The pathophysiology of chronic subdural haematoma and the role of the dexamethasone

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Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

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Abstract: The pathophysiology of chronic subdural haematoma and the role of the dexamethasone

Chronic subdural haematoma (CSDH) is an encapsulated collection of blood and fluid overlying the surface of the brain, which is often treated with surgical drainage to prevent cerebral compression. Despite treatment it can recur, requiring further surgery and causing significant morbidity.

Historically CSDH formation was considered to occur due to the aging and expansion of acute bleeding following head trauma. However, within this thesis the natural progression of CSDH was assessed with serial imaging following trauma and highlighted the novel process of CSDH formation from a normal CT. This was hypothesised to occur through injury initiating inflammation within the subdural space which drives formation of a hypervascular membrane responsible for fluid and blood accumulation over time. This theory was investigated by examining 68 CSDH samples for the presence of selected inflammatory markers and haemoglobin concentration. All pro-inflammatory markers were significantly elevated in CSDH fluid compared to venous blood, particularly vascular endothelial growth factor (VEGF). Haemoglobin degradation products were quantified with UV-Vis spectroscopy and more recent haemorrhage indicated an increased CSDH recurrence risk.

This work was performed alongside the Dex-CSDH trial, a randomised, placebo-controlled trial of the potent anti-inflammatory dexamethasone. Blinded interim results of the trial are discussed, but the main focus are the scientific, mechanistic sub-studies. A method for detecting dexamethasone in CSDH and blood was developed using high performance liquid chromatography (HPLC), and suggested there is no accumulation of the drug within the CSDH space. However, many of the inflammatory markers were reduced post-operatively following dexamethasone treatment. Volumetric and density analysis of pre-operative and post-operative imaging was also performed and may aid prediction of which patients are more likely to have a poor outcome or recurrence. This could help identify patients in the future who would benefit most from adjuvant therapies such as dexamethasone.

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I dedicate this to my mother, Susan Robson, who will sadly never be able to read this but is the source of all my ambition.

Abbreviations

ASDH	Acute Subdural Haematoma
BBB	Blood Brain Barrier
BCR	Bicaudate Ratio
BH	Burr Hole
CAS	Cerebral Atrophy Scale
CBF	Cerebral Blood Flow
CSDH	Chronic Subdural Haematoma
CSDH-AT	Chronis Subdural Haematoma - Acute Transformed
CSDH-DN	Chronic Subdural Haematoma - De Novo
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
Dex	Dexamethasone
FDP	Fibrin/Fibrinogen degradation products
HIF	Hypoxia-inducible factor
HPLC	High Performance Liquid Chromatography
HU	Hounsfield Units
IL	Interleukin
IP	Interferon gamma-induced protein
IT	Intra-thecal
MC	Mini Craniotomy
MCP	Monocyte Chemoattractant Protein
MIP	Macrophage Inflammatory Protein
MMP	Matrix Metalloprotease
MRI	Magnetic Resonance Imaging
PGE	Prostaglandin E
RP-HPLC	Reverse-phase High Performance Liquid Chromatography
RVA	Residual Volume of Air
RVF	Residual Volume of Fluid
SDG	Subdural Hygroma

SSD	Subdural Space Depth
TBI	Traumatic Brain Injury
TCD	Transcranial Doppler
TNF	Tumour Necrosis Factor
TRV	Total Residual Volume
TPA	Tissue Plasminogen Activator
VEGF	Vascular Endothelial Growth Factor

Contents

Chapter 1: Introduction

1.1 Background of Chronic Subdural Haematoma

A Chronic Subdural Haematoma (CSDH) is an encapsulated collection of fluid and blood overlying the brain surface. Despite a large body of research on this condition since the early 19th century, the process of how it is initiated and develops is still relatively unknown. A range of theories have been developed over the years but still doctors and scientists have varying views on the underlying pathophysiological processes that occur. It is well recognised to affect predominately the elderly population and probably have a delayed association with head trauma. Whilst there are reports of small CSDHs resolving, it is well established that they generally continue to enlarge over time. Once growing, the CSDH will expand until either treated or patient death from raised intracranial pressure. Thus, it is a source of significant morbidity and mortality in the elderly and through better understanding of the pathophysiological process, prompt diagnosis and appropriate treatment algorithms can be adopted. Currently, surgical drainage of CSDH forms the mainstay of treatment, but there is growing interest in pharmacological therapies, such as dexamethasone, which can be used in combination with surgery or as stand-alone treatment to encourage reabsorption and resolution of a CSDH.

CSDH is considered a common entity in neurosurgical practice, and has an incidence of around 7.32-8.2 per 100,000 per annum in the peak demographic of over 65-year-olds (Asghar, 2002; Foelholm & Waltimo, 1975). The reported mean patient age in the literature is in the range of 68-77 years old and there is a clear predilection for men, with a ratio of around 1.7-2.9:1 of men to women (Baechli, Nordmann, Bucher, & Gratzl, 2004; Brennan et al., 2017; Gelabert-Gonzalez, Iglesias-Pais, Garcia-Allut, & Martinez-Rumbo, 2005; Goto et al., 2015; Santarius & Hutchinson, 2009; M. Wada et al., 2014). The reason for the gender bias is unclear, but as life expectancy has continued to rise, so does the number and age of patients under-going neurosurgical treatment for conditions such as this (Whitehouse, Jeyaretna, Wright, & Whitfield, 2016). The most common presenting symptoms include cognitive impairment, gait disturbance, limb weakness and headaches (Brennan et al., 2017; Santarius & Hutchinson, 2009). Such symptoms can lead to the suspicion of stroke, although

the onset is usually more insidious, and computed tomography (CT) imaging of the head is diagnostic (Figure 1.1A).

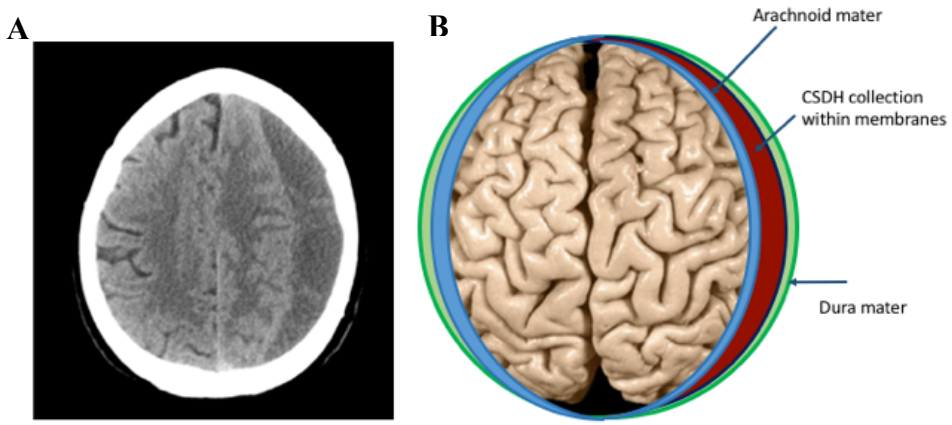

Figure 1.1; anatomical location of CSDH: **(A)** CT image of CSDH showing area of low density (dark grey) on the right side of the image, **(B)** corresponding graphic highlighting layers overlying the brain involved in CSDH.

The earliest report of CSDH was an autopsy finding by Johannes Wepfer in 1657, and was followed by several other reports which developed into an understanding that CSDH involved a blood clot beneath the dura, surrounded by membranes (D'Errico & German, 1930). It was Virchow, in 1857, who described the pathophysiology of CSDH as "pachymeningitis haemorrhagica interna", or chronic inflammation of the dura, leading to fibrin exudation and growth of new capillaries (D'Errico & German, 1930). This was counteracted by the theory that traumatic head injury caused bleeding from bridging veins overlying the brain which resulted in CSDH (Markwalder, 1981; Putnam & Cushing, 1925; Trotter, 1914; Yamashima & Friede, 1984). More recently, there is growing evidence to suggest that both inflammation and trauma may be important in CSDH pathophysiology, but the mechanisms occurring are still obscure. Indeed, each CSDH may form through different processes rather than there being one homogenous disease entity. The aim of this thesis is to interrogate the possible processes in CSDH formation and ascertain the main underlying drivers. The overarching hypothesis is that inflammation is crucial to the development of CSDH and that dexamethasone, an anti-inflammatory drug, is a treatment option which could lead to less requirement for surgical intervention. A greater understanding of the inflammatory processes occurring in CSDH could be applied clinically to help predict the natural course of CSDH in each patient and tailor treatment, using pharmacological and/or

surgical management where appropriate. The clinical data presented herein was obtained from patients recruited to a UK multi-centre randomised control trial of dexamethasone in the treatment of CSDH patients, namely the Dex-CSDH trial, which is detailed later.

1.1.1 The subdural space

In order to investigate the pathophysiology of a CSDH an understanding of the anatomy of the meninges is required. The brain is covered by three layers of cerebral meninges: the pia mater (which closely invests the brain cortex), the arachnoid mater (which contains cerebrospinal fluid (CSF) bathing the brain) and finally dura mater (the tough outer shell of fibrous tissue separating the brain from the skull). In the normal physiological state the dura and arachnoid mater are closely adherent, however their morphology is such that they are prone to separation in pathological circumstances (Moskala et al., 2007). A CSDH allows a space to develop between these layers, the so-called "subdural space" and new membranes form within this space, encapsulating blood and fluid (Figure 1.1B).

