect of prophylactic transluminal balloon angioplasty on cerebral vasospasm and outcome in patients with Fisher grade III subarachnoid hemorrhage: results of a phase II multicenter, randomized, clinical trial. Stroke 2008;39:1759-65.

72. Biondi A, Ricciardi GK, Puybasset L, et al. Intra-arterial nimodipine for the treatment of symptomatic cerebral vasospasm after aneurysmal subarachnoid hemorrhage: preliminary results. Ajnr 2004;25:1067-76.

73. Oran I, Cinar C. Continuous intra-arterial infusion of nimodipine during embolization of cerebral aneurysms associated with vasospasm. Ajnr 2008;29:291-5.

74. Cho WS, Kang HS, Kim JE, et al. Intra-arterial nimodipine infusion for cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. Interv Neuroradiol 2011;17:169-78.

75. Hanggi D, Turowski B, Beseoglu K, Yong M, Steiger HJ. Intra-arterial nimodipine for severe cerebral vasospasm after aneurysmal subarachnoid hemorrhage: influence on clinical course and cerebral perfusion. Ajnr 2008;29:1053-60.

76. Doukas A, Petridis AK, Barth H, Jansen O, Mehdorn HM. Continuous intra-arterial infusion of nimodipine at the onset of resistant vasospasm in aneurysmal subarachnoidal haemorrhage. Technical report. Neurol Res 2011;33:290-4.

77. Wolf S, Martin H, Landscheidt JF, Rodiek SO, Schurer L, Lumenta CB. Continuous selective intraarterial infusion of nimodipine for therapy of refractory cerebral vasospasm. Neurocrit Care 2010;12:346-51.

78. Frontera JA, Gowda A, Grilo C, et al. Recurrent vasospasm after endovascular treatment in subarachnoid hemorrhage. Acta Neurochir Suppl 2011;110:117-22.

79. Kasuya H, Onda H, Sasahara A, Takeshita M, Hori T. Application of nicardipine prolonged-release implants: analysis of 97 consecutive patients

with acute subarachnoid hemorrhage. Neurosurgery 2005;56:895-902; discussion 895-902.

80. Barth M, Capelle HH, Weidauer S, et al. Effect of nicardipine prolonged-release implants on cerebral vasospasm and clinical outcome after severe aneurysmal subarachnoid hemorrhage: a prospective, randomized, double-blind phase IIa study. Stroke; a journal of cerebral circulation 2007;38:330-6.

81. Barth M, Thome C, Schmiedek P, Weiss C, Kasuya H, Vajkoczy P. Characterization of functional outcome and quality of life following subarachnoid hemorrhage in patients treated with and without nicardipine prolonged-release implants. Journal of neurosurgery 2009;110:955-60.

82. Chou SH, Smith EE, Badjatia N, et al. A randomized, double-blind, placebo-controlled pilot study of simvastatin in aneurysmal subarachnoid hemorrhage. Stroke; a journal of cerebral circulation 2008;39:2891-3.

83. Lynch JR, Wang H, McGirt MJ, et al. Simvastatin reduces vasospasm after aneurysmal subarachnoid hemorrhage: results of a pilot randomized clinical trial. Stroke 2005;36:2024-6.

84. Tseng MY, Czosnyka M, Richards H, Pickard JD, Kirkpatrick PJ. Effects of acute treatment with pravastatin on cerebral vasospasm, autoregulation, and delayed ischemic deficits after aneurysmal subarachnoid hemorrhage: a phase II randomized placebo-controlled trial. Stroke 2005;36:1627-32.

85. Kramer AH, Gurka MJ, Nathan B, Dumont AS, Kassell NF, Bleck TP. Statin use was not associated with less vasospasm or improved outcome

after subarachnoid hemorrhage. Neurosurgery 2008;62:422-7; discussion 7-30.

86. Kerz T, Victor A, Beyer C, Trapp I, Heid F, Reisch R. A case control study of statin and magnesium administration in patients after aneurysmal subarachnoid hemorrhage: incidence of delayed cerebral ischemia and mortality. Neurological research 2008;30:893-7.

87. Vergouwen MD, Meijers JC, Geskus RB, et al. Biologic effects of simvastatin in patients with aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled randomized trial. J Cereb Blood Flow Metab 2009;29:1444-53.

88. Sillberg VA, Wells GA, Perry JJ. Do statins improve outcomes and reduce the incidence of vasospasm after aneurysmal subarachnoid hemorrhage: a meta-analysis. Stroke 2008;39:2622-6.

89. Trimble JL, Kockler DR. Statin treatment of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. The Annals of pharmacotherapy 2007;41:2019-23.

90. Vergouwen MD, de Haan RJ, Vermeulen M, Roos YB. Effect of Statin Treatment on Vasospasm, Delayed Cerebral Ischemia, and Functional Outcome in Patients With Aneurysmal Subarachnoid Hemorrhage. A Systematic Review and Meta-Analysis Update. Stroke; a journal of cerebral circulation 2009.

91. McGirt MJ, Blessing R, Alexander MJ, et al. Risk of cerebral vasopasm after subarachnoid hemorrhage reduced by statin therapy: A multivariate analysis of an institutional experience. J Neurosurg 2006;105:671-4.

92. Parra A, Kreiter KT, Williams S, et al. Effect of prior statin use on functional outcome and delayed vasospasm after acute aneurysmal subarachnoid hemorrhage: a matched controlled cohort study. Neurosurgery 2005;56:476-84; discussion -84.

93. Moskowitz SI, Ahrens C, Provencio JJ, Chow M, Rasmussen PA. Prehemorrhage statin use and the risk of vasospasm after aneurysmal subarachnoid hemorrhage. Surgical neurology 2008.

94. Singhal AB, Topcuoglu MA, Dorer DJ, Ogilvy CS, Carter BS, Koroshetz WJ. SSRI and statin use increases the risk for vasospasm after subarachnoid hemorrhage. Neurology 2005;64:1008-13.

95. van den Bergh WM, Algra A, van der Sprenkel JW, Tulleken CA, Rinkel GJ. Hypomagnesemia after aneurysmal subarachnoid hemorrhage. Neurosurgery 2003;52:276-81; discussion 81-2.

96. van den Bergh WM, Algra A, van Kooten F, et al. Magnesium sulfate in aneurysmal subarachnoid hemorrhage: a randomized controlled trial. Stroke; a journal of cerebral circulation 2005;36:1011-5.

97. Westermaier T, Stetter C, Vince GH, et al. Prophylactic intravenous magnesium sulfate for treatment of aneurysmal subarachnoid hemorrhage: a randomized, placebo-controlled, clinical study. Crit Care Med 2010;38:1284-90.

98. Dorhout Mees SM. Magnesium in aneurysmal subarachnoid hemorrhage (MASH II) phase III clinical trial MASH-II study group. Int J Stroke 2008;3:63-5.

99. Ng WH, Moochhala S, Yeo TT, Ong PL, Ng PY. Nitric oxide and subarachnoid hemorrhage: elevated level in cerebrospinal fluid and their implications. Neurosurgery 2001;49:622-6; discussion 6-7.

100. Woszczyk A, Deinsberger W, Boker DK. Nitric oxide metabolites in cisternal CSF correlate with cerebral vasospasm in patients with a subarachnoid haemorrhage. Acta neurochirurgica 2003;145:257-63; discussion 63-4.

101. Pluta RM, Oldfield EH. Analysis of nitric oxide (NO) in cerebral vasospasm after aneursymal bleeding. Reviews on recent clinical trials 2007;2:59-67.

102. Suzuki M, Asahara H, Endo S, et al. Increased levels of nitrite/nitrate in the cerebrospinal fluid of patients with subarachnoid hemorrhage. Neurosurg Rev 1999;22:96-8.

103. Raabe A, Zimmermann M, Setzer M, Vatter H, Berkefeld J, Seifert V. Effect of intraventricular sodium nitroprusside on cerebral hemodynamics and oxygenation in poor-grade aneurysm patients with severe, medically refractory vasospasm. Neurosurgery 2002;50:1006-13; discussion 13-4.

104. Thomas JE, Rosenwasser RH, Armonda RA, Harrop J, Mitchell W, Galaria I. Safety of intrathecal sodium nitroprusside for the treatment and prevention of refractory cerebral vasospasm and ischemia in humans. Stroke; a journal of cerebral circulation 1999;30:1409-16.

105. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nature medicine 2003;9:1498-505.

106. Chow M, Dumont AS, Kassell NF. Endothelin receptor antagonists and cerebral vasospasm: an update. Neurosurgery 2002;51:1333-41; discussion 42.

107. Fujimori A, Yanagisawa M, Saito A, et al. Endothelin in plasma and cerebrospinal fluid of patients with subarachnoid haemorrhage. Lancet 1990;336:633.

108. Kastner S, Oertel MF, Scharbrodt W, Krause M, Boker DK, Deinsberger W. Endothelin-1 in plasma, cisternal CSF and microdialysate following aneurysmal SAH. Acta neurochirurgica 2005;147:1271-9; discussion 9.

109. Kessler IM, Pacheco YG, Lozzi SP, de Araujo AS, Jr., Onishi FJ, de Mello PA. Endothelin-1 levels in plasma and cerebrospinal fluid of patients with cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Surgical neurology 2005;64 Suppl 1:S1:2-5; discussion S1:5.

110. Macdonald RL, Kassell NF, Mayer S, et al. Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. Stroke; a journal of cerebral circulation 2008;39:3015-21.

111. Pilitsis JG, Coplin WM, O'Regan MH, et al. Free fatty acids in human cerebrospinal fluid following subarachnoid hemorrhage and their potential role in vasospasm: a preliminary observation. J Neurosurg 2002;97:272-9.

112. Asaeda M, Sakamoto M, Kurosaki M, et al. A non-enzymatic derived arachidonyl peroxide, 8-iso-prostaglandin F2 alpha, in cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage participates in the pathogenesis of delayed cerebral vasospasm. Neurosci Lett 2005;373:222-5.

113. Jang YG, Ilodigwe D, Macdonald RL. Metaanalysis of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage. Neurocritical care 2009;10:141-7.

114. Zhang S, Wang L, Liu M, Wu B. Tirilazad for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev 2010:CD006778.

115. Kranc KR, Pyne GJ, Tao L, et al. Oxidative degradation of bilirubin produces vasoactive compounds. European journal of biochemistry / FEBS 2000;267:7094-101.

116. Clark JF, Sharp FR. Bilirubin oxidation products (BOXes) and their role in cerebral vasospasm after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2006;26:1223-33.

117. Pyne-Geithman GJ, Morgan CJ, Wagner K, et al. Bilirubin production and oxidation in CSF of patients with cerebral vasospasm after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2005;25:1070-7.

118. Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472-8.

119. Ohkuma H, Manabe H, Tanaka M, Suzuki S. Impact of cerebral microcirculatory changes on cerebral blood flow during cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 2000;31:1621-7.

120. Stein SC, Levine JM, Nagpal S, LeRoux PD. Vasospasm as the sole cause of cerebral ischemia: how strong is the evidence? Neurosurg Focus 2006;21:E2.

121. Heistad DD. What's new in the cerebral microcirculation? Landis Award lecture. Microcirculation 2001;8:365-75.

122. Rowe JG, Soper N, Ouwerkerk R, Kerr RS, Radda GK, Rajagopalan B. Delayed ischaemia after subarachnoid haemorrhage: a role for small vessel changes. J Neurol Neurosurg Psychiatry 1995;59:451-2.