In 1946 Inglis performed a histological analysis of the lining of the subdural space and identified a layer of specialised, modified connective tissue cells (Inglis, 1946). These cells had two essential roles; they could phagocytose, and they could develop into fibro-cellular connective tissue. This latter role explains the source of the fibrous membrane which forms around the CSDH. A further histological study has named these "dural border cells" and confirmed their ability to form CSDH membranes (Figure 1.2) (Haines, 1991).

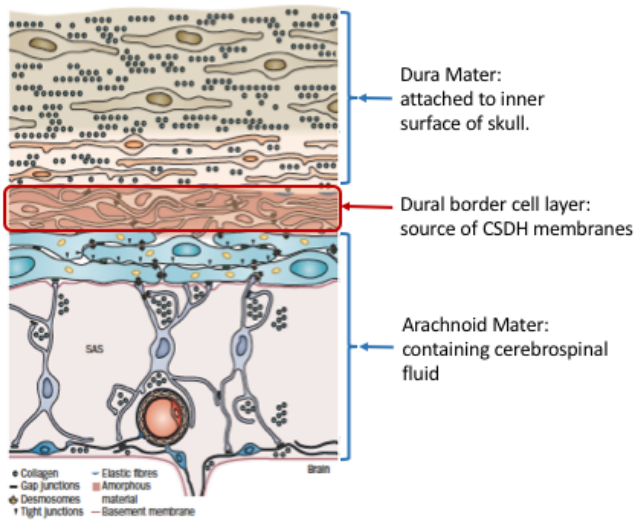

Figure 1.2; representation of dural border cell layer involved in CSDH formation (reproduced from (Kolias, Chari, Santarius, & Hutchinson, 2014).

Although there is the potential to create a subdural space in any brain, the risk is greatest in the elderly, thus explaining the demographic affected by CSDH. This can be explained by cerebral atrophy (shrinkage), which is ubiquitous in the aging population (Fox & Schott, 2004; Giorgio et al., 2010). As the brain atrophies it will sag inwards, applying a gravitational force on the dural border cells. This is likely to mean that minimal further force, such as trivial trauma or head movement, could lead to separation of these cells and that this physical disruption acts as a stimulus for creating subdural membranes. A recent review on the causation of CSDH suggested that it would not be possible for a CSDH to occur unless there is a pre-existing capacious subdural space (as seen with cerebral atrophy) (K. Lee, 2016). Hence, the authors suggested rebranding CSDH as a "degenerative" disease, rather than a traumatic one.

Several studies have assessed the degree of brain atrophy on baseline trauma scans prior to the development of a CSDH. One showed that the brain atrophy index (CSF volume as a percentage of total intracranial volume) was significantly higher in the baseline scans compared to age-matched controls (Jeong et al., 2016). Two further studies have identified significantly greater subdural depths (measured from cortex to skull) in baseline scans compared to other head injury controls (Han et al., 2014; Ju et al., 2015). However, there is some conflicting data with one study identifying no significant relationship between measures of cerebral atrophy and CSDH formation (J. J. Lee, Won, Y., Yang, T., Kim, S., Choi, C. S., Yang, J., 2015).

Cerebral atrophy and the potential subdural space do appear to be contributive factors in the developing a CSDH, but cannot be the entire story. If that were the case then many more elderly patients would develop a CSDH, and, as cerebral atrophy is constant and progressive, one would expect all CSDHs to be recurrent and persistent. Whereas in reality, the majority of CSDHs resolve following adequate treatment and late recurrence is rare. Overall, it is difficult to quantify the degree to which cerebral atrophy contributes to CSDH pathophysiology, but this will be explored further in chapter two.

1.1.2 The role of trauma: transformation from ASDH and SDG

As previously mentioned, many authors support the theory that the pathogenesis of a CSDH is solely secondary to trauma, with tearing of cerebral bridging veins, which are encompassed

within the dura mater as they traverse from the brain surface to the dural-venous sinuses (Markwalder, 1981; Ommaya & Yarnell, 1969). This is supported by evidence that these veins are particularly vulnerable as they enter the subdural space, where the vessel wall is thinner and less well supported (Yamashima & Friede, 1984). However, this theory is questionable, as even a slow venous haemorrhage would accumulate sufficiently quickly for a patient to become clinically symptomatic within hours to days at most; rather than four to seven weeks, which is the mean time from trauma to onset of CSDH symptoms (Gelabert-Gonzalez et al., 2005; Stroobandt, Fransen, Thauvoy, & Menard, 1995). Further to this, only approximately 60% of CSDH patients report any recent trauma and it has been argued that the pattern of blood spanning the cerebral convexities is inconsistent with a source of bleeding entirely from bridging veins, which neighbour the medial venous sinuses (D'Abbondanza & Loch Macdonald, 2014; Gelabert-Gonzalez et al., 2005; Nakaguchi, Tanishima, & Yoshimasu, 2001; Santarius & Hutchinson, 2009). Finally, although a CSDH can contain areas of acute haemorrhage, many are almost entirely "old" haematoma, seen as homogenous hypodensity on CT (Figure 1.1A). This is not in-keeping with the idea of continuous acute venous haemorrhage as a source of CSDH expansion over time, which would be exemplified by a significant layer of hyperdensity within all CSDHs.

This is not to say that acute haemorrhage from bridging veins is not involved in at least *some* cases of CSDH. High acceleration injuries lead to sufficient strain on the bridging veins to cause rupture and rapid accumulation of blood leading to an acute subdural haematoma (ASDH) (Crooks, 1991). Although ASDH occurs in the same anatomical space as CSDH, it is a very different entity with more severe trauma resulting in the rapid accumulation of blood. This results in more severe levels of associated brain injury, high levels of coma and mortality (Bullock et al., 2006). The demographics of patients affected are also markedly different, with only 4% of ASDHs aged over 60, compared with 88% of CSDHs (Asan, 2018). Despite the different patient populations affected there is clearly some cross over between these conditions, with a proportion of ASDH later becoming CSDH. This occurs in patients who have conservative (non-operative) management of an ASDH, either because the collection is small enough or the patient considered too unfit for surgery. Elderly patients, in particularly, can also tolerate relatively large ASDHs, with limited neurological impact, and therefore surgery will likely be avoided if the patient is clinically alert and stable. This relates back to the high proportion of cerebral atrophy in the elderly, allowing more space in the

intracranial compartment to compensate for even relatively large haematomas. Following conservative management, an ASDH can form a CSDH over weeks to months, whilst others spontaneously resolve (Laviv, 2014; K. B. Lee, WK; Doh, JW; Bae, HG; Yun, IG, 1998).

The literature on the rate of transformation from ASDH to CSDH is extremely varied and is likely to depend largely on the inclusion criteria involved in the study. Lee et al. reported on 177 conservatively managed ASDHs with a mean age of 60.84, showing only 16 (9%) developed a CSDH within 3 months (J. J. Lee, Won, Y., Yang, T., Kim, S., Choi, C. S., Yang, J., 2015). Laviv et al. looked only at low velocity ASDHs in patients with a mean age of 77.5, with 43/95 (45%) developing subsequent CSDH at mean of 23.3 days (Laviv, 2014). Further studies have described 18-21% of ASDHs transforming into CSDHs (Izumihara, Yamashita, & Murakami, 2013; Lucke-Wold, Turner, Josiah, Knotts, & Bhatia, 2016). Follow-up imaging may also be helpful, as around 25% of ASDHs showed expansion at one month and 73% of these formed a subsequent CSDH (Lucke-Wold et al., 2016). Age and gender do not appear to influence likelihood of CSDH formation after ASDH, but increased size and associated midline shift of ASDH do (Laviv, 2014; J. J. Lee, Won, Y., Yang, T., Kim, S., Choi, C. S., Yang, J., 2015; Mathew, Oluoch-Olunya, Condon, & Bullock, 1993). This may be because larger ASDHs take longer to resolve and therefore are more likely to develop neomembranes and transform into chronic collections. Inglis reported that haemorrhage in the subdural space could initiate proliferation of the dural border cells, hence why an ASDH can stimulate growth into a CSDH (Inglis, 1946). However, many animal studies have shown that the presence of blood alone, in the subdural space, is not sufficient to initiate progression to a CSDH, so there must be other factors at play (D'Abbondanza & Loch Macdonald, 2014; D'Errico & German, 1930; Goodell & Mealey, 1963).

It is important to understand that whilst only a limited proportion of ASDHs become CSDHs, reciprocally only a small number of CSDHs were ever ASDHs. This is a relatively new concept to some, who believe that all CSDHs were ASDHs to start with. However, chapter two reviews a series of CSDH patients who were scanned at the time of recent trauma, showing that only a proportion had evidence of ASDH. Such data can only now be collected due to the increase in easy-access CT scanning in all emergency departments, and the low threshold for imaging elderly patients experiencing traumatic injury.

For patients who do not have an ASDH, an alternative route to formation of a CSDH by subdural hygroma (SDG) has been suggested (K. Lee, 1998, 2004; K. Lee, Bae, Park, & Yun, 1994; K. B. Lee, WK; Doh, JW; Bae, HG; Yun, IG, 1998; Liu, Bakker, & Groen, 2014; Murata, 1993; Ohno et al., 1987; S. H. Park et al., 2008). SDG, also called subdural effusion, is an accumulation of modified CSF in the subdural space, which also requires separation of the dura-arachnoid interface (at the dural border cell layer) (K. Lee, 1998; K. B. Lee, WK; Doh, JW; Bae, HG; Yun, IG, 1998; Liu et al., 2014). Many consider brain atrophy and SDG synonymous, however although the former can cause the latter, SDG is a defined pathological condition which can cause cerebral compression requiring operative management (K. Lee, 1998; K. Lee et al., 1994; Liu et al., 2014). The morphology of the skull and as such the varying dependent role of gravity has also been suggested to influence SDG development (K. Lee, 2004).