123. Weidauer S, Vatter H, Beck J, et al. Focal laminar cortical infarcts following aneurysmal subarachnoid haemorrhage. Neuroradiology 2008;50:1-8.

124. Rabinstein AA, Friedman JA, Weigand SD, et al. Predictors of cerebral infarction in aneurysmal subarachnoid hemorrhage. Stroke 2004;35:1862-6.

125. Neil-Dwyer G, Lang DA, Doshi B, Gerber CJ, Smith PW. Delayed cerebral ischaemia: the pathological substrate. Acta neurochirurgica 1994;131:137-45.

126. Stoltenberg-Didinger G. Neuropathology of subarachnoid hemorrhage, in Bederson JB (ed): Subarachnoid Hemorrhage: Pathophysiology and Management. Park Ridge, IL1997.

127. Yundt KD, Grubb RL, Jr., Diringer MN, Powers WJ. Autoregulatory vasodilation of parenchymal vessels is impaired during cerebral vasospasm. J Cereb Blood Flow Metab 1998;18:419-24.

128. Jaeger M, Schuhmann MU, Soehle M, Nagel C, Meixensberger J. Continuous monitoring of cerebrovascular autoregulation after subarachnoid hemorrhage by brain tissue oxygen pressure reactivity and its relation to delayed cerebral infarction. Stroke 2007;38:981-6.

129. Lang EW, Diehl RR, Mehdorn HM. Cerebral autoregulation testing after aneurysmal subarachnoid hemorrhage: the phase relationship between

arterial blood pressure and cerebral blood flow velocity. Critical care medicine 2001;29:158-63.

130. Soehle M, Czosnyka M, Pickard JD, Kirkpatrick PJ. Continuous assessment of cerebral autoregulation in subarachnoid hemorrhage. Anesthesia and analgesia 2004;98:1133-9, table of contents.

131. Minhas PS, Menon DK, Smielewski P, et al. Positron emission tomographic cerebral perfusion disturbances and transcranial Doppler findings among patients with neurological deterioration after subarachnoid hemorrhage. Neurosurgery 2003;52:1017-22; discussion 22-4.

132. Akopov S, Sercombe R, Seylaz J. Cerebrovascular reactivity: role of endothelium/platelet/leukocyte interactions. Cerebrovascular and brain metabolism reviews 1996;8:11-94.

133. del Zoppo GJ. Microvascular responses to cerebral ischemia/inflammation. Annals of the New York Academy of Sciences 1997;823:132-47.

134. Hirashima Y, Hamada H, Kurimoto M, Origasa H, Endo S. Decrease in platelet count as an independent risk factor for symptomatic vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2005;102:882-7.

135. Schebesch KM, Woertgen C, Brawanski A, Rothoerl RD. A study of possible correlation between subarachnoid haemorrhage related vasospasm and the post-bleed blood platelet count chart in a Caucasian population. Acta neurochirurgica 2007;149:387-91.

136. Dorhout Mees SM, van den Bergh WM, Algra A, Rinkel GJ. Antiplatelet Therapy in Aneurysmal Subarachnoid Hemorrhage. Stroke 2008.

137. Ilveskero S, Juvela S, Siironen J, Lassila R. D-dimer predicts outcome after aneurysmal subarachnoid hemorrhage: no effect of thromboprophylaxis on coagulation activity. Neurosurgery 2005;57:16-24; discussion 16-24.

138. Juvela S, Siironen J. D-dimer as an independent predictor for poor outcome after aneurysmal subarachnoid hemorrhage. Stroke 2006;37:1451-6.

139. Peltonen S, Juvela S, Kaste M, Lassila R. Hemostasis and fibrinolysis activation after subarachnoid hemorrhage. J Neurosurg 1997;87:207-14.

140. Nina P, Schisano G, Chiappetta F, et al. A study of blood coagulation and fibrinolytic system in spontaneous subarachnoid hemorrhage. Correlation with hunt-hess grade and outcome. Surgical neurology 2001;55:197-203.

141. Fujii Y, Takeuchi S, Sasaki O, Minakawa T, Koike T, Tanaka R. Serial changes of hemostasis in aneurysmal subarachnoid hemorrhage with special reference to delayed ischemic neurological deficits. J Neurosurg 1997;86:594-602.

142. Kasuya H, Shimizu T, Takakura K. Thrombin activity in CSF after SAH is correlated with the degree of SAH the persistence of subarachnoid clot and the development of vasospasm. Acta neurochirurgica 1998;140:579-84.

143. Park S, Yamaguchi M, Zhou C, Calvert JW, Tang J, Zhang JH. Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. Stroke 2004;35:2412-7.

144. Cahill J, Calvert JW, Solaroglu I, Zhang JH. Vasospasm and p53-induced apoptosis in an experimental model of subarachnoid hemorrhage. Stroke 2006;37:1868-74.

145. Gao C, Liu X, Liu W, et al. Anti-apoptotic and neuroprotective effects of Tetramethylpyrazine following subarachnoid hemorrhage in rats. Auton Neurosci 2008;141:22-30.

146. Iseda K, Ono S, Onoda K, et al. Antivasospastic and antiinflammatory effects of caspase inhibitor in experimental subarachnoid hemorrhage. J Neurosurg 2007;107:128-35.

147. Zhou C, Yamaguchi M, Colohan AR, Zhang JH. Role of p53 and apoptosis in cerebral vasospasm after experimental subarachnoid hemorrhage. J Cereb Blood Flow Metab 2005;25:572-82.

148. Leao A. Spreading Depression of Activity in the Cerebral Cortex. J Neurophysiol 1944;7:359-90.

149. Dreier JP, Major S, Manning A, et al. Cortical spreading ischaemia is a novel process involved in ischaemic damage in patients with aneurysmal subarachnoid haemorrhage. Brain 2009;132:1866-81.

150. Shin HK, Dunn AK, Jones PB, Boas DA, Moskowitz MA, Ayata C. Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. J Cereb Blood Flow Metab 2006;26:1018-30.

151. Dreier JP, Ebert N, Priller J, et al. Products of hemolysis in the subarachnoid space inducing spreading ischemia in the cortex and focal necrosis in rats: a model for delayed ischemic neurological deficits after subarachnoid hemorrhage? J Neurosurg 2000;93:658-66.

152. Dreier JP, Korner K, Ebert N, et al. Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by N-nitro-L-arginine induces cortical spreading ischemia when K+ is increased in the subarachnoid space. J Cereb Blood Flow Metab 1998;18:978-90.

153. Dreier JP, Woitzik J, Fabricius M, et al. Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations. Brain 2006;129:3224-37.

154. Hijdra A, Van Gijn J, Stefanko S, Van Dongen KJ, Vermeulen M, Van Crevel H. Delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: clinicoanatomic correlations. Neurology 1986;36:329-33.

155. Kassell NF, Torner JC, Haley EC, Jr., Jane JA, Adams HP, Kongable GL. The International Cooperative Study on the Timing of Aneurysm Surgery. Part 1: Overall management results. J Neurosurg 1990;73:18-36.

156. Klimo P, Jr., Kestle JR, MacDonald JD, Schmidt RH. Marked reduction of cerebral vasospasm with lumbar drainage of cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 2004;100:215-24.

157. MacDonald RL. Randomized Trial of Clazosentan for Prevention of Vasosopasm After
Aneurysmal Subarachnoid Hemorrhage. Stroke 2007;38:462.

158. Haley EC, Jr., Kassell NF, Apperson-Hansen C, Maile MH, Alves WM. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in North America. J Neurosurg 1997;86:467-74.

159. Al-Tamimi YZ, Orsi NM, Quinn AC, Homer-Vanniasinkam S, Ross SA. A review of delayed ischemic neurologic deficit following aneurysmal subarachnoid hemorrhage: historical overview, current treatment, and pathophysiology. World neurosurgery;73:654-67.

160. Friedman JA, Goerss SJ, Meyer FB, et al. Volumetric quantification of Fisher Grade 3 aneurysmal subarachnoid hemorrhage: a novel method to

predict symptomatic vasospasm on admission computerized tomography scans. J Neurosurg 2002;97:401-7.

161. Hijdra A, van Gijn J, Nagelkerke NJ, Vermeulen M, van Crevel H. Prediction of delayed cerebral ischemia, rebleeding, and outcome after aneurysmal subarachnoid hemorrhage. Stroke; a journal of cerebral circulation 1988;19:1250-6.

162. Kistler JP, Crowell RM, Davis KR, et al. The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: a prospective study. Neurology 1983;33:424-36.

163. Kawakami Y, Shimamura Y. Cisternal drainage after early operation of ruptured intracranial aneurysm. Neurosurgery 1987;20:8-14.

164. Findlay JM, Weir BK, Kassell NF, Disney LB, Grace MG. Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. Journal of neurosurgery 1991;75:181-8.

165. Hanggi D, Liersch J, Turowski B, Yong M, Steiger HJ. The effect of lumboventricular lavage and simultaneous low-frequency head-motion therapy after severe subarachnoid hemorrhage: results of a single center prospective Phase II trial. Journal of neurosurgery 2008;108:1192-9.

166. Kraemer JL, Gobbato PL, Andrade-Souza YM. Third ventriculostomy through the lamina terminalis for intracranial pressure monitoring after aneurysm surgery: technical note. Arq Neuropsiquiatr 2002;60:932-4.

167. Sato J, Sato O, Kamitani H, Kanazawa I, Kokunai T. [Intracranial surgery and postoperative management of patients with ruptured aneurysm in acute and subacute stage--basal cisternal drainage and lumbar subarachnoid drainage (author's transl)]. Neurol Med Chir (Tokyo) 1979;19:173-9.

168. Kasuya H, Shimizu T, Kagawa M. The effect of continuous drainage of cerebrospinal fluid in patients with subarachnoid hemorrhage: a retrospective analysis of 108 patients. Neurosurgery 1991;28:56-9.

169. Yamada K, Yoshimura S, Enomoto Y, Yamakawa H, Iwama T. Effectiveness of combining continuous cerebrospinal drainage and intermittent intrathecal urokinase injection therapy in preventing symptomatic vasospasm following aneurysmal subarachnoid haemorrhage. British journal of neurosurgery 2008;22:649-53.

170. Usui M, Saito N, Hoya K, Todo T. Vasospasm prevention with postoperative intrathecal thrombolytic therapy: a retrospective comparison of urokinase, tissue plasminogen activator, and cisternal drainage alone. Neurosurgery 1994;34:235-44; discussion 44-5.

171. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage. Outcome in 217 patients. Surgical neurology 2000;53:110-7; discussion 7-8.

172. Inagawa T, Kamiya K, Matsuda Y. Effect of continuous cisternal drainage on cerebral vasospasm. Acta neurochirurgica 1991;112:28-36.

173. Amin-Hanjani S, Ogilvy CS, Barker FG, 2nd. Does intracisternal thrombolysis prevent vasospasm after aneurysmal subarachnoid hemorrhage? A meta-analysis. Neurosurgery 2004;54:326-34; discussion 34-5.

174. Kwon OY, Kim YJ, Kim YJ, Cho CS, Lee SK, Cho MK. The Utility and Benefits of External Lumbar CSF Drainage after Endovascular Coiling on

Aneurysmal Subarachnoid Hemorrhage. Journal of Korean Neurosurgical Society 2008;43:281-7.

175. Selman WR. Vasospasm. J Neurosurg 2004;100:208-9; discussion 9.

176. Keyrouz SG, Diringer MN. Clinical review: Prevention and therapy of vasospasm in subarachnoid hemorrhage. Crit Care 2007;11:220.

177. Vergouwen MD, Vermeulen M, van Gijn J, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. Stroke; a journal of cerebral circulation 2010;41:2391-5.

178. Wilson JT, Hareendran A, Grant M, et al. Improving the assessment of outcomes in stroke: use of a structured interview to assign grades on the modified Rankin Scale. Stroke 2002;33:2243-6.

179. Ochiai H, Yamakawa Y. Continuous lumbar drainage for the preoperative management of patients with aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 2001;41:576-80; discussion 81.

180. Heuer GG, Smith MJ, Elliott JP, Winn HR, LeRoux PD. Relationship between intracranial pressure and other clinical variables in patients with aneurysmal subarachnoid hemorrhage. Journal of neurosurgery 2004;101:408-16.

181. Auer LM, Mokry M. Disturbed cerebrospinal fluid circulation after subarachnoid hemorrhage and acute aneurysm surgery. Neurosurgery 1990;26:804-8; discussion 8-9.

182. Heinsoo M, Eelmae J, Kuklane M, Tomberg T, Tikk A, Asser T. The possible role of CSF hydrodynamic parameters following in management of SAH patients. Acta neurochirurgica 1998;71:13-5.

183. Gambardella G, De Blasi F, Caruso G, Zema A, Turiano F, Collufio D. Intracranial pressure, cerebral perfusion pressure, and SPECT in the management of patients with SAH Hunt and Hess grades I-II. Acta neurochirurgica 1998;71:215-8.

184. Le Roux PD, Elliot JP, Newell DW, Grady MS, Winn HR. The incidence of surgical complications is similar in good and poor grade patients undergoing repair of ruptured anterior circulation aneurysms: a retrospective review of 355 patients. Neurosurgery 1996;38:887-93; discussion 93-5.

185. Fukuhara T, Douville CM, Eliott JP, Newell DW, Winn HR. Relationship between intracranial pressure and the development of vasospasm after aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1998;38:710-5; discussion 6-7.

186. Kapadia FN, Jha AN. Simultaneous lumbar and intraventricular manometry to evaluate the role and safety of lumbar puncture in raised intracranial pressure following subarachnoid haemorrhage. British journal of neurosurgery 1996;10:585-7.

187. Tuettenberg J, Czabanka M, Horn P, et al. Clinical evaluation of the safety and efficacy of lumbar cerebrospinal fluid drainage for the treatment of refractory increased intracranial pressure. Journal of neurosurgery 2009;110:1200-8.

188. Bloch J, Regli L. Brain stem and cerebellar dysfunction after lumbar spinal fluid drainage: case report. Journal of neurology, neurosurgery, and psychiatry 2003;74:992-4.

189. Fountas KN, Kapsalaki EZ, Machinis T, Karampelas I, Smisson HF, Robinson JS. Review of the literature regarding the relationship of rebleeding

and external ventricular drainage in patients with subarachnoid hemorrhage of aneurysmal origin. Neurosurgical review 2006;29:14-8; discussion 9-20.

190. Ruijs AC, Dirven CM, Algra A, Beijer I, Vandertop WP, Rinkel G. The risk of rebleeding after external lumbar drainage in patients with untreated ruptured cerebral aneurysms. Acta neurochirurgica 2005;147:1157-61; discussion 61-2.

191. Grady RE, Horlocker TT, Brown RD, Maxson PM, Schroeder DR. Neurologic complications after placement of cerebrospinal fluid drainage catheters and needles in anesthetized patients: implications for regional anesthesia. Mayo Perioperative Outcomes Group. Anesthesia and analgesia 1999;88:388-92.

192. Coplin WM, Avellino AM, Kim DK, Winn HR, Grady MS. Bacterial meningitis associated with lumbar drains: a retrospective cohort study. Journal of neurology, neurosurgery, and psychiatry 1999;67:468-73.

193. Ohwaki K, Yano E, Nakagomi T, Tamura A. Relationship between shunt-dependent hydrocephalus after subarachnoid haemorrhage and duration of cerebrospinal fluid drainage. British journal of neurosurgery 2004;18:130-4.

194. Upton ML, Weller RO. The morphology of cerebrospinal fluid drainage pathways in human arachnoid granulations. Journal of neurosurgery 1985;63:867-75.

195. Rinkel GJ, Wijdicks EF, Vermeulen M, Tans JT, Hasan D, van Gijn J. Acute hydrocephalus in nonaneurysmal perimesencephalic hemorrhage: evidence of CSF block at the tentorial hiatus. Neurology 1992;42:1805-7.

196. Kreiter KT, Mayer SA, Howard G, et al. Sample size estimates for clinical trials of vasospasm in subarachnoid hemorrhage. Stroke; a journal of cerebral circulation 2009;40:2362-7.

197. Dumont AS, Dumont RJ, Chow MM, et al. Cerebral vasospasm after subarachnoid hemorrhage: putative role of inflammation. Neurosurgery 2003;53:123-33; discussion 33-5.

198. Baird AE. The forgotten lymphocyte: immunity and stroke. Circulation 2006;113:2035-6.

199. Dumont AS, Kassell NF. Vasospasm. J Neurosurg 2005;103:1-3; discussion

200. Hutchinson PJ, O'Connell MT, Rothwell NJ, et al. Inflammation in human brain injury: intracerebral concentrations of IL-1alpha, IL-1beta, and their endogenous inhibitor IL-1ra. J Neurotrauma 2007;24:1545-57.

201. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. British journal of pharmacology 2006;147 Suppl 1:S232-40.

202. Medawar P. Immunity to homologous grafted skin: Part III—The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 1948;29:58-69.

203. Orsi NM. Cytokine networks in the establishment and maintenance of pregnancy. Hum Fertil (Camb) 2008;11:222-30.

204. Clatterbuck RE, Oshiro EM, Hoffman PA, Dietsch GN, Pardoll DM, Tamargo RJ. Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. J Neurosurg 2002;97:676-82.

205. Argaw AT, Zhang Y, Snyder BJ, et al. IL-1beta regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. J Immunol 2006;177:5574-84.

206. Chapman KZ, Dale VQ, Denes A, et al. A rapid and transient peripheral inflammatory response precedes brain inflammation after experimental stroke. J Cereb Blood Flow Metab 2009;29:1764-8.

207. Chuaqui R, Tapia J. Histologic assessment of the age of recent brain infarcts in man. J Neuropathol Exp Neurol 1993;52:481-9.

208. Grau AJ, Sigmund R, Hacke W. Modification of platelet aggregation by leukocytes in acute ischemic stroke. Stroke 1994;25:2149-52.

209. Wang WZ, Olsson T, Kostulas V, Hojeberg B, Ekre HP, Link H. Myelin antigen reactive T cells in cerebrovascular diseases. Clin Exp Immunol 1992;88:157-62.

210. Kao TK, Ou YC, Kuo JS, et al. Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. Neurochem Int 2006;48:166-76.

211. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. Nat Rev Immunol 2005;5:629-40.

212. Azzimondi G, Bassein L, Nonino F, et al. Fever in acute stroke worsens prognosis. A prospective study. Stroke 1995;26:2040-3.

213. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. Nat Rev Neurosci 2001;2:734-44.

214. Sozen T, Tsuchiyama R, Hasegawa Y, et al. Role of interleukin-1beta in early brain injury after subarachnoid hemorrhage in mice. Stroke 2009;40:2519-25.

215. Jedrzejowska-Szypulka H, Straszak G, Larysz-Brysz M, et al. Interleukin-1beta plays a role in the activation of peripheral leukocytes after blood-brain barrier rupture in the course of subarachnoid hemorrhage. Curr Neurovasc Res 2010;7:39-48.

216. Castellanos M, Leira R, Serena J, et al. Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. Stroke; a journal of cerebral circulation 2003;34:40-6.

217. Castillo J, Rodriguez I. Biochemical changes and inflammatory response as markers for brain ischaemia: molecular markers of diagnostic utility and prognosis in human clinical practice. Cerebrovasc Dis 2004;17 Suppl 1:7-18.

218. Sironi L, Banfi C, Brioschi M, et al. Activation of NF-kB and ERK1/2 after permanent focal ischemia is abolished by simvastatin treatment. Neurobiol Dis 2006;22:445-51.

219. Schoch B, Regel JP, Wichert M, Gasser T, Volbracht L, Stolke D. Analysis of intrathecal interleukin-6 as a potential predictive factor for vasospasm in subarachnoid hemorrhage. Neurosurgery 2007;60:828-36; discussion -36.

220. Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. Curr Opin Crit Care 2002;8:101-5.

221. Dziedzic T, Bartus S, Klimkowicz A, Motyl M, Slowik A, Szczudlik A. Intracerebral hemorrhage triggers interleukin-6 and interleukin-10 release in blood. Stroke; a journal of cerebral circulation 2002;33:2334-5.

222. Waje-Andreassen U, Krakenes J, Ulvestad E, et al. IL-6: an early marker for outcome in acute ischemic stroke. Acta Neurol Scand 2005;111:360-5.

223. Oto J, Suzue A, Inui D, et al. Plasma proinflammatory and anti-inflammatory cytokine and catecholamine concentrations as predictors of neurological outcome in acute stroke patients. Journal of anesthesia 2008;22:207-12.

224. Worthmann H, Tryc AB, Goldbecker A, et al. The temporal profile of inflammatory markers and mediators in blood after acute ischemic stroke differs depending on stroke outcome. Cerebrovasc Dis 2010;30:85-92.

225. Tarkowski E, Rosengren L, Blomstrand C, et al. Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. Stroke 1995;26:1393-8.

226. Welsh P, Lowe GD, Chalmers J, et al. Associations of proinflammatory cytokines with the risk of recurrent stroke. Stroke; a journal of cerebral circulation 2008;39:2226-30.

227. Chiaretti A, Genovese O, Aloe L, et al. Interleukin 1beta and interleukin 6 relationship with paediatric head trauma severity and outcome. Childs Nerv Syst 2005;21:185-93; discussion 94.

228. Hayakata T, Shiozaki T, Tasaki O, et al. Changes in CSF S100B and cytokine concentrations in early-phase severe traumatic brain injury. Shock 2004;22:102-7.

229. Kossmann T, Hans VH, Imhof HG, et al. Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. Shock 1995;4:311-7.

230. Winter CD, Pringle AK, Clough GF, Church MK. Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury. Brain 2004;127:315-20.

231. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest 2000;80:617-53.

232. Shimakura A, Kamanaka Y, Ikeda Y, Kondo K, Suzuki Y, Umemura K. Neutrophil elastase inhibition reduces cerebral ischemic damage in the middle cerebral artery occlusion. Brain Res 2000;858:55-60.

233. Matsuo Y, Onodera H, Shiga Y, et al. Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. Stroke; a journal of cerebral circulation 1994;25:1469-75.

234. Garau A, Bertini R, Colotta F, et al. Neuroprotection with the CXCL8 inhibitor repertaxin in transient brain ischemia. Cytokine 2005;30:125-31.