SDGs have been shown to form following traumatic head injury and it is conceivable that trauma cleaves open the dural border cells without causing haemorrhage but allowing accumulation of CSF in the subdural space. This could then activate the cascade of events (see later discussion on inflammation) leading to subsequent formation of a CSDH. Ohno et al. reviewed 715 head injuries over five years and showed that 6% of patients developed a SDG within one week of trauma, although this increased to 30% in those aged over 65 (Ohno et al., 1987). Another study found that only 1.5% of patients admitted with a head injury developed a SDG that was not there on admission (Ahn et al., 2016). Between 12-47% of SDGs have been reported to expand rather than resolve, and as such develop into a CSDH (Ahn et al., 2016; K. Lee, 2004; Liu et al., 2014; Murata, 1993; Ohno et al., 1987; S. H. Park et al., 2008). Conversely, when looking at the number of patients with CSDHs, 15% (24 out of 160) have been found to have a preceding diagnosis of a SDG (S. H. Park et al., 2008). The mean time interval from traumatic SDG to CSDH diagnosis is around seven to eight weeks and risk factors include male gender and bilateral SDGs (Ahn et al., 2016; S. H. Park et al., 2008). In the case where MRI is performed, diffuse pachymeningeal enhancement on MRI is more common in patients whose SDGs become CSDHs, and therefore may be a prognostic indicator (Ahn et al., 2016).

There is some biochemical data to support the theory of CSDHs developing from SDGs. Kristopf. et al showed beta-trace protein (bTP), a marker for CSF, was present in 94% of

CSDHs, whilst Park found it in 100% of 31 CSDHs (Kristof, Grimm, & Stoffel-Wagner, 2008; K. S. Park, Park, Hwang, Kim, & Hwang, 2015). However, this was contradicted by Jafari et al, who measured an alternative marker of CSF (beta-2-transferrin) and found this was only present in 24% of patients (Jafari, Gesner, Koziol, Rotoli, & Hubschmann, 2017). This difference may be explained by an exogenous source of bTP, as it is also present in plasma and is increased in pathologies such as renal failure.

A recent paper identified the presence of increased "CSF spaces" ipsilateral to subsequent CSDH locations on the original trauma scans, but only when the scans were reviewed retrospectively (Olivero, Wang, Farahvar, Kim, & Wang, 2017). Therefore, this may represent a source of missed opportunity in identifying patients at risk for developing a CSDH after head injury. As with an ASDH, the lack of prior imaging in all CSDH patients makes it is impossible to define exactly how many CSDHs originate as SDGs and to what extent this crosses over with cerebral atrophy. During chapter two and seven imaging correlations for SDG and cerebral atrophy in relation to CSDH pathology are explored.

1.1.3 CSDH membranes; angiogenesis and fibrinolysis.

A summary of factors discussed thus far, which contribute to CSDH formation including age (predisposing to cerebral atrophy) and trauma (leading to ASDHs and SDGs) are shown in Figure 1.3. Pharmacological anti-aggregation (AA) and anti-coagulation (AC) therapies are also potential risk factors for increasing bleeding risk, although some AAs such as aspirin have anti-inflammatory properties which may also be relevant (see section 1.4.3). The final common stem of the pathway is inflammation, which is discussed in section 1.2, but a crucial source of inflammation, and bleeding, are the CSDH membranes, discussed here.

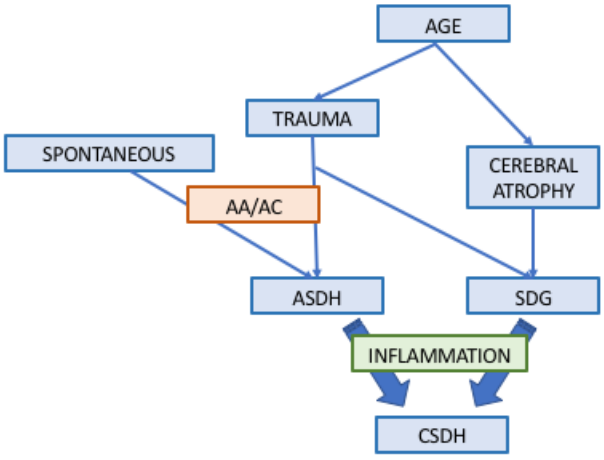

Figure 1.3; processes involved in CSDH pathophysiology.

As previously discussed, the CSDH membranes form between the normally closely opposed dura mater and arachnoid mater (Figure 1.1 & 1.2). Proliferation of dural border cells form two new membranes enclosing the subdural cavity, which fills with fluid and haematoma. High concentrations of type-1 (PICP) and type-3 (PIIINP) procollagen are present in this fluid and suggest a fibro-proliferative process occurs, similar to that seen in wound healing (Heula, Sajanti, & Majamaa, 2009; Sajanti & Majamaa, 2003). This process is likely to occur as part of an attempt to repair the separated and damaged dural border cells. However, the collagen synthesis outweighs its breakdown, leading to the continuous formation of a collagen matrix and hence formation of the new CSDH membranes, or neomembranes (Heula et al., 2009). Internal and external membranes are formed, which relate to the layers contiguous with the arachnoid and dura mater respectively.

The internal membrane is reported as containing collagen and fibroblasts only, and is therefore relatively non-functional with respect to driving CSDH growth (Sato & Suzuki, 1975). One histological study using a scanning electron microscope (SEM) identified occasional blood vessels in the internal membrane, but even these seem to disappear with maturation of the CSDH (Moskala et al., 2007). The external membrane, however, is considered more crucial in driving CSDH growth. It contains layers of fibroblasts and collagen fibers with the addition of inflammatory cells such as neutrophils, lymphocytes and macrophages (Hara, Tamaki, Aoyagi, & Ohno, 2009; Moskala et al., 2007; Nanko et al., 2009; Shono et al., 2001). Importantly there are numerous highly permeable capillaries with thin walls containing thin or absent basement membrane and lacking smooth muscle cells and pericytes (Moskala et al., 2007; Yamashima, Yamamoto, & Friede, 1983). Gap junctions are also abundant within the capillary walls, allowing continued migration of erythrocytes, leucocytes and plasma from these vessels into the CSDH cavity (Sato & Suzuki, 1975; Yamashima et al., 1983).

Development of new vessels (angiogenesis) within the CSDH membranes, is mediated by several markers. This includes angiopoietins (Ang); a group of growth factors involved in regulating angiogenesis and vascular permeability. They have been reported to play an important role in permeability of normal brain membranes, the so-called blood-brain-barrier (BBB), and therefore it is unsurprising that they may also be implicated in CSDH membrane permeability (Helmy, De Simoni, Guilfoyle, Carpenter, & Hutchinson, 2011). The two

ligands, Ang-1 and Ang-2, have the same tyrosine kinase receptor (Tie-2) but opposing effects on angiogenesis (Jones, Iljin, Dumont, & Alitalo, 2001). Higher levels of Ang-1 are present in mature vascular beds with stable microvascular networks and low permeability, as seen in normal brain tissue (Hohenstein, Erber, Schilling, & Weigel, 2005). In contrast, the CSDH outer membrane has been shown to over-express Ang-2 mRNA, which is likely to represent on-going angiogenesis with destabilization of blood vessel structure (Hohenstein et al., 2005). Vascular endothelial growth factor (VEGF) is another important angiogenic growth factor (discussed in more detail in section 1.2), which can modulate Ang-2, with both elevated at the time of neovascularization following ischaemia (Zhang et al., 2002). Hence, Ang-2 and VEGF over-expression may be the driving force for the new, fragile vessel formation in the CSDH membrane. This network of fragile vessels is the primary source of haemorrhage and serum protein exudation contributing to progressive CSDH growth (Fujisawa, Nomura, Tsuchida, & Ito, 1998; Tokmak, Iplikcioglu, Bek, Gokduman, & Erdal, 2007).

Haemorrhage is an essential part of CSDH formation and involves two key biomolecular processes; coagulation and fibrinolysis. Activation of the coagulation cascade involves converting prothrombin to thrombin, the latter of which can then cleave fibrinogen into fibrin, allowing clot formation (Figure 1.4) (Chapin & Hajjar, 2015). Fibrinolysis is the converse process, whereby fibrin is broken down into fibrin degradation products (FDPs) and clot breakdown facilitated. Plasmin is required to activate fibrinolysis; it is formed from plasminogen by tissue plasminogen activator (tPA) (Chapin & Hajjar, 2015).

A study utilising Cr-labelled erythrocytes showed that CSDHs contain, on average, 6.7% acute haemorrhage, and that all CSDHs contain at least some active haemorrhage, which is highest in patients presenting with worse neurological deficits and higher density on CT (Ito, Yamamoto, Saito, Ikeda, & Hisada, 1987). High levels of FDPs in CSDH fluid are thought to represent excessive fibrinolysis, promoting continued haemorrhage (Heula, Ohlmeier, Sajanti, & Majamaa, 2013; Ito, Yamamoto, Komai, & Mizukoshi, 1976; Nomura, Kashiwagi, Fujisawa, Ito, & Nakamura, 1994b; Shim, Park, Hyun, Park, & Yoon, 2007). The levels of fibrin and FDPs are also higher in certain CSDH subtypes; mixed density and layered (Nomura, Kashiwagi, Fujisawa, Ito, & Nakamura, 1994a). This may signify different bleeding frequencies in CSDHs over time, causing haematoma growth to vary and different

patterns of blood to appear on imaging. These patterns and their possible relevance to CSDH pathology and prognosis are discussed in the imaging chapter, chapter seven.