235. Villa P, Triulzi S, Cavalieri B, et al. The interleukin-8 (IL-8/CXCL8) receptor inhibitor reparixin improves neurological deficits and reduces long-term inflammation in permanent and transient cerebral ischemia in rats. Molecular medicine (Cambridge, Mass 2007;13:125-33.

236. Al-Bahrani A, Taha S, Shaath H, Bakhiet M. TNF-alpha and IL-8 in acute stroke and the modulation of these cytokines by antiplatelet agents. Curr Neurovasc Res 2007;4:31-7.

237. Grau AJ, Reis A, Buggle F, et al. Monocyte function and plasma levels of interleukin-8 in acute ischemic stroke. J Neurol Sci 2001;192:41-7.

238. Kostulas N, Kivisakk P, Huang Y, Matusevicius D, Kostulas V, Link H. Ischemic stroke is associated with a systemic increase of blood mononuclear cells expressing interleukin-8 mRNA. Stroke 1998;29:462-6.

239. Wang Y, Zhong M, Tan XX, et al. Expression change of interleukin-8 gene in rabbit basilar artery after subarachnoid hemorrhage. Neuroscience bulletin 2007;23:151-5.

240. Di Sabatino A, Calarota SA, Vidali F, Macdonald TT, Corazza GR. Role of IL-15 in immune-mediated and infectious diseases. Cytokine Growth Factor Rev 2011;22:19-33.

241. Kivisakk P, Matusevicius D, He B, Soderstrom M, Fredrikson S, Link H. IL-15 mRNA expression is up-regulated in blood and cerebrospinal fluid mononuclear cells in multiple sclerosis (MS). Clin Exp Immunol 1998;111:193-7.

242. Baranzini SE, Elfstrom C, Chang SY, et al. Transcriptional analysis of multiple sclerosis brain lesions reveals a complex pattern of cytokine expression. J Immunol 2000;165:6576-82.

243. Rentzos M, Cambouri C, Rombos A, et al. IL-15 is elevated in serum and cerebrospinal fluid of patients with multiple sclerosis. J Neurol Sci 2006;241:25-9.

244. Maslinska D, Laure-Kamionowska M, Kaliszek A, Makarewicz D. Proinflammatory cytokines in injured rat brain following perinatal asphyxia. Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences 2002;40:177-82.

245. Huang SH, Frydas S, Kempuraj D, et al. Interleukin-17 and the interleukin-17 family member network. Allergy Asthma Proc 2004;25:17-21.

246. Witowski J, Ksiazek K, Jorres A. Interleukin-17: a mediator of inflammatory responses. Cell Mol Life Sci 2004;61:567-79.

247. Kebir H, Kreymborg K, Ifergan I, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nature medicine 2007;13:1173-5.

248. Lv M, Liu Y, Zhang J, et al. Roles of inflammation response in microglia cell through Toll-like receptors 2/interleukin-23/interleukin-17 pathway in cerebral ischemia/reperfusion injury. Neuroscience 2011;176:162-72.

249. Shichita T, Sugiyama Y, Ooboshi H, et al. Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. Nat Med 2009;15:946-50.

250. Li GZ, Zhong D, Yang LM, et al. Expression of interleukin-17 in ischemic brain tissue. Scand J Immunol 2005;62:481-6.

251. Wang DD, Zhao YF, Wang GY, et al. IL-17 potentiates neuronal injury induced by oxygen-glucose deprivation and affects neuronal IL-17 receptor expression. J Neuroimmunol 2009;212:17-25.

252. Fukui O, Kinugasa Y, Fukuda A, et al. Post-ischemic hypothermia reduced IL-18 expression and suppressed microglial activation in the immature brain. Brain Res 2006;1121:35-45.

253. Menge T, Jander S, Stoll G. Induction of the proinflammatory cytokine interleukin-18 by axonal injury. J Neurosci Res 2001;65:332-9.

254. Schmidt OI, Morganti-Kossmann MC, Heyde CE, et al. Tumor necrosis factor-mediated inhibition of interleukin-18 in the brain: a clinical and experimental study in head-injured patients and in a murine model of closed head injury. J Neuroinflammation 2004;1:13.

255. Yatsiv I, Morganti-Kossmann MC, Perez D, et al. Elevated intracranial IL-18 in humans and mice after traumatic brain injury and evidence of neuroprotective effects of IL-18-binding protein after experimental closed head injury. J Cereb Blood Flow Metab 2002;22:971-8.

256. Wheeler RD, Boutin H, Touzani O, Luheshi GN, Takeda K, Rothwell NJ. No role for interleukin-18 in acute murine stroke-induced brain injury. J Cereb Blood Flow Metab 2003;23:531-5.

257. Jander S, Schroeter M, Stoll G. Interleukin-18 expression after focal ischemia of the rat brain: association with the late-stage inflammatory response. J Cereb Blood Flow Metab 2002;22:62-70.

258. Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H. Interleukin-18 involvement in hypoxic-ischemic brain injury. J Neurosci 2002;22:5910-9.

259. Yuen CM, Chiu CA, Chang LT, et al. Level and value of interleukin-18 after acute ischemic stroke. Circ J 2007;71:1691-6.

260. Zhang N, Yu JT, Yu NN, et al. Interleukin-18 promoter polymorphisms and risk of ischemic stroke. Brain Res Bull 2010;81:590-4.

261. Haile WB, Echeverry R, Wu J, Yepes M. The interaction between tumor necrosis factor-like weak inducer of apoptosis and its receptor fibroblast growth factor-inducible 14 promotes the recruitment of neutrophils into the ischemic brain. J Cereb Blood Flow Metab 2010;30:1147-56.

262. Kim GH, Kellner CP, Hahn DK, et al. Monocyte chemoattractant protein-1 predicts outcome and vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2008;109:38-43.

263. Minami M, Satoh M. Chemokines and their receptors in the brain: pathophysiological roles in ischemic brain injury. Life Sci 2003;74:321-7.

264. Kim JS, Gautam SC, Chopp M, et al. Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. J Neuroimmunol 1995;56:127-34.

265. Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. J Cereb Blood Flow Metab 2002;22:308-17.

266. Schilling M, Strecker JK, Schabitz WR, Ringelstein EB, Kiefer R. Effects of monocyte chemoattractant protein 1 on blood-borne cell recruitment after transient focal cerebral ischemia in mice. Neuroscience 2009;161:806-12.

267. Kumai Y, Ooboshi H, Takada J, et al. Anti-monocyte chemoattractant protein-1 gene therapy protects against focal brain ischemia in hypertensive rats. J Cereb Blood Flow Metab 2004;24:1359-68.

268. Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. J Cereb Blood Flow Metab 2007;27:1213-24.

269. Chen Y, Hallenbeck JM, Ruetzler C, et al. Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. J Cereb Blood Flow Metab 2003;23:748-55.

270. Losy J, Zaremba J. Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. Stroke; a journal of cerebral circulation 2001;32:2695-6.

271. Onda H, Kasuya H, Takakura K, et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J Cereb Blood Flow Metab 1999;19:1279-88.

272. Lu H, Shi JX, Chen HL, Hang CH, Wang HD, Yin HX. Expression of monocyte chemoattractant protein-1 in the cerebral artery after experimental subarachnoid hemorrhage. Brain Res 2009;1262:73-80.

273. Wang Z, Zuo G, Shi XY, Zhang J, Fang Q, Chen G. Progesterone administration modulates cortical TLR4/NF-kappaB signaling pathway after subarachnoid hemorrhage in male rats. Mediators Inflamm 2011;2011:848309.

274. Wirrig C, Hunter I, Mathieson FA, Nixon GF. Sphingosylphosphorylcholine is a proinflammatory mediator in cerebral arteries. J Cereb Blood Flow Metab 2011;31:212-21.

275. Morganti-Kossmann MC, Rancan M, Otto VI, Stahel PF, Kossmann T. Role of cerebral inflammation after traumatic brain injury: a revisited concept. Shock 2001;16:165-77.

276. Tomimoto H, Akiguchi I, Wakita H, et al. Glial expression of cytokines in the brains of cerebrovascular disease patients. Acta Neuropathol 1996;92:281-7.

277. Zaremba J, Skrobanski P, Losy J. Tumour necrosis factor-alpha is increased in the cerebrospinal fluid and serum of ischaemic stroke patients

and correlates with the volume of evolving brain infarct. Biomed Pharmacother 2001;55:258-63.

278. Croll SD, Goodman JH, Scharfman HE. Vascular endothelial growth factor (VEGF) in seizures: a double-edged sword. Adv Exp Med Biol 2004;548:57-68.

279. Ogunshola OO, Antic A, Donoghue MJ, et al. Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. J Biol Chem 2002;277:11410-5.

280. Skold MK, Risling M, Holmin S. Inhibition of vascular endothelial growth factor receptor 2 activity in experimental brain contusions aggravates injury outcome and leads to early increased neuronal and glial degeneration. Eur J Neurosci 2006;23:21-34.

281. Chow J, Ogunshola O, Fan SY, Li Y, Ment LR, Madri JA. Astrocyte-derived VEGF mediates survival and tube stabilization of hypoxic brain microvascular endothelial cells in vitro. Brain Res Dev Brain Res 2001;130:123-32.

282. Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH. Signaling pathways for early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2004;24:916-25.

283. Vila N, Castillo J, Davalos A, Esteve A, Planas AM, Chamorro A. Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. Stroke 2003;34:671-5.

284. Pelidou SH, Kostulas N, Matusevicius D, Kivisakk P, Kostulas V, Link H. High levels of IL-10 secreting cells are present in blood in cerebrovascular diseases. Eur J Neurol 1999;6:437-42.

285. Katsuno M, Yokota H, Yamamoto Y, Teramoto A. Increased regional interleukin-4 during the acute stage of severe intracranial disorders. Neurol Med Chir (Tokyo) 2006;46:471-4; discussion 4-5.

286. Saud K, Herrera-Molina R, Von Bernhardi R. Pro- and anti-inflammatory cytokines regulate the ERK pathway: implication of the timing for the activation of microglial cells. Neurotoxicity research 2005;8:277-87.

287. Perini F, Morra M, Alecci M, Galloni E, Marchi M, Toso V. Temporal profile of serum anti-inflammatory and pro-inflammatory interleukins in acute ischemic stroke patients. Neurol Sci 2001;22:289-96.

288. Bachis A, Colangelo AM, Vicini S, et al. Interleukin-10 prevents glutamate-mediated cerebellar granule cell death by blocking caspase-3-like activity. J Neurosci 2001;21:3104-12.

289. Frenkel D, Huang Z, Maron R, et al. Nasal vaccination with myelin oligodendrocyte glycoprotein reduces stroke size by inducing IL-10-producing CD4+ T cells. J Immunol 2003;171:6549-55.

290. de Bilbao F, Arsenijevic D, Moll T, et al. In vivo over-expression of interleukin-10 increases resistance to focal brain ischemia in mice. Journal of neurochemistry 2009;110:12-22.

291. Londono D, Carvajal J, Arguelles-Grande C, Marques A, Cadavid D. Interleukin 10 protects the brain microcirculation from spirochetal injury. J Neuropathol Exp Neurol 2008;67:976-83.

292. Ooboshi H, Ibayashi S, Shichita T, et al. Postischemic gene transfer of interleukin-10 protects against both focal and global brain ischemia. Circulation 2005;111:913-9.

293. Nayak AR, Kashyap RS, Purohit HJ, Kabra D, Taori GM, Daginawala HF. Evaluation of the inflammatory response in sera from acute ischemic stroke patients by measurement of IL-2 and IL-10. Inflamm Res 2009;58:687-91.

294. Chang LT, Yuen CM, Liou CW, et al. Link between interleukin-10 level and outcome after ischemic stroke. Neuroimmunomodulation;17:223-8.

295. van Exel E, Gussekloo J, de Craen AJ, Bootsma-van der Wiel A, Frolich M, Westendorp RG. Inflammation and stroke: the Leiden 85-Plus Study. Stroke 2002;33:1135-8.

296. Munshi A, Rajeshwar K, Kaul S, et al. Interleukin-10-1082 promoter polymorphism and ischemic stroke risk in a South Indian population. Cytokine 2010;52:221-4.

297. Chamorro A, Amaro S, Vargas M, et al. Interleukin 10, monocytes and increased risk of early infection in ischaemic stroke. J Neurol Neurosurg Psychiatry 2006;77:1279-81.

298. Cyktor JC, Turner J. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. Infect Immun 2011;79:2964-73.

299. Fassbender K, Hodapp B, Rossol S, et al. Inflammatory cytokines in subarachnoid haemorrhage: association with abnormal blood flow velocities in basal cerebral arteries. J Neurol Neurosurg Psychiatry 2001;70:534-7.

300. Gaetani P, Tartara F, Pignatti P, Tancioni F, Rodriguez y Baena R, De Benedetti F. Cisternal CSF levels of cytokines after subarachnoid hemorrhage. Neurological research 1998;20:337-42.

301. Kikuchi T, Okuda Y, Kaito N, Abe T. Cytokine production in cerebrospinal fluid after subarachnoid haemorrhage. Neurological research 1995;17:106-8.

302. Kwon KY, Jeon BC. Cytokine levels in cerebrospinal fluid and delayed ischemic deficits in patients with aneurysmal subarachnoid hemorrhage. J Korean Med Sci 2001;16:774-80.

303. Mathiesen T, Andersson B, Loftenius A, von Holst H. Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage. J Neurosurg 1993;78:562-7.

304. Mathiesen T, Edner G, Ulfarsson E, Andersson B. Cerebrospinal fluid interleukin-1 receptor antagonist and tumor necrosis factor-alpha following subarachnoid hemorrhage. J Neurosurg 1997;87:215-20.

305. Osuka K, Suzuki Y, Tanazawa T, et al. Interleukin-6 and development of vasospasm after subarachnoid haemorrhage. Acta neurochirurgica 1998;140:943-51.

306. Hendryk S, Jarzab B, Josko J. Increase of the IL-1 beta and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH. Neuro Endocrinol Lett 2004;25:141-7.

307. Killer M, Arthur A, Al-Schameri AR, et al. Cytokine and growth factor concentration in cerebrospinal fluid from patients with hydrocephalus following endovascular embolization of unruptured aneurysms in comparison with other types of hydrocephalus. Neurochem Res 2010;35:1652-8.

308. Muroi C, Frei K, El Beltagy M, Cesnulis E, Yonekawa Y, Keller E. Combined therapeutic hypothermia and barbiturate coma reduces interleukin-

6 in the cerebrospinal fluid after aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol 2008;20:193-8.

309. Mellergard P, Aneman O, Sjogren F, Saberg C, Hillman J. Differences in cerebral extracellular response of interleukin-1beta, interleukin-6, and interleukin-10 after subarachnoid hemorrhage or severe head trauma in humans. Neurosurgery 2011;68:12-9; discussion 9.

310. Nakahara T, Tsuruta R, Kaneko T, et al. High-mobility group box 1 protein in CSF of patients with subarachnoid hemorrhage. Neurocritical care 2009;11:362-8.

311. Ni W, Gu YX, Song DL, Leng B, Li PL, Mao Y. The relationship between IL-6 in CSF and occurrence of vasospasm after subarachnoid hemorrhage. Acta Neurochir Suppl 2011;110:203-8.

312. Sarrafzadeh A, Schlenk F, Gericke C, Vajkoczy P. Relevance of cerebral interleukin-6 after aneurysmal subarachnoid hemorrhage. Neurocrit Care 2010;13:339-46.

313. Graetz D, Nagel A, Schlenk F, Sakowitz O, Vajkoczy P, Sarrafzadeh A. High ICP as trigger of proinflammatory IL-6 cytokine activation in aneurysmal subarachnoid hemorrhage. Neurol Res 2010;32:728-35.

314. Hillman J, Aneman O, Persson M, Andersson C, Dabrosin C, Mellergard P. Variations in the response of interleukins in neurosurgical intensive care patients monitored using intracerebral microdialysis. J Neurosurg 2007;106:820-5.

315. Frijns CJ, Fijnheer R, Algra A, van Mourik JA, van Gijn J, Rinkel GJ. Early circulating levels of endothelial cell activation markers in aneurysmal

subarachnoid haemorrhage: associations with cerebral ischaemic events and outcome. J Neurol Neurosurg Psychiatry 2006;77:77-83.

316. Frijns CJ, Kasius KM, Algra A, Fijnheer R, Rinkel GJ. Endothelial cell activation markers and delayed cerebral ischaemia in patients with subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 2006;77:863-7.

317. Mack WJ, Mocco J, Hoh DJ, et al. Outcome prediction with serum intercellular adhesion molecule-1 levels after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;96:71-5.

318. McGirt MJ, Lynch JR, Blessing R, Warner DS, Friedman AH, Laskowitz DT. Serum von Willebrand factor, matrix metalloproteinase-9, and vascular endothelial growth factor levels predict the onset of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Neurosurgery 2002;51:1128-34; discussion 34-5.

319. Mocco J, Mack WJ, Kim GH, et al. Rise in serum soluble intercellular adhesion molecule-1 levels with vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;97:537-41.

320. Nissen JJ, Mantle D, Gregson B, Mendelow AD. Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage: the immunoglobulin and selectin superfamilies. Journal of neurology, neurosurgery, and psychiatry 2001;71:329-33.

321. Fontanella M, Rainero I, Gallone S, et al. Interleukin-1 cluster gene polymorphisms and aneurysmal subarachnoid hemorrhage. Neurosurgery 2010;66:1058-62; discussion 62-3.

322. Fontanella M, Rainero I, Gallone S, et al. Interleukin 6 gene polymorphisms are not associated with aneurysmal subarachnoid haemorrhage in an Italian population. J Neurol Neurosurg Psychiatry 2008;79:471-3.

323. McColgan P, Thant KZ, Sharma P. The genetics of sporadic ruptured and unruptured intracranial aneurysms: a genetic meta-analysis of 8 genes and 13 polymorphisms in approximately 20,000 individuals. J Neurosurg 2010;112:714-21.

324. Ruigrok YM, Slooter AJ, Bardoel A, Frijns CJ, Rinkel GJ, Wijmenga C. Genes and outcome after aneurysmal subarachnoid haemorrhage. J Neurol 2005;252:417-22.

325. Nam DH, Kim JS, Hong SC, et al. Expression of interleukin-1 beta in lipopolysaccharide stimulated monocytes derived from patients with aneurysmal subarachnoid hemorrhage is correlated with cerebral vasospasm. Neurosci Lett 2001;312:41-4.

326. Frijns CJ, Rinkel GJ, Castigliego D, Van Gijn J, Sixma JJ, Fijnheer R. Endothelial cell activation after subarachnoid hemorrhage. Neurosurgery 2002;50:1223-9; discussion 9-30.

327. Rousseaux P, Scherpereel B, Bernard MH, Graftieaux JP, Guyot JF. Fever and cerebral vasospasm in ruptured intracranial aneurysms. Surgical neurology 1980;14:459-65.

328. Weir B, Disney L, Grace M, Roberts P. Daily trends in white blood cell count and temperature after subarachnoid hemorrhage from aneurysm. Neurosurgery 1989;25:161-5.

329. Neil-Dwyer G, Cruickshank J. The blood leucocyte count and its prognostic significance in subarachnoid haemorrhage. Brain 1974;97:79-86.

330. Spallone A, Acqui M, Pastore FS, Guidetti B. Relationship between leukocytosis and ischemic complications following aneurysmal subarachnoid hemorrhage. Surgical neurology 1987;27:253-8.

331. Niikawa S, Hara S, Ohe N, Miwa Y, Ohkuma A. Correlation between blood parameters and symptomatic vasospasm in subarachnoid hemorrhage patients. Neurologia medico-chirurgica 1997;37:881-4; discussion 4-5.

332. Maiuri F, Gallicchio B, Donati P, Carandente M. The blood leukocyte count and its prognostic significance in subarachnoid hemorrhage. Journal of neurosurgical sciences 1987;31:45-8.

333. McGirt MJ, Mavropoulos JC, McGirt LY, et al. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2003;98:1222-6.

334. Gallia GL, Tamargo RJ. Leukocyte-endothelial cell interactions in chronic vasospasm after subarachnoid hemorrhage. Neurological research 2006;28:750-8.

335. Pellettieri L, Carlson CA, Lindholm L. Is the vasospasm following subarachnoidal hemorrhage an immunoreactive disease? Experientia 1981;37:1170-1.

336. Mathiesen T, Lefvert AK. Cerebrospinal fluid and blood lymphocyte subpopulations following subarachnoid haemorrhage. British journal of neurosurgery 1996;10:89-92.

337. Dhar R, Diringer MN. The burden of the systemic inflammatory response predicts vasospasm and outcome after subarachnoid hemorrhage. Neurocritical care 2008;8:404-12.

338. Yoshimoto Y, Tanaka Y, Hoya K. Acute systemic inflammatory response syndrome in subarachnoid hemorrhage. Stroke; a journal of cerebral circulation 2001;32:1989-93.

339. Naredi S, Lambert G, Friberg P, et al. Sympathetic activation and inflammatory response in patients with subarachnoid haemorrhage. Intensive care medicine 2006;32:1955-61.

340. Kasuya H, Shimizu T. Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. J Neurosurg 1989;71:741-6.

341. Pellettieri L, Nilsson B, Carlsson CA, Nilsson U. Serum immunocomplexes in patients with subarachnoid hemorrhage. Neurosurgery 1986;19:767-71.

342. Lindsberg PJ, Ohman J, Lehto T, et al. Complement activation in the central nervous system following blood-brain barrier damage in man. Annals of neurology 1996;40:587-96.

343. Mack WJ, Ducruet AF, Hickman ZL, et al. Early plasma complement C3a levels correlate with functional outcome after aneurysmal subarachnoid hemorrhage. Neurosurgery 2007;61:255-60; discussion 60-1.

344. Komotar RJ, Zacharia BE, Valhora R, Mocco J, Connolly ES, Jr. Advances in vasospasm treatment and prevention. Journal of the neurological sciences 2007;261:134-42.

345. Vignali DA. Multiplexed particle-based flow cytometric assays. Journal of immunological methods 2000;243:243-55.

346. Kato H, Kawaguchi M, Inoue S, Hirai K, Furuya H. The effects of beta-adrenoceptor antagonists on proinflammatory cytokine concentrations after subarachnoid hemorrhage in rats. Anesth Analg 2009;108:288-95.

347. !!! INVALID CITATION !!!

348. Garcia JH, Liu KF, Ho KL. Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. Stroke; a journal of cerebral circulation 1995;26:636-42; discussion 43.