Higher levels of plasminogen at initial operation have been identified in patients subsequently experiencing a CSDH recurrence (Weir & Gordon, 1983). This suggests that markers of hyper-fibrinolysis may be able to predict those at highest risk of recurrence and therefore help guide treatment and/or follow-up. Similar findings have shown that higher levels of tPA at initial surgery indicate a higher risk of recurrence (Katano, Kamiya, Mase, Tanikawa, & Yamada, 2006). The tPA has been shown to be produced by blood vessel walls in the CSDH external membrane, and soluble tPA can diffuse directly into the CSDH cavity (Ito, Komai, & Yamamoto, 1978). Thrombomodulin also enhances fibrinolysis, and has been found in high levels in CSDH fluid, again with a suggested source from the vessel endothelium within the membranes, and a possible link to increased recurrence rates in certain CSDH sub-types on imaging (Kitazono et al., 2012; Murakami et al., 2002).

Paradoxically, the hyperfibrinolytic mechanism causing CSDH growth has also been exploited as a treatment during CSDH surgical evacuation, with tPA infusion into the subdural space used to aid haemtoma liquefaction and washout (Neils et al., 2012). This led to substantially increased post-operative drainage volumes and significantly lower recurrence rates, but was only performed on 15 patients so is not well validated. The success of this treatment is likely to hinge on the very short half-life of most tPA drugs. Thus, short-term fibrinolysis whilst the CSDH is under-going drained may be helpful whilst more chronic hyperfibrinolysis only contributes to CSDH growth.

The external membrane shows evolutionary changes over time with inflammation causing progression to a more mature, so-called “scar” membrane (Gandhoke, Kaif, Choi, Williamson, & Nakaji, 2013; Moskala et al., 2007). This type of membrane is associated with higher haemtoma thickness and attenuation on CT and suggests that CSDH growth and haemorrhage increases as the membranes mature and organise (Gandhoke et al., 2013). Asan et al. showed that CSDHs containing acute or subacute components expand the most frequently and rapidly, thus exemplifying this late stage of escalating haemorrhage and growth (Asan, 2018). Protein exudation is also at its highest rate in patients with worse neurology and mixed density CSDH, and therefore likely occurs in tandem with the

progressive haemorrhage (Tokmak et al., 2007). Analysis of CSDH contents, including ratio of acute haemorrhage and correlations with findings on imaging will be explored in chapters three (CSDH composition) and seven (imaging).

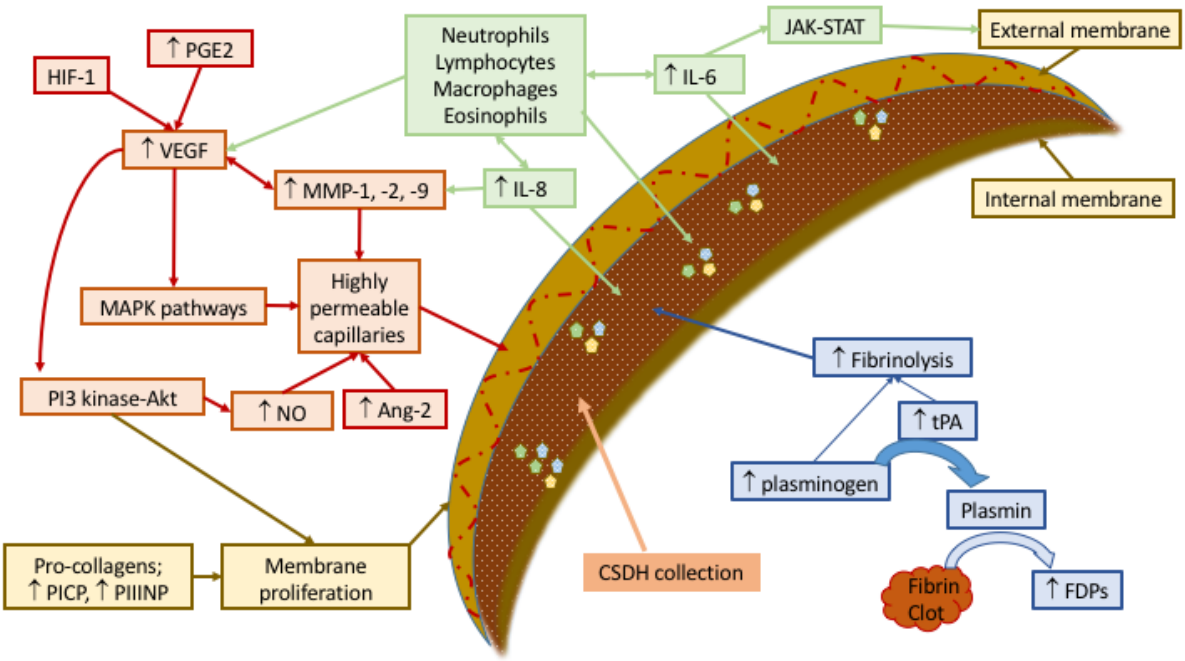

Figure 1.4; CSDH membranes, angiogenic, fibrinolytic and inflammatory processes in CSDH formation.

1.2 Inflammatory markers of interest in CSDH

This thesis aims to investigate the hypothesis that the linchpin of CSDH formation is inflammation. A range of inflammatory mediators that may potentiate this are shown in Table 1.1, and are the topic of interest in this thesis. CSDH expansion has been postulated to occur due to the continued cycle created by chronic inflammation which creates a hypoxic environment, stimulating further mediator production (Tsutsumi et al., 2017).

Table 1.1; key inflammatory mediators that may play a role in CSDH inflammation

Inflammatory mediators	Finding in CSDH	References
Vascular Endothelial Growth Factor (VEGF)	Pro-angiogenic factor. Very high levels in CSDH fluid and mRNA in membranes and neutrophils.	(Hara et al., 2009; Hohenstein et al., 2005; Hong et al., 2009; Hua et al., 2016; Kalamatianos et al., 2013; Nanko et al., 2009; Shono et al., 2001; Weigel, Schilling, & Schmiedek, 2001)
Matrix Metalloproteases (MMPs)	Present in outer membrane and CSDH fluid. Contributes to poor capillary integrity.	(Hua et al., 2016; Jung et al., 2006; Nakagawa, Kodera, & Kubota, 2000)
Interleukin-1 α and β	Pro-inflammatory markers important in acute TBI with very limited investigation in CSDH to date.	(Hutchinson et al., 2007; Pripp & Stanisic, 2014)
Interleukin-6 and 8	Pro-inflammatory markers elevated in CSDH fluid, particularly in recurrent CSDH.	(Frati et al., 2004; Hong et al., 2009; Kitazono et al., 2012; Pripp & Stanisic, 2014)
Interleukin-10	Anti-inflammatory marker elevated in CSDH, higher levels may help prevent recurrence.	(Pripp & Stanisic, 2014; Stanisic, Aasen, et al., 2012; T. Wada et al., 2006)
TNF-α	Reduced levels in CSDH.	(Stanisic, Aasen, et al., 2012)
Chemokines; MCP-1 and IP-10	Raised in CSDH fluid, likely to contribute to pro-inflammatory.	(Stanisic, Lyngstadaas, et al., 2012)

(IP = Interferon-gamma-induced protein, MCP = monocyte chemoattractant protein, TNF = tumour necrosis factor)

1.2.1 Vascular endothelial growth factor (VEGF)

VEGF is a family of seven growth factors which are the most important pro-angiogenic factors, and are involved in potentiating microvascular permeability (Hoeben et al., 2004). All members of the VEGF family share a common domain and share two tyrosine kinase receptors (VEGF-R), but for the purpose of this study it is considered as one factor (Hoeben et al., 2004). There is a wealth of evidence that VEGF and VEGF-R are found in significantly higher concentrations in CSDH fluid compared with peripheral blood and CSF (Hara et al., 2009; Hohenstein et al., 2005; Hua et al., 2016; Kalamatianos et al., 2013; Nanko et al., 2009; Shono et al., 2001; Weigel et al., 2001). The difference in CSDH levels of VEGF can be more than 28 times that found in serum, with example serum levels of 355 pg/mL compared to 10,277 pg/mL in the CSDH fluid (Shono et al., 2001). This difference is higher than that seen with any of the subsequent inflammatory markers discussed.

The source of this VEGF is debated but studies have shown it may be produced by neutrophils from within the CSDH fluid, infiltrating macrophages or the vascular endothelial cells within the CSDH membrane (Hohenstein et al., 2005; Nanko et al., 2009; Shono et al., 2001). Either way, an excess of VEGF is capable of inducing angiogenesis and excessive vascular permeability which may contribute to the on-going rebleeding implicated in CSDH growth (Shono et al., 2001). This is supported to some degree by correlations between VEGF concentrations and CSDH imaging-subtypes which are thought to reflect increased re-bleeding (Hua et al., 2016; Nanko et al., 2009). Further to this, higher levels of VEGF expression in the external membrane has been associated with a higher probability of CSDH recurrence (Hong et al., 2009). The external membrane has also provided information on the potential downstream signaling pathways involved, with Ras-Raf-MEK-ERK molecules identified as important in the pathway of VEGF-induced angiogenesis and disruption of endothelial gap junctions (Osuka et al., 2012).

Other factors directly related to VEGF are also likely to be important, such as prostaglandin E (PGE2), which is synthesised from arachidonic acid by cyclooxygenase (COX)-2 and regulates VEGF expression (Hoeben et al., 2004). High PGE2 levels have been found in CSDH fluid, and correlated to the time interval from trauma, suggesting it might be a useful marker for monitoring escalating inflammation in CSDH (Hara et al., 2009). Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor integral to the body's response to

hypoxia, which also regulates VEGF and VEGF-R gene expression (Hoeben et al., 2004). Positive staining for this factor has been found in CSDH membranes and correlates with VEGF concentration, so may also be a potential target for assessing the inflammatory response (Nanko et al., 2009).

Although VEGF has a pro-angiogenic and potentially pro-inflammatory role it also has a contrasting role in promoting wound repair and neuroprotection, with a role in neurogenesis and recovery from traumatic brain injury (TBI) (Helmy et al., 2011; Hoeben et al., 2004; Thau-Zuchman, Shohami, Alexandrovich, & Leker, 2010). This exemplifies the paradoxical roles some mediators have in both potentially harmful inflammation and beneficial repair mechanisms, making them a challenging therapeutic target.

1.2.2 Matrix metalloproteinases (MMPs)

MMPs are a family of proteolytic enzymes responsible for digesting the extracellular matrix and are released by endothelial cells during the early stages of vessel growth (Burbridge et al., 2002). They play a critical role in angiogenesis, and their inhibition leads to suppressed angiogenic response with fewer and shortened blood vessels (Burbridge et al., 2002). Increased MMP expression is seen in almost all human diseases involving inflammation, and contributes to the inflammatory processes by modulating other mediators (e.g. cytokines and chemokines) (Manicone & McGuire, 2008). MMP-proteolysis of endothelial cell junctional proteins also alters barrier permeability, aiding infiltration of inflammatory cells into otherwise privileged compartments such as across the blood-brain-barrier (BBB) (Manicone & McGuire, 2008). High MMP-9 and MMP-2 expression have been identified in haemorrhagic brain tumours with associated basement membrane disruption (Jung et al., 2006). It was suggested that these MMPs, alongside VEGF, were responsible for the instability of newly formed blood vessels in the tumour, leading to a higher risk of haemorrhage. High levels of MMP-9 have also been identified in the peripheral blood, CSF and in peri-contusional brain fluid in acute TBI patients, and may be contributing to BBB breakdown and hence brain oedema (Gong et al., 2012; Grossetete, Phelps, Arko, Yonas, & Rosenberg, 2009; Guilfoyle et al., 2015)

Two studies examining MMPs in CSDH membranes and fluid have highlighted MMP-1, -2 and -9 as being present and likely factors contributing to the formation of fragile, leaky

capillaries causing haemorrhage into CSDHs (Hua et al., 2016; Nakagawa et al., 2000). The levels of MMP-2 and -9 also correlated with VEGF concentration, suggesting a combined angiogenic process (Hua et al., 2016).

1.2.3 Cytokines

Cytokines are a crucial part of the body's immune response. They are small, inducible proteins that are released by all cell types involved in the inflammatory response. Different families of cytokines are described, based on their structure and receptor-type, with chemokines being a sub-family specifically involved in leukocyte recruitment. It is difficult to know the relevance of each individual cytokine as it is well recognised that they work in cascades, influence one another, can compete for receptors and act antagonistically or synergistically. A gross analysis of pro- and anti-inflammatory cytokines in CSDH, shows they are both raised in CSDH fluid compared with serum, but that the balance is significantly more pro- than anti-inflammatory (Pripp & Stanisic, 2014; Stanisic, Aasen, et al., 2012). It is important to consider the balance of both pro- and anti-inflammatory molecules, and how this changes over time. Not all inflammatory markers are detrimental all of the time and certain markers which are perceived to be harmful may be required in reparative processes at later time points. However, there is evidence that elevated levels of many cytokines (such as IL-1, IL-6, IL-8 and TNF-α) have been associated with worse outcomes following TBI, therefore they may also be valuable in predicting outcome in CSDH (Rodney, Osier, & Gill, 2018).

Interleukin 1 (IL-1)

IL-1 was the first cytokine to be discovered, and although 11 different molecules compose the family (IL-1α, IL1-β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-1Ra, IL-36Ra, IL-38, IL-37), often only IL-1α and -1β are considered when referring to IL-1 (Garlanda, Dinarello, & Mantovani, 2013). These two ligands have very similar biological properties which are considered pro-inflammatory and they act on the same receptor, IL-1R1.

IL-1α mediates the early phase of inflammation and it is present as a precursor in astrocytes, which is released when cell death via necrosis occurs (Garlanda et al., 2013). It is then immediately active and behaves as an "alarmin" for the inflammatory cascade of cytokines and chemokines to begin. IL-1β is also a precursor but requires cleavage with caspase-1 to release the activated form into the extra-cellular space (Garlanda et al., 2013). It is produced

by haematopoetic cells such as blood monocytes, macrophages, dendritic cells and brain microglia. Both play a role in the adaptive immune response by enhancing the function of B and T cell subsets and activate neutrophils, monocytes and macrophages as part of the innate immune response (Sims & Smith, 2010). The IL-1R1 receptor can be blocked by binding of IL-1ra which inhibits the actions on IL-1 and therefore potentially has an anti-inflammatory effect (Hutchinson et al., 2007; Rothwell & Luheshi, 2000). IL-1 activity can also be reduced by binding it to an alternate receptor, IL-1RII, which is a decoy receptor with no onward signaling, and has been shown to be induced by dexamethasone, exerting an anti-inflammatory effect (Colotta et al., 1993).

When assessing the cerebral response to TBI, IL-1α and IL1-β and their receptor activation has been considered important. Mouse studies have demonstrated that inhibition of IL-1β can results in reduced microglial activation and infiltration with neutrophils and T cells, which may relate to reduction in hemispheric tissue loss and cognitive decline (Clausen et al., 2009). This is supported by human TBI studies which have correlated high levels of IL-1ra with a more favourable neurological outcome than patients with low levels (Hutchinson et al., 2007). IL-1ra/IL-1β ratio was also significantly higher in patients with a favourable outcome and it is suggested that this ratio may relate to the balance between the patients pro- and anti-inflammatory state. Early clinical and pre-clinical studies have supported IL-1ra as a potentially protective treatment against brain injury from conditions such as stroke and subarachnoid haemorrhage, which drives continued interest in this molecule (Brough, Rothwell, & Allan, 2015). Despite this, there has been relatively little focus on IL-1 in CSDH. Only one study has measured IL-1β and perhaps surprisingly the levels were significantly lower in CSDH fluid when compared with serum (Pripp & Stanisic, 2014). This study also looked at IL-1ra, and found no significant difference in levels when comparing serum and CSDH fluid. No studies have reviewed IL-1α. If inflammation is considered more critical than trauma in the formation of a CSDH, then this may explain why the role of IL-1 is very different, this will be explored further in the neurochemistry work in this thesis.

Interleukin 6 and 8 (IL-6 and IL-8)

IL-6 and IL-8 are being discussed together because in many conditions their production is coordinated, possibly due to a common signaling pathway (Kishimoto, Akira, & Taga, 1992).

IL-6 is an important inflammatory cytokine secreted by a variety of cells including fibroblasts, monocytes and endothelial cells (Benveniste, 1998). It has a key role in the acute response to inflammation, promoting B and T cell differentiation, platelet production, acute phase protein induction and can enhance leukocyte recruitment by upregulating chemokines adhesion molecules (Benveniste, 1998; Kishimoto et al., 1992). It is released in response to soft tissue trauma and haemorrhage, and dysregulated IL-6 has been linked to autoimmune diseases and cancer (Ayala et al., 1991; Kishimoto et al., 1992). It is also recognised to have some neurotropic and neuroprotective effects, particularly within the field of TBI (Benveniste, 1998; Helmy et al., 2011).

IL-8 is from a family of small, polypeptide cytokines that have pro-inflammatory and reparative properties (Oppenheim, Zachariae, Mukaida, & Matsushima, 1991). It is classically recognised as a neutrophil-chemoattractant, with IL-8 acting as a stimulus to draw neutrophils, and other inflammatory cells carrying the IL-8 receptor, to the site of IL-8 release (Oppenheim et al., 1991). Conversely, it has also been shown to act in an anti-inflammatory manner, limiting the extent of leucocyte adhesion to vessel walls at sites of inflammation (Gimbrone et al., 1989). It is certainly known to be able to modulate its own receptor expression, and this may be important in controlling response to inflammation. More latterly IL-8 has also been recognised to play a key role in angiogenesis, with production from endothelial cells, leucocytes and fibroblasts resulting in capillary tube formation, endothelial cell proliferation and MMP-2 release (Li et al., 2005).