349. Muroi C, Mink S, Seule M, Bellut D, Fandino J, Keller E. Monitoring of the inflammatory response after aneurysmal subarachnoid haemorrhage in the clinical setting: review of literature and report of preliminary clinical experience. Acta Neurochir Suppl 2011;110:191-6.

350. Mellergard P, Aneman O, Sjogren F, Pettersson P, Hillman J. Changes in extracellular concentrations of some cytokines, chemokines, and neurotrophic factors after insertion of intracerebral microdialysis catheters in neurosurgical patients. Neurosurgery 2008;62:151-7; discussion 7-8.

Statement of individual contribution

Yahia Al-Tamimi (YA) was the priniciple investigator and conductor of this research. Deepti Bhargava (DB) acted as a research assistant in the conduct of the trial during the period 2008-2010. The diagnosis of DIND was made prospectively by the multidisciplinary team caring for the patient: this included a vascular neurosurgeon (of which there are four), several neurointensivists and a neuroradiologist. The two investigators involved in the management of the trial (YA and DB) did not make this diagnosis. Neurological examination in this setting was performed by a number of clinicians completely independent of the trial and repeated several times daily (in addition to standard hourly nursing observations). Only after an established diagnosis did the investigators make a prospective independent assessment of neurology to confirm this diagnosis (there were no instances of disagreement at this stage). Retrospective verification at the end of the trial by the investigators was performed with prospectively collected clinical data (without any reference to treatment allocation), blood results and radiological images. No disagreements did occur between this retrospective assessment and the prospective assessments. The Modified Rankin Score at day 10 was performed by YA/DB following a short discussion with the physiotherapist involved with the care of a particular patient. This was subsequently verified by the investigators (there were no instances of disagreement between these two assessments). For those few patients discharged before day 10, an assessment of the MRS was performed over the phone in a similar fashion to

the six-month MRS. The six-month MRS was obtained by YA and DB. In order to minimise bias, the investigator was blinded to treatment allocation and where possible, the task was performed by the investigator who was not directly involved in recruiting patients.

The data monitoring committee consisted of two vascular neurosurgeons not involved with the trial (Mr A Tyagi and Mr N Phillips, Leeds Teaching Hospital) and the trial statistician (Mr R Feltbower). The trial statistician performed the interim analysis. The aims of the interim analyses at 40 patients and 100 patients were to highlight any safety issues and to demonstrate obvious benefit/detriment of trial intervention respectively.

The second part of this study (multiplex analysis) was performed by YA and Dr Uma Ekbote. YA collected all the samples and acted a laboratory assistant to Dr Ekbote who led the analysis.

Appendices

Appendix One

Lumbar drainage of cerebrospinal fluid following aneurysmal subarachnoid haemorrhage: A prospective, randomised and controlled trial (LUMAS)

Trial Protocol

Chief Investigator:

Mr Yahia Al-Tamimi

Senior Investigator

Mr Stuart Ross

Introduction

Aneurysmal subarachnoid haemorrhage affects between six and eight per 100,000 population per year. Despite modern intervention the mortality and morbidity rate remains high both from the initial haemorrhage and from the associated complications of subarachnoid haemorrhage. The two major neurological complications (excluding re-haemorrhage) seen in patients with subarachnoid haemorrhage are cerebral vasospasm and hydrocephalus.

Cerebral Vasospasm

Cerebral vasospasm is characterised by delayed neurological deficit and/or impairment of the level of consciousness occurring after the third or fourth day post-haemorrhage. The period in which cerebral vasospasm may occur extends from the third to the twenty-first day post-bleed, but is most commonly seen between days three and fourteen. Although seen in up to 70% of angiograms, clinically apparent cerebral vasospasm occurs in around 30% of patients in large series. Of these, as many as 50% will develop a permanent cerebral infarction with 15-20% of patients suffering severe disability or death. The cause of cerebral vasospasm is unknown, but many workers believe that the presence of a cerebrospinal fluid (CSF borne spasmodic agent derived from the breakdown products of subarachnoid blood is responsible). Several candidate agents have been suggested including catecholamines, serotonin, prostaglandins and oxyhaemoglobin released during red-cell lysis.

A number of factors predicting the likelihood of developing cerebral vasospasm have been identified. Of these the volume of blood seen on initial CT scan has the greatest predictive value.

The diagnosis of cerebral vasospasm is made by clinical evaluation in conjunction with supportive investigations including CT scan and, where available, trans-cranial Doppler ultrasonography of the large intracranial vessels. However the investigation of choice to demonstrate vasospasm is cerebral angiography.

Early recognition of incipient vasospasm enables aggressive treatment to be given which consists of hypervolaemia, hypertension and haemodilution (HHH). This three-pronged approach is aimed at providing adequate cerebral perfusion. Supplementary oxygen and control of sodium balance (patients with cerebral vasospasm are prone to centrally-mediated natriuresis) are important adjuncts to HHH therapy.

A recent retrospective study has suggested that continuous drainage of CSF via a lumbar drain has a significant impact on patients with cerebral vasospasm, reducing the incidence and severity of the condition and improving overall outcome. The suggested mechanism by which this benefit is achieved is removal of the presumed CSF borne spasmogen. This research group compared the practice of two vascular neurosurgeons during the period of 1994 and 2003. One surgeon routinely placed an intra-operative

lumbar drain during clipping or endovascular coiling of an aneurysm (unless contraindicated), whilst the second surgeon did not. Data was collected on 167 patients.

All patients received standardised care and were placed into two groups, those that had lumbar drainage (number of patients 81) and those that did not (control group, number of patients 86). Outcome measures included the development of clinically evident vasospasm, the need for endovascular interventions (including angioplasty and/or intra-arterial papaverine) and the development of vasospasm-related infarction. Results were in favour of lumbar drainage. There was a statistically significant reduction in the incidence of clinically symptomatic vasospasm (from 51% to 17%), need for endovascular therapy (from 45% to 17%) and vasospasm-related infarction (from 27% to 7%). There was also a statistically significant improvement in disposition at discharge, length of in-patient stay and Glasgow outcome scores.

Although a robust retrospective study, there were certain limitations. The study had a degree of selection bias with a tendency of higher Fisher grades (appendix 1.3) not to have lumbar drainage. Logistic regression analysis and subgroup analyses showed that following correction for this bias, results were still in favour of lumbar drainage. Other limitations of the study include the long period in which patients presented to the department (9 years) and the inherent limitations to retrospectively analysing case notes. Other features that could be addressed in a future study include quantification of the amount

of CSF drainage (including the amount intraoperative drainage) and the use of lamina terminalis fenestration during the operation and the implications this would have in the outcome measures. These factors have not been adequately addressed in this study.

Study Aims

The aim of this study is to determine whether continuous lumbar drainage of CSF reduces the incidence and severity of clinical vasospasm and leads to improved long-term outcome. Lumbar drainage of CSF is a cheap, safe procedure performed under local anaesthesia and is routinely used in patients undergoing surgery for intracranial aneurysms to facilitate surgery. There are a number of contraindications to lumbar drain insertion, which are outlined in the exclusion criteria for this study.

Study Design and Methodology

This study is a prospective, randomised, case-controlled study in which patients will receive standard therapy or standard therapy plus lumbar drainage following initial CT scan.

Written informed consent will be sought prior to recruitment into the study. Written assent from the patient's relatives will be sought in those cases where confusion prevents written informed consent being obtained from the patient.

Patients with CT evident subarachnoid blood corresponding to Fisher Grades 2, 3 and 4 (appendix 1.3) will be randomised to standard therapy or standard therapy with the addition of continuous lumbar drainage of CSF at 5-10 mL per hour until the CSF shows no evidence of xanthochromia or day 10 post-insertion. Patients will be entered into the study as soon as possible following haemorrhage, but not after 96 hours post-haemorrhage day (i.e. prior to the onset of vasospasm).

Patients may withdraw from the study at any time and will continue with routine management.

Should the lumbar drain fall out prematurely the patient will be asked whether they wish to continue in the study and, if so, the lumbar drain will be reinserted and management will continue as before.

Patients showing signs of lumbar drain infection will have the drain removed, a sample of CSF and the drain tip sent to the microbiology laboratory and appropriate antibiotic therapy instituted. Appendix one demonstrates the flow diagram for patient recruitment.

Inclusion / Exclusion Criteria

Inclusion criteria

Aneurysmal subarachnoid haemorrhage.

Recruitment prior to 96 hours post-haemorrhage.

Written informed consent or relative assent given.

WFNS grade 1-3 (Appendix 1.2).

Fisher grade 2, 3 and 4 (without space occupying haematoma) on initial CT scan (Appendix 1.3).

No significant intraventricular haemorrhage, space occupying haematoma or other contra-indication to lumbar puncture.

Exclusion criteria

Non-aneurysmal subarachnoid haemorrhage.

Delayed presentation / recruitment (after 96 hours post-haemorrhage)

Written informed consent or relative assent denied or unobtainable.

WFNS grade 4 or 5 (Appendix 1.2).

Fisher grade 1 on initial CT scan (Appendix 1.3).

Intraventricular haematoma obstructing ventricular outflow.

Intracranial haematoma with mass effect.

Bleeding diathesis.

Outcome Measures

Primary Outcome Measure

Development of clinical vasospasm (new delayed neurological deficit and / or impairment of consciousness without other cause). This is defined as a drop of one motor point or two eye/verbral points of the Glasgow Coma Score and/or a new focal neurological deficit, seen at least 96 hours post haemorrhage.

Secondary Outcome Measures

Modified Rankin score at day 10 and six months post-discharge.

Development of completed stroke clinically and radiologically (when computerised tomography is performed as part of routine clinical care).

CSF Infection.

Prevalence of cerebrospinal fluid shunting.

Special Note on the Trial Protocol

If a patient has presented greater than four days following the initial haemorrhage, any neurological deficit present may be a delayed ischaemic neurological deficit and thus must be excluded from the trial. Should the lumbar drain fall out prematurely the patient will be asked whether they wish to continue in the study and, if so, the lumbar drain will be re-inserted and management will continue as before. Patients showing signs of lumbar drain infection will have the drain removed, a sample of CSF and the drain tip sent to the microbiology laboratory and appropriate antibiotic therapy instituted.

In those patients that present with or develop raised intracranial pressure secondary to hydrocephalus, if indicated, an external ventricular drain would be inserted regardless of trial status. If subsequently randomised to the study arm of the trial, a lumbar drain would be inserted and cerebrospinal fluid drainage gradually weaned off the external ventricular drain into the lumbar drain. The external ventricular drain can subsequently be removed. Those patients with an external ventricular drain that are subsequently randomised

to the control arm of the trial shall be analysed as a separate group of controls.

Those patients that develop raised cerebrospinal fluid pressure whilst a lumbar drain is in situ would have their drain readjusted to be pressure dependant and not volume dependant (i.e. the pursuit of cerebrospinal fluid drainage of 5-10 ml/hour would no longer be appropriate and the drain would be sent to maintain normal ventricular pressure (15 cm/water above zygoma)).

Although primarily a clinical study with clinical outcome measures, transcranial Doppler may be used to aid diagnosis of radiological vasospasm. This will act as a guide to therapy only. Increase flow velocity is not a pre-requisite for the diagnosis of delayed ischaemic neurological deficit.

Statistical Analysis

The neurosurgical unit at Leeds General Infirmary treated 313 patients with acute subarachnoid haemorrhage in the three years 1997-2000. Of these 243 patients would have been suitable for this study on the basis of their admission clinical status.