Both IL-6 and IL-8 have been identified as being significantly elevated in CSDH fluid (Frati et al., 2004; Hong et al., 2009; Kitazono et al., 2012; Stanisic, Aasen, et al., 2012). The JAK-STAT transcription factors have been identified in external membranes as the potential down-stream signaling pathway regulated by the IL-6 (Osuka et al., 2016; Osuka et al., 2013). Most significantly, high levels of both IL-6 and IL-8 have been correlated with increased risk of CSDH recurrence, and the related imaging features of recurrence (Frati et al., 2004; Hong et al., 2009). As discussed, whilst these cytokines can be considered pro-inflammatory, they also have protective functions. Therefore, it is difficult to know whether high levels in patients with recurrence signifies that they are the driving force for CSDH expansion or whether they are indicative of the protective response occurring in reaction to an expanding CSDH. A lower risk of recurrence has been correlated with increased levels of

anti-inflammatory cytokines (i.e. IL-10) (Pripp & Stanisic, 2014; Stanisic, Aasen, et al., 2012). Therefore, the overall balance of cytokines may be useful in predicting the risk of recurrence. Cytokine patterns have also been correlated with the time interval since injury and it may be this, and hence the stage of the inflammatory cycle, that is actually important in predicting recurrence (Pripp & Stanisic, 2014; Stanisic, Aasen, et al., 2012).

Interleukin 10 (IL-10)

Considered an anti-inflammatory cytokine, IL-10 has a role in deactivating T cells, monocytes and macrophages and reducing subsequent pro-inflammatory cytokines production (e.g. IL-1) (Moore, O'Garra, de Waal Malefyt, Vieira, & Mosmann, 1993; Seymour & Henderson, 2001). In one study on CSDH patients, the majority were found to have low (<60 pg/ml) rather than high (>200 pg/ml) concentrations of IL-10 (T. Wada et al., 2006). The lower concentrations were also associated with lower concentrations of IL-6 and -8 suggesting there is a reciprocal response of anti-to pro-inflammatory cytokines in the body's attempt to control inflammation. Some reports have suggested consistently raised levels of IL-10, as well as the anti-inflammatory cytokine IL-13, when comparing CSDH fluid with serum (Kitazono et al., 2012; Stanisic, Aasen, et al., 2012). As cytokines are known to vary in their temporal profile, the likelihood is that levels will vary between patients depending on the stage of inflammation at the time of sampling. Different inclusion criteria for studies and time patterns from trauma to surgery might be important. Observing the relative levels of cytokines at more than one time-point would be helpful in clarifying the patterns occurring. This thesis aims to obtain such data for the first time, with inflammatory marker analysis at multiple time points from operation up to three days post-operatively (see chapters five and six).

Tumour Necrosis Factor (TNF-α)

TNF-α is widely accepted as a pro-inflammatory cytokine which has two receptors, one of which is also linked to the activation of nuclear factor kappa B (NFκB), a family of transcription factor proteins involved in apoptosis (Casault et al., 2018) and other inflammatory processes. As well as relating to cellular apoptosis and necrosis, TNF-α has been identified to have a range of effects specifically related to TBI, including changes in endothelial permability, cerebral oedema and translocation of leucocytes (Casault et al.,

2018). The resultant affect appears to be an increased mortality and poor outcome in TBI patients who have elevated levels of TNF-α (Casault et al., 2018).

Only one study has assessed TNF-α in CSDH fluid and found reduced levels compared to serum, but very little is known about its interaction with other cytokines and how this relates to outcome in CSDH (Stanisic, Aasen, et al., 2012).

1.2.4 Chemokines

Chemokines are small proteins, often released in response to cytokines, which are responsible for trafficking of cells such as monocytes, neutrophils and lymphocytes, to the site on inflammation (Deshmane, Kremlev, Amini, & Sawaya, 2009).

Monocyte chemoattractant protein-1 (MCP-1), also called Chemokine ligand 2 (CCL2), as the name suggests is responsible for attracting monocytes and can be produced by cell types such as endothelial and fibroblasts but mostly by the monocytes themselves (Deshmane et al., 2009). The activity of monocytes can be varied and includes macrophage-mediated angiogenesis, which may also be relevant to CSDH pathophysiology (Deshmane et al., 2009). Raised levels of MCP-1 have been identified in human CSF in the early stages following TBI, and mouse models suggest the associated macrophage accumulation with MCP-1 may contribute to secondary brain injury (Semple, Bye, Rancan, Ziebell, & Morganti-Kossmann, 2010). Other mouse studies looking at different types of cortical brain injury have shown rapid accumulation of macrophages after injury with associated increased levels of MCP-1, Interferon gamma-induced protein-10 (IP-10) and macrophage inflammatory protein (MIP-1) mRNA expression (Hausmann et al., 1998). IP-10, also called chemokine ligand 10 (CXCL10), can attract both monocytes and T lymphocytes, whilst MIP-1α and β, also known as chemokine ligand 3 (CCL3), attract different types of lymphocyte to the site of inflammation (Schall, 1993; Taub et al., 1993).

Only one study has assessed MCP-1 and IP-10 in CSDH and found both to be in higher concentrations in CSDH compared to serum, but little is known about how they interact with other chemokines and cytokines (Stanisic, Lyngstadaas, et al., 2012). No previous studies have investigated MIP-1 in CSDH, therefore this will be a novel marker.

1.3 Imaging and CSDH recurrence

Application of CT in clinical CSDH diagnosis is ubiquitous, therefore whilst other imaging techniques such as magnetic resonance imaging (MRI) may provide more detailed information (Hosoda, Tamaki, Masumura, Matsumoto, & Maeda, 1987), CT has more clinical relevance. Overall, CT is a reliable tool for visualising a CSDH and provides information on the volume, density and impact (mass effect) of the CSDH on underlying brain (Figure 1.5).

Figure 1.5; example CT of CSDH, areas of high density (white) and low density (black) and mass effect exerted on underlying brain causing compression of normal brain structures.

On normal CT imaging the structures found within the intracranial cavity include grey matter, white matter and cerebrospinal fluid (CSF). Each of these have different densities on CT, as measured in Hounsfield units (HU) (Cala, Thickbroom, Black, Collins, & Mastaglia, 1981). In general blood appears as "hyperdense", or white, in relation to surrounding tissues, but the density gradually decreases as clotted blood ages, and can eventually overlap with that of grey matter, making distinguishable boundaries difficult (Table 1.2) (Ito et al., 1984; Nowinski et al., 2014). This commonly occurs in CSDH, where the haematoma can be classified as "isodense" and hence be of a very similar density to underlying grey and white matter. Very high density areas can also represent either acute haemorrhage or membranes, which can also appear white, but usually have more of a "trabecular" pattern, as seen in Figure 1.5.

Table 1.2; density of white matter, grey matter and haematoma on CT.

Substance	**Mean x-ray absorption density (HU)**	**Reference**
White matter	28 - 30	(Cala et al., 1981; Nowinski et al., 2014)
Grey matter	33 - 37	(Cala et al., 1981; Nowinski et al., 2014)
IVH/ICH in acute stroke patients	59 (Range 25-88); 60 day one to 53 day eight+	Nowinski et al. 2014
CSDH	15 - 50	(Ito et al., 1984)

(IVH = intraventricular haemorrhage, ICH = intracerebral haemorrhage).

Several previous studies have described how the pattern of density within a CSDH on CT relates to different stages of pathological growth and therefore recurrence risk (Huang, Lin, Lu, & Chen, 2014; Jack, O'Kelly, McDougall, & Findlay, 2015; Nakaguchi et al., 2001; Ohba, Kinoshita, Nakagawa, & Murakami, 2013; Stanisic & Pripp, 2017; Yamamoto et al., 2003; Yan, Yang, & Huang, 2018). However, the classification of density patterns is entirely subjective and varied between published studies making comparison of the findings challenging, and at times causing conflicting results. With a large cohort of patients recruited to the Dex-CSDH study with CT imaging available, this provided the opportunity to assess density patterns in detail and relate the findings to an objective computed-automated quantification of density. This is discussed in further detail in Chapter seven.

Imaging findings can also reflect the histology of CSDH membranes and relate to the clinical state of the patient at presentation. One study showed how more mature membranes (with scarring) correlated with hypodense haematomas, whilst immature membranes had more acute bleeding (hyperdensity) and was associated with a worse clinical state (Gandhoke et al., 2013). The authors postulated that more immature membranes represented an earlier stage in the inflammatory cycle which was more rapidly expanding, hence the associated worse clinical presentation (Gandhoke et al., 2013).

Finally, imaging is necessary to diagnose reaccumulation, or recurrence, of a CSDH, rates of which have been reported to range from 6.1% - 22%, (Baechli et al., 2004; Berghauser Pont, Dammers, Schouten, Lingsma, & Dirven, 2012; Brennan et al., 2017; Gelabert-Gonzalez et

al., 2005; Goto et al., 2015; Santarius & Hutchinson, 2009; Stroobandt et al., 1995), with an average time period to recurrence around 35 days in one series on 414 patients (Goto et al., 2015). Application of a post-operative subdural drainage has been a significant factor in reducing recurrence rates in the last decade, reducing them to an average of 9% from a recent national UK audit (Almenawer et al., 2014; Brennan et al., 2017; Liu et al., 2014; Santarius & Hutchinson, 2009). Several studies have shown a higher recurrence rate in older patients (i.e >70 years old) compared with younger patients (Gelabert-Gonzalez et al., 2005; Stroobandt et al., 1995). The cause of this is not well defined but may be related to poorer brain re-expansion in the elderly. Certainly, higher residual post-operative volumes, which could be considered a corollary of poor brain re-expansion, have been linked to a higher recurrence risk (Stavrinou et al., 2017; Yan et al., 2018).