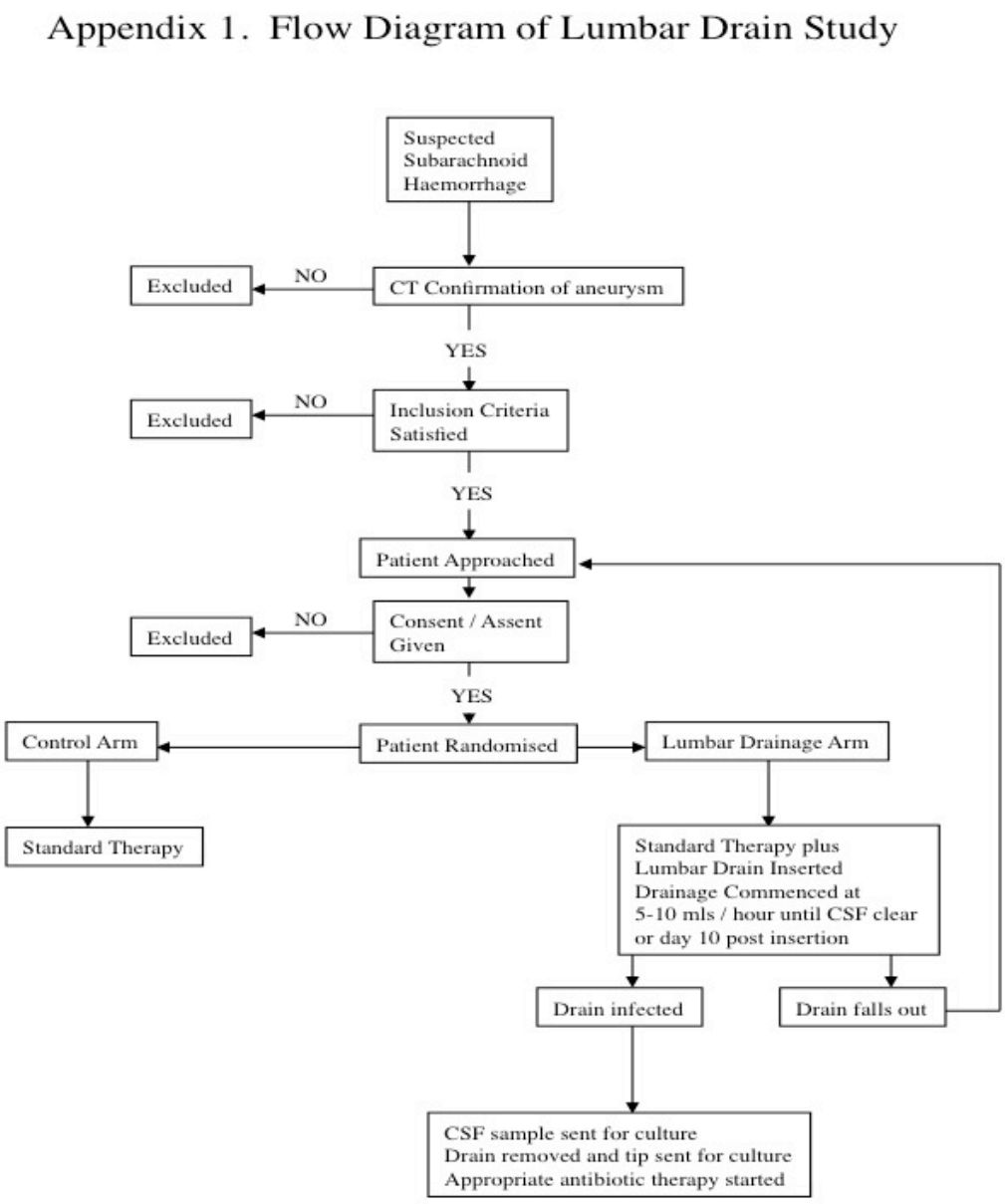
The power calculation based on the hypothesis that lumbar drainage will result in a twenty percent reduction in the incidence of vasospasm requires

the recruitment of 105 patients to each arm of the study to achieve 85% power (appendix 1.4).

Conclusion

Aneurysmal subarachnoid haemorrhage remains an important cause of mortality and morbidity in neurosurgical practice. Cerebral infarction secondary to the onset of cerebral vasospasm is the main cause of mortality and morbidity in those patients who survive the initial haemorrhage. Cerebral vasospasm is thought to result from CSF borne blood breakdown products. Recent retrospective studies have suggested that continuous lumbar drainage of CSF reduces the incidence and severity of cerebral vasospasm and results in improved outcome. No prospective randomised study has been performed to address this question.

Appendix 1.1-Flow diagram of patient recruitment



Appendix 1.2 – World Federation of Neurosurgeons Grading of Subarachnoid Haemorrhage

WFNS Grade	Glasgow Coma Score	Motor Deficit
1	15	None
2	13-14	None
3	13-14	Present
4	7-12	None / Present
5	3-6	None / Present

Appendix 1.3 – Fisher classification of Subarachnoid Haemorrhage

Fisher Grade	Description of CT appearance
1	No blood detected
2	Diffuse deposition of subarachnoid blood, no clots, no layers of blood greater than 1mm
3	Localised clots and/or vertical layers of blood 1mm or greater in thickness
4	Diffuse or no subarachnoid blood, but intracerebral or intraventricular clots are present
3+4	Both dense subarachnoid blood and intracerebral/intraventricular haemorrhages of 5 ml or more (greater than 2cm)

Appendix 1.4-Power calculation for the trial

Incidence of DIND		30 %	25 %	20 %	15 %	10 %
Absolute Change		-10 %	-15 %	-20 %	-25%	-30%
Power	80 %	375	165	90	60	40
	85 %	430	190	**105**	65	45
	90 %	500	215	120	73	50

Appendix 1.5-Modified Rankin Score

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

Appendix Two

Consent Form

Spinal Fluid Drainage in Subarachnoid Bleed

Patient Identification number [ ]

I have read the attached Patient Information Sheet

I have had the opportunity to ask questions and have received answers to my satisfaction

I understand that I may not gain any personal benefit from participation in this study

The additional risk to me above that covered in the consent for my operation has been explained to my satisfaction

I understand that all information relating to my case will be stored within a secure database and that if presented or published all personal details will be withheld

I understand that I may withdraw my consent at any time without affecting my routine treatment or legal rights

I understand that that cerebrospinal fluid and venous blood that is removed as part of this study may be stored anonymously for further biochemical analysis and disposed of accordingly

Signed Date

I confirm that I have explained the purpose of the study and am satisfied that the patient has an adequate understanding of the study

Signed Date

The Leeds Teaching Hospitals NHS Trust

Patient Information Sheet

Spinal Fluid Drainage in Subarachnoid Bleed

You are being invited to take part in a **RESEARCH STUDY**. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Background and Purpose of the Study

We are trying to find out whether drainage of spinal fluid results in better outcome for patients with subarachnoid haemorrhage. There is evidence to show from a previous study that inserting a spinal drain following subarachnoid haemorrhage has reduced the complication rate following this type of haemorrhage. The main complication is a process called vasospasm which is serious and poorly understood. It is associated with neurological deterioration and disability. There is evidence that spinal drainage may reduce the chance of this occurring. However, this has yet to be proven with a detailed and effective study like this one we are performing.

Why have I been chosen?

We hope to include all suitable patients with subarachnoid haemorrhage in the Neurosurgery Department in Leeds.

Do I have to take part?

Participation is entirely voluntary, you may withdraw from the study at any time without having to give reasons and your routine hospital care and legal rights will not be affected. If you decide to take part you will be given this information sheet to keep and will be asked to sign a consent form.

What will happen to me if I take part?

If you decide to take part, we will place you into one of two groups (called group 1 or group 2). If placed in group 1, you will be given normal therapy and treatment. Normal treatment consists of keeping you on a special ward where there are many nurses available to look after you. We will place a small plastic tube into your vein (called a cannula) in order to give you fluid therapy directly into your blood. This will ensure you receive adequate amounts of water and salt. We will take regular blood tests (possibly daily) to keep a close eye on salt levels in your blood. You will be given tablets every 4 hours to help your brain cope with this haemorrhage. We may feel the need to place further small tubes (similar to the cannula in your vein) in your neck and wrist to help monitor you closely.

If we decided to place you in group 2, you will receive all the standard therapy described above in addition to a spinal drainage tube which we place in your lower back. This will be attached to a bag to collect spinal fluid. This may stay in your back for 1-2 weeks. This procedure is carried out under local anaesthetic and although application of this local anaesthetic may be slightly uncomfortable, the procedure should be painless. You will be

asked to turn to your side with your legs curled up. Your lower back will be cleaned with disinfectant and a small needle will be used to inject some local anaesthetic. The procedure should take no longer than twenty to thirty minutes. Once the drain is inserted, you will be allowed to move as your condition allows.

The way we decide which group you will be placed in is on a random basis. This is like tossing a coin to decide the group. If it lands on heads, you will be placed in group 1 and if it lands on tails, you will be placed in group 2. Another way to understand this is to think of a roulette wheel. If the ball lands on red, you are put in group 1 and if the ball lands on black, you are put in group 2. This means there is an equal chance that you will be placed in either group 1 or group 2. This method ensures that the study is scientifically accurate.

Are there any benefits from taking part?

There may be no benefit to you from participation in this study. We are investigating whether or not spinal drainage does improve the patient's outcome following subarachnoid haemorrhage.

Are there any risks?

There is a very small risk of infection in the spinal fluid (we estimate this to be less than one percent). Although this complication is not often seen, we treat it very seriously. We will continually assess for signs of infection including inspection of the drain site and sending spinal fluid samples to the laboratory to look for infection. We collect this from the draining bag and so it will not require any further needles or injections. If there is any sign of infection we will remove the drain and treat appropriately (with antibiotics if necessary).

Will my taking part in this study be kept confidential?

All information collected in the study is confidential. You will not be able to be identified at any time including during presentation or publication of the study results.

What will happen to the results of the study?

We hope to publish the results in a medical journal. You will not be able to be identified from published results.

Who is organizing and funding the research?

The Neurosurgical Department at Leeds General Infirmary.

Who has reviewed the study?

The study has been reviewed by the Leeds (West) Research Ethics Committee.

Contact for further information

Mr Yahia Al-Tamimi (Lead Investigator)

Neurosurgery Dept, Leeds General Infirmary

Mr Stuart Ross (Consultant Neurosurgeon)

Neurosurgery Dept, Leeds General Infirmary

Thank you for your participation

Date: Number:

Appendix Three

Assent Form

Spinal Fluid Drainage in Subarachnoid Bleed

Patient Identification number [ ]

I have read the attached Relative Information Sheet

I have had the opportunity to ask questions and have received answers to my satisfaction

I understand that the patient (my relative) may not gain any personal benefit from participation in this study

The additional risk to the patient above that covered in the consent for his/her operation has been explained to my satisfaction

I understand that all information relating to the patient's case will be stored within a secure database and that if presented or published all personal details will be withheld

I understand that I may withdraw my assent on behalf of the patient at any time without affecting

the patient's routine treatment or legal rights

I understand that cerebrospinal fluid and venous blood that is removed from the patient as part of this study may be stored anonymously for further biochemical analysis and disposed of accordingly

Signed Date

I confirm that I have explained the purpose of the study and am satisfied that the relative has an adequate understanding of the study and is able to give informed assent on behalf of the patient

Signed Date

Relative Information Sheet

The Leeds Teaching Hospitals NHS Trust

Spinal Fluid Drainage in Subarachnoid Bleed

Your relative (the patient) is being invited to take part in a **RESEARCH STUDY**. In view of the patient's impaired ability to provide consent for this, you are being asked on their behalf to give permission for the patient to take part in this study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends, relatives and the patient's GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish the patient to take part.