There is some debate about whether routine post-operative imaging is helpful in predicting CSDH recurrence. Some clinicians advocate a routine post-operative CT to get a baseline understanding of the extent of CSDH evacuation and rule-out complications, whilst others suggest that it is unnecessary radiation with no practical implications unless the patient has clinically deteriorated. Residual CSDH on post-operative imaging is common place, seen in 43-78% of patients, is normally resolved by day 40 post-operatively, and is not correlated with clinically significant recurrence or outcome (Goto et al., 2015; Markwalder, 1981).

1.4 Pharmacological treatments and CSDH

For patients who become symptomatic from CSDH, which can range from headaches, to neurological deficits and finally coma, surgery is often considered the mainstay of treatment. In cases where patients are critically unwell they require emergency evacuation of the CSDH to resolve the acute decompensation due to raised intracranial pressure. However, there is a large cohort of patients who are clinically stable with appearances of CSDH found on imaging who would be appropriate for a more conservative treatment pathway, such as a drug therapy. There is also the risk of CSDH recurrence in the surgically treated patients, and therefore the opportunity to implement additional peri-operative therapies targeted at preventing recurrence and further surgery. Scientists have sought to discover drugs which influence the inflammatory pathophysiological processes involved in CSDH discussed earlier, in the hope that they can provide a conservative and potentially preventative treatment option for CSDH. These therapies will be further discussed further here.

1.4.1 Dexamethasone

Steroids have long been used in the context of CSDH, and in 1974 it was summarised that steroids aid the resolution of CSDH, supporting medical management rather than surgery in some cases (Bender, 1974). Dexamethasone is a synthetic version of naturally-occurring corticosteroid hormone, and was first made in 1958 and heralded as a potent anti-inflammatory drug (Arth et al., 1958). In 1976 Glover and Labadie used an experimental rat model of CSDH to show that dexamethasone could inhibit the formation of an expanding encapsulated haematoma from implanted blood clot, which did form in 47% of the untreated rats (Glover & Labadie, 1976).They suggested that dexamethasone inhibited the inflammatory response and hence the process driving membrane development, the latter of which was an essential source of continued haemorrhage allowing CSDH growth.

In 2005, Sun. et al showed that dexamethasone could be successfully employed as a conservative treatment option, with 25/26 patients who received dexamethasone and no surgery experiencing complete CSDH resolution (Sun, Boet, & Poon, 2005). Several other studies have supported these findings of successful conservative management but also shown reductions in recurrence in surgical patients treated additionally with dexamethasone (Berghauser Pont, Dammers, et al., 2012; Delgado-Lopez et al., 2009; Dran, Berthier, Fontaine, Rasenrarijao, & Paquis, 2007; Qian, Yang, Sun, & Sun, 2017; Thotakura &

Marabathina, 2015). However, further level one evidence is still needed to support the implementation of dexamethasone in improving long term outcome for CSDH patients (Berghauser Pont, Dirven, Dippel, Verweij, & Dammers, 2012).

It is well recognised that the anti-inflammatory effect of corticosteroids is mediated through gene expression, altering transcription of inflammatory proteins such as cytokines and chemokines (Barnes, 1998; Czock, Keller, Rasche, & Haussler, 2005; Dietrich, Rao, Pastorino, & Kesari, 2011; Dinarello, 2010). Other signaling pathways involved in inflammation and membrane function are also targeted and there is a significant effect on differentiation and function of immune cells, such as B and T cells, dendritic cells and macrophages (Coutinho & Chapman, 2011; Czock et al., 2005; Dietrich et al., 2011). Some clinical studies have identified mediators that reflect the anti-inflammatory mechanisms of steroids in the brain, such as tuberculous meningitis patients showing decreased MMP-9 levels in CSF following administration of corticosteroids (Green et al., 2009). Cerebral extracellular fluid (brain microdialysate) in brain tumour patients receiving dexamethasone has also shown significantly increased concentrations of anti-inflammatory mediators such as IL-1ra and tissue inhibitor of metalloproteinase-1 (TIMP-1) (Marcus, Carpenter, Price, & Hutchinson, 2010).

Despite the role of dexamethasone as an anti-inflammatory, since the 1960s its primary application in neurosurgery has been in the treatment of cerebral oedema (Maxwell RE, 1972). Originally the focus was on cerebral oedema secondary to brain tumours but also included oedema following TBI or haemorrhage (French, 1966; Maxwell RE, 1972). Whilst its efficacy in improving oedema related to tumours has remained, it benefits vary depending on the underlying pathology and it is now considered harmful in the context of TBI (Edwards et al., 2005). Steroids continue to be used to reduce oedema in other conditions such as spinal cord compression and high-altitude pulmonary and cerebral oedema (Siegal, 1995; Stream & Grissom, 2008).

The suggested mechanism of action of dexamethasone in reducing cerebral oedema is a reduction in vascular permeability at the BBB through modification of capillary endothelial cells and regulation of tight junctions via expression of the occludin gene (Andersen, Haselgrove, Doenstrup, Astrup, & Gyldensted, 1993; Andersen & Jensen, 1998; Forster et

al., 2005; Ostergaard et al., 1999). This all leads to "tightening" of the BBB and hence difficulty for fluid, but also other inflammatory cells such as leucocytes, to enter the brain. It is plausible that dexamethasone has the same effect on the vascular endothelium of the "leaky" blood vessels seen in CSDH membranes, thereby reducing fluid exudation and bleeding and allowing resolution of the collection. The anti-inflammatory effect of dexamethasone is a more commonly accepted explanation for its role in CSDH, but as this has never been tested at a molecular level, the true mechanism remains unknown.

One of the major drawbacks of steroid therapy is the significant side-effect profile seen with systemic application, a challenge which may out-weigh the benefits of its use (Prud'homme, Mathieu, Marcotte, & Cottin, 2016; Weissman, Dufer, Vogel, & Abeloff, 1987). Brain tumour studies have shown that the toxic effects of dexamethasone are dose-related, and lower daily doses (4 mg compared to 16 mg) and courses shorter than 28 days reduce this risk significantly (Vecht, Hovestadt, Verbiest, van Vliet, & van Putten, 1994). Dosing must also take into consideration the pharmacokinetic and dynamic properties of dexamethasone. It has very good oral bioavailability (76-90%) and high potency but a relatively short plasma half-life of 4-4.7 hours (Czock et al., 2005; Meikle & Tyler, 1977). It only takes 1-1.5 hours to reach its peak plasma concentration, hence the rapid clinical effect, but is considered a long-acting steroid with a biological half-life of 36-54 hours (Czock et al., 2005; Nicolaides NC, 2018). Several trials to establish the evidence for dexamethasone efficacy, and appropriate administration and dosing, for CSDH treatment are on-going throughout the world, including that which is discussed throughout this PhD; the Dex-CSDH trial (Emich et al., 2014; Henaux, Le Reste, Laviolle, & Morandi, 2017; Kolias et al., 2018; Miah et al., 2018).

1.4.2 Other drugs investigated as CSDH therapies

Dexamethasone is not the only drug that has been used as a treatment for CSDH, and several others are currently under investigation but are yet to show as much promise. These are not investigated in this thesis, but the data on inflammatory pathways may be useful in understanding the mechanisms of actions of these alternative drugs.

Atorvastatin, classically known as a cholesterol-lowering drug, has been suggested to have a range of properties relevant to CSDH such as anti-angiogenesis (inhibiting VEGF and IL-8), anti-inflammatory (reducing TNF-α and MCP-1) and even fibrogenic effects by reducing

collagen deposition (Araujo, Rocha, Mendes, & Andrade, 2010; Dulak et al., 2005). Recent publication of a large study on 196 Chinese patients randomised to 20mg atorvastatin or placebo found reduced haematoma size and surgical intervention rates in the atorvastatin arm (Jiang et al., 2018). This study was limited by a large list of exclusions and only recruited patients with a mild-moderate CSDH from out-patient clinics, many of whom would never have needed any treatment, as exemplified by only a 23.5% surgical intervention rate in the placebo arm. A further retrospective, observational study has suggested that peri-operative administration of atorvastatin may reduce recurrence (Tang et al., 2018), but well-designed randomised studies are needed to fully understand if atorvastin if useful in large, symptomatic CSDH.

Angiotensin Converting Enzyme (ACE) inhibitors may also help regulate some inflammatory markers as part of their primary application in coronary artery disease, where inflammation is involved in plaque rupture (da Silva, Fraga-Silva, Stergiopulos, Montecucco, & Mach, 2015). One early study suggested this could lead to lower recurrence rates in CSDH, possibly through reduction in VEGF (Weigel, Hohenstein, Schlickum, Weiss, & Schilling, 2007). However, several further studies have reported contradictory findings with similar or even increased recurrence rates in patients on ACE inhibitors (Bartek et al., 2018; Neidert et al., 2016; Poulsen, Munthe, Soe, & Halle, 2014)

Finally, the anti-fibrinolytic drug tranexamic acid has been shown to reduce death due to haemorrhage in acute trauma patients, and therefore may also be relevant to CSDH (Shakur et al., 2010). One small study reviewed 21 CSDH patients treated with tranexamic acid, where only three patients required surgery, and all had complete resolution of the CSDH (Kageyama, Toyooka, Tsuzuki, & Oka, 2013). Currently there is an on-going prospective trial assessing tranexamic acid in CSDH management and the results of this are keenly awaited (Iorio-Morin, Blanchard, Richer, & Mathieu, 2016).