Thank you for reading this.

Background and Purpose of the Study

We are trying to find out whether drainage of spinal fluid results in better outcome for patients with subarachnoid haemorrhage. There is evidence to show from a previous study that inserting a spinal drain following subarachnoid haemorrhage has reduced the complication rate following this type of haemorrhage. The main complication is a process called vasospasm which is serious and poorly understood. It is associated with neurological deterioration and disability. There is evidence that spinal drainage may reduce the chance of this occurring. However, this has yet to be proven with a detailed randomised study like this one we are performing.

Why has the patient been chosen?

We hope to include all suitable patients with subarachnoid haemorrhage in the Neurosurgery Department in Leeds.

Does the patient have to take part?

Participation is entirely voluntary, the patient may withdraw from the study at any time without having to give reasons and his/her routine hospital care and legal rights will not be affected. If unable to make this decision, you can withdraw the patient from the study acting in his/her best interest. If you decide to allow the patient to take part you will be given this information sheet to keep and will be asked to sign a consent form.

What will happen to the patient if he/she takes part

If the patient takes part, we will place him/her place into one of two groups (called group 1 or group 2). If placed in group 1, he/she will be given normal therapy and treatment. Normal

treatment consists of keeping the patient on a special ward where there are many nurses available to look after him/her. We will place a small plastic tube into his/her vein (called a cannula) in order to give fluid therapy directly into the blood. This will ensure that adequate amounts of water and salt are received. We will take regular blood tests (possibly daily) to keep a close eye on salt levels in the blood. The patient will be given tablets every 4 hours to help their brain cope with this haemorrhage. We may feel the need to place further small tubes (similar to the cannula in the vein) in his/her neck and wrist to help monitor more closely.

If we decided to place the patient in group 2, he/she will receive all the standard therapy described above in addition to a spinal drainage tube which we place in the lower back. This will be attached to a bag to collect spinal fluid. This may stay in the back for 1-2 weeks. This procedure is carried out under local anaesthetic and although application of this local anaesthetic may be slightly uncomfortable, the procedure should be painless. The patient will be turned to the side with their legs curled up. The lower back will be cleaned with disinfectant and a small needle will be used to inject some local anaesthetic. The procedure should take no longer than twenty to thirty minutes. Once the drain is inserted, the patient will be allowed to move as their condition allows.

The way we decide which group the patient will be placed in is on a random basis. This is like tossing a coin to decide the group. If it lands on heads, he/she will be placed in group 1 and if it lands on tails, he/she will be placed in group 2. Another way to understand this is to think of a roulette wheel. If the ball lands on red, the patient is put in group 1 and if the ball lands on black, he/she will be put in group 2. This means there is an equal chance of being placed in either group 1 or group 2. This method ensures that the study is scientifically accurate.

Are there any benefits from taking part?

There may be no benefit to the patient from participation in this study. We are investigating whether or not spinal drainage does improve the patient's outcome following subarachnoid haemorrhage.

Are there any risks?

There is a very small risk of infection in the spinal fluid (we estimate this to be less than one percent). Although this complication is not often seen, we treat it very seriously. We will continually assess for signs of infection including inspection of the drain site and sending spinal fluid samples to the laboratory to look for infection. We collect this from the draining bag and so it will not require any further needles or injections. If there is any sign of infection we will remove the drain and treat appropriately (with antibiotics if necessary).

Will the patient's taking part in this study be kept confidential?

All information collected in the study is confidential. The patient will not be able to be identified at any time including during presentation or publication of the study results.

What will happen to the results of the study?

We hope to publish the results in a medical journal. The patient will not be able to be identified from published results.

Who is organizing and funding the research?

The Neurosurgical Department at Leeds General Infirmary.

Who has reviewed the study?

The study has been reviewed by the Leeds (West) Research Ethics Committee. This committee has given their approval for this study.

Contact for further information

Mr Yahia Al-Tamimi (Lead Investigator)

Neurosurgery Dept, Leeds General Infirmary

Mr Stuart Ross (Consultant Neurosurgeon)

Neurosurgery Dept, Leeds General Infirmary

Thank you for your participation

Date: Number:

Appendix Four

Registration

Booklet for any Subarachnoid Haemorrhage patient eligible to enter the Lumbar Drain Trial

Name of Patient……
Hospital Number……..

When to use this document: on admission of all SAH patient regardless of inclusion into trial)

Demographics

Name **Admission Date**
Hospital Number **DOB**
Sex **Address and Post code**

Date of Bleed:
Date of Admission:

Referring Hospital ……..

Admitting Ward: ITU HDU Ward

Diagnosis

CT: blood in the following regions:

Supracallosal	Prepontine
Interhemispheric	Third Ventricle
Convexity	Fourth Ventricle
Sylvian	Lateral Ventricles
Basal Cistern	Posterior Fossa
Perimesencephalic	Other ……

Is there ventriculomegaly? Yes No

Haematoma present? Yes No

If so, where?

Sylvian
Subfrontal
Posterior Fossa
Subdural haematoma

mm

GCS when bleed: E M V

GCS at admission: E M V

WFNS grade: 1 2 3 4 5

Fisher grade: 1 2 3 4 3+4

Hunt and Hess: 0 1 2 3 4 5

Investigation

CT-Angiogram: Date ……./……/…….

Aneurysm:	R	L		R	L
Pericallosal			Anterior Communicating		
Carotid Bifurcation			Posterior Communicating		
Opthalmic			Cavernous		
M1			M2		
PICA			BasilarTip		
Basilar Trunk			Other		
None					

Formal Angiogram: Date ……./……./…….

Aneurysm:	R	L		R	L
Pericallosal			Anterior Communicating		
Carotid Bifurcation			Posterior Communicating		
Opthalmic			Cavernous		
M1			M2		
PICA			BasilarTip		
Basilar Trunk			Other		
None					

Unruptured aneurysm? Yes No

Prior sentinel bleeds? Yes No

Presenting features

Headache		**Glucose**		**Lactate**	
Meningism		**Na**		**FiO_2**	
Altered consciousness		**K**		**PO_2**	
Vomiting		**Urea**		**PCO_2**	
Diplopia		**Creatinine**		**pH**	
Confusion		**Lactate**		**Pulse**	
Motor Deficit		**Albumin**		**BP**	
Dysphasia		**Hb**		**Resp Rate**	
Temperature		**Wcc**		**Pupil reactivity-L**	
		Platelets		**Pupil size-L**	
		APTT		**Pupil reactivity-R**	
		PT		**Pupil size-R**	
		INR			

Past Medical History

DM HTN IHD MI PVD
Other ……

Previous Intracranial haemorrhage Stroke

Family History of aneurysmal bleed …….. Smoker Y N

Examination Findings L R

Cranial Nerve deficits: ……. …….

Upper Limb

Tone

Power Shoulder Abduction ……. …….
Shoulder Adduction ……. …….
Elbow Flexion ……. …….
Elbow Extension ……. …….
Wrist Flexion ……. …….
Wrist Extension ……. …….
Grip ……. …….
Sensation ……. …….
Reflexes Biceps ……. …….
Triceps ……. …….
Supinator ……. …….
Co-ordination/Gait (impaired/normal) ……. …….

Lower Limb		**L**	**R**
Tone			
Power	Hip Flexion	…….	…….
	Hip Extension	…….	…….
	Knee Flexion	…….	…….
	Knee Extension	…….	…….
	Ankle Flexion	…….	…….
	Ankle Extension	…….	…….
	Toe Extension	…….	…….
Sensation		…….	…….
Reflexes	Knee	…….	…….
	Ankle	…….	…….
Co-ordination/Gait (impaired/normal)		…….	…….

<u>Treatment</u>

Coiling Date ……/……/……
Clipping Date ……/……/……
EVD Date ……/……/……

Intra-operative rupture? Yes No
Temporary Clip Yes No
Clip 1: Time (min)…….. Clip 2: Time (min)……..

Post op complication ……..

Post op neurological deterioration? Yes No

Anaesthetic Problems ……..

<u>Randomisation to Lumbar Drain Trial</u>

Randomised to lumbar drain trial? Yes No

If NO, why not?……..

If YES, randomised to: Drain Control
Date ……/……/……

If drain inserted: Opening pressure ……..
Closing pressure ……..
CSF colour ……..

Date Removed ……./……/……..

Delayed Neurological Deficit? Yes No
Shunt Dependant? Yes No
Date of discharge from high dependency……../……../……

Date neurosurgical input discontinued……../……../……

Date of discharge as neurosurgical input……../……../……..

Place of discharge……

Notes

……..

……..

Appendix Five

Daily Progress

Booklet for Subarachnoid Haemorrhage patient included in the Lumbar Drain Trial

Name of Patient……
Hospital Number……..

Randomised to: Lumbar Drain

Control Arm

When to use this document: to collect information on a daily basis. Day 0 is day of haemorrhage. Day 1 is the following day etc. This is regardless of date of admission.

Daily progress sheet: Day 1

Glasgow Coma Score **Motor Examination**

Best …… M V E Normal X4

Worst …….. M V E Weak arm L…….R…….

Weak leg

L…….R…….

Cranial Nerves

Pupil size (mm) L…….R……. Other CNS Defect

L…….R……

Pupil reactive? (tick for yes) Describe defect

……

Orientation **Speech**

Full Fluent

Partial Dysphasic

None None

Can't assess Intubated/trach

Airway: Normal Adjuvant Tracheostomy ET tube

Ventilation-number of hours of this support (max 24 hours):

SIMV……. CPAP/BIPAP……. Wall CPAP……. None…….

FiO_2 ……. PO_2 ……. PCO_2 ……. pH …….

MAP (periods >60 mins tick boxes and write number of hours at this MAP):

0-60 …… 60-80 …… 80-100 …… 100-120 …… 120-140 …… >140 …….

Inotrope use? Y N Which one?…….. How much?……..

CVP 0-4 …… 4-8 …… 8-12 …… 12-16 …… 16-20 …… >20 …….

Infection Screen:

Max reproducible temp……CRP……WCC……Neut……Lymp……

CSF EVD: WCC……. RBC……. PM (%)……. Gram stain……..Org ……..

Volume/24 hours…….. Colour……..

CSF Lumbar: WCC……. RBC……. PM (%)……. Gram stain……..Org ……..

Volume/24 hours…… Colour ……..

Fluid intake: N. Saline …….. ml Gelofusin…….ml Voluven……..ml
NG/Oral……..... ml Other: Type…….. Volume……..ml
Magnesium……/24 h Statin? Y N Which one?…….....
Fluid Output: Urine/24 hours …..... L Other……..... L

Other results:
Na……. K……. Urea……. Creatinine……. Hb……. Platelets…….
Serum osmolality……... Urine osmolality……... Urine Na: Spot…….. 24 h……..
Glucose……. Magnesium……..
Albumin……APTT……PT……..INR……..
Drain wound concerns.....
CT/CT-A/P findings……
TCD ……
……