1.4.3 Anti-aggregant and anti-coagulant drugs

The use of anti-aggregant (AA) medications, such as aspirin and clopidogrel, and anti-coagulant (AC) medications, such as warfarin and novel oral anti-coagulants (NOACs) are widespread within the elderly population.

Some studies report that AA/ACs do not increase the likelihood of an ASDH transforming into a CSDH, especially if coagulation is corrected at the time of ASDH (J. J. Lee, Won, Y., Yang, T., Kim, S., Choi, C. S., Yang, J., 2015; Lucke-Wold et al., 2016). Although patients given prothrombin complex concentrate do show faster resolution of their ASDH (Lucke-Wold et al., 2016). However, other studies suggest that AA and ACs are risk factors for CSDH development and can increased haematoma growth in both ASDHs and CSDHs (Asan, 2018; Laviv, 2014; Yang & Huang, 2017). Interestingly, the risk of CSDH appears to be lower for aspirin, which has some anti-inflammatory function as a cyclooxygenase inhibitor, compared with other AAs (clopidogrel) and ACs (De Bonis et al., 2013).

Whether AA and ACs increase the likelihood of CSDH recurrence and when they should be restarted is much debated, and a randomised trial in this area is needed (Aspegren, Astrand, Lundgren, & Romner, 2013; Fornebo et al., 2017; Gonugunta & Buxton, 2001; Nathan, Goodarzi, Jette, Gallagher, & Holroyd-Leduc, 2017; Poon & Al-Shahi Salman, 2018; Wang et al., 2017). The use of these mediations is reported in all patients recruited to the Dex-CSDH trial, and this data is referred to throughout the sub-study analyses to assess their role in CSDH pathophysiology.

1.5 The Dex-CSDH trial

The lack of high-quality, robust data to determine whether dexamethasone is an efficacious treatment for CSDH has led to wide variations in practice. As with all pharmacological therapies, one must weigh up the risks of the treatment with the potential benefits, and although the former are well known for steroids, the latter have not been well-established. Therefore, the Dex-CSDH trial was designed by a panel of experts to answer the question of whether giving dexamethasone to patients with a CSDH would improve their outcome at 6-months. The details of the trial are discussed at length in chapter eight, but in essence it is a randomised, placebo-controlled trial of dexamethasone for patients admitted to a neurosurgical unit with a CSDH. The investigational medicinal product (IMP), dexamethasone or placebo, would be given as a weaning course over two weeks and the patients monitored closely for 30 days for potential side-effects (electronic Medicines Compendium). The trial was not prescriptive other treatment for the CSDH; therefore, the IMP could be given as either a stand-alone treatment or in combination with surgery. The inclusion criteria were deliberately broad to allow maximum inclusion of CSDH patients, unlike many CSDH trials which appear to have long exclusion lists and thus suffer with selection bias. The aim was for the trial to be pragmatic and therefore highly relevant and translatable to neurosurgical clinical practice.

Many specialists contributed to the design and approval of this trial, and my role was to establish and run the trial throughout my PhD. As the Dex-CSDH trial research fellow I have been intimately involved in the day-to-day running of the trial, including site initiations, site queries, local recruitment, safety reporting and trial oversight. However, the clinical data produced from patients recruited to the trial is not a product of my own work, but rather that of the many clinical research nurses, neurosurgeons and other clinical trials staff involved at multiple sites across the UK. The final results of the trial are not presented here as the study will not complete until May 2019; although some blinded pilot data is discussed in chapter eight. During the running of the trial I established some independent sub-studies which ran at the sponsor site only (Cambridge). These sub-studies were my own design, with the support of supervisors, and were ethically approved to run in parallel with the main study. The findings from these sub-studies are the main focus of this thesis, and it is hoped that they will provide some scientific understanding for the final results of the Dex-CSDH trial.

1.6 Thesis outline

This thesis combines neurochemical analysis, imaging and clinical data to build a picture of the true pathophysiological events that take place during the development of CSDH. The purpose of this is to aid scientific understanding of the condition such that identification of primary and recurrent CSDH is more efficient, treatment options are better applied (particularly pharmacological agents such as dexamethasone) and ultimately patient outcomes improved.

Chapter two explores the theory of two different developmental origins of CSDH, a distinction which is increasingly apparent with frequent imaging of elderly patients following head trauma. These different origins may have implications for the natural history of each CSDH and guide our understanding of the underlying biological processes occurring. Chapter three details the specific components within a CSDH, attempting to understand the age of the blood present and how this can be used to predict outcome following surgical drainage.

Chapter four is primarily a methodological chapter which involved developing a process for analysing CSDH for the presence of dexamethasone to determine whether it enters the subdural space.

In chapter five the primary hypothesis underlying the pathophysiology of CSDH is explored through examination of inflammatory markers within CSDH fluid. Intra-operative and post-operative CSDH samples are interrogated for their inflammatory profiles and how this relates to CSDH recurrence. This links in closely to the findings discussed in chapter six, which relate the inflammatory profiles to dexamethasone exposure and other clinical factors.

Chapter seven is an extensive review of the CT imaging characteristics of CSDH, how they are best defined, measured and what clinical relevance they have.

Chapter eight includes my own reflections on the design and methodology related to the Dex-CSDH trial and some blinded data from the pilot period and early stage of the trial.

Finally, chapter nine concludes the findings from all the chapters, bringing together an overarching theory on how CSDH forms, recurs and can be treated. This involves considerations for future research on CSDH and how both clinical and research practice can be optimised in this field.

1.7 References

Ahn, J. H., Jun, H. S., Kim, J. H., Oh, J. K., Song, J. H., & Chang, I. B. (2016). Analysis of Risk Factor for the Development of Chronic Subdural Hematoma in Patients with Traumatic Subdural Hygroma. *J Korean Neurosurg Soc, 59*(6), 622-627. doi:10.3340/jkns.2016.59.6.622

Almenawer, S. A., Farrokhyar, F., Hong, C., Alhazzani, W., Manoranjan, B., Yarascavitch, B., . . . Singh, S. (2014). Chronic subdural hematoma management: a systematic review and meta-analysis of 34,829 patients. *Ann Surg, 259*(3), 449-457. doi:10.1097/SLA.0000000000000255

Andersen, C., Haselgrove, J. C., Doenstrup, S., Astrup, J., & Gyldensted, C. (1993). Resorption of peritumoural oedema in cerebral gliomas during dexamethasone treatment evaluated by NMR relaxation time imaging. *Acta Neurochir (Wien), 122*(3-4), 218-224.

Andersen, C., & Jensen, F. T. (1998). Differences in blood-tumour-barrier leakage of human intracranial tumours: quantitative monitoring of vasogenic oedema and its response to glucocorticoid treatment. *Acta Neurochir (Wien), 140*(9), 919-924.

Araujo, F. A., Rocha, M. A., Mendes, J. B., & Andrade, S. P. (2010). Atorvastatin inhibits inflammatory angiogenesis in mice through down regulation of VEGF, TNF-alpha and TGF-beta1. *Biomed Pharmacother, 64*(1), 29-34. doi:10.1016/j.biopha.2009.03.003

Arth, G. E., Johnston, D. B. R., Fried, J., Spooncer, W. W., Hoff, D. R., & Sarett, L. H. (1958). 16-Methylated steriods. I. 16 α -methylated analogs of cortisone, a new group of anti-inflammatory steroids. *Journal of the American Chemical Society, 80*(12), 3160-3161. doi:10.1021/ja01545a061

Asan, Z. (2018). Growth Potential of Subdural Hematomas Under Clinical Observation: Which Subdural Hematomas Tend to Grow and Why They Do. *World Neurosurg, 113*, e598-e603. doi:10.1016/j.wneu.2018.02.106

Asghar. (2002). Chronic subdural haematoma in the elderly—a North Wales experience. *J R Soc Med, 95*(6), 290-292.

Aspegren, O. P., Astrand, R., Lundgren, M. I., & Romner, B. (2013). Anticoagulation therapy a risk factor for the development of chronic subdural hematoma. *Clin Neurol Neurosurg, 115*(7), 981-984. doi:10.1016/j.clineuro.2012.10.008

Ayala, A., Wang, P., Ba, Z. F., Perrin, M. M., Ertel, W., & Chaudry, I. H. (1991). Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. *Am J Physiol, 260*(1 Pt 2), R167-171. doi:10.1152/ajpregu.1991.260.1.R167

Baechli, H., Nordmann, A., Bucher, H. C., & Gratzl, O. (2004). Demographics and prevalent risk factors of chronic subdural haematoma: results of a large single-center cohort study. *Neurosurg Rev, 27*(4), 263-266. doi:10.1007/s10143-004-0337-6

Barnes, P. J. (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond), 94*(6), 557-572.

Bartek, J., Jr., Sjavik, K., Schaible, S., Gulati, S., Solheim, O., Forander, P., & Jakola, A. S. (2018). The Role of Angiotensin-Converting Enzyme Inhibitors in Patients with Chronic Subdural Hematoma: A Scandinavian Population-Based Multicenter Study. *World Neurosurg, 113*, e555-e560. doi:10.1016/j.wneu.2018.02.094

Bender. (1974). Nonsurgical Treatment of Subdural Hematomas. *Arch Neurol, 31*.

Benveniste, E. N. (1998). Cytokine actions in the central nervous system. *Cytokine Growth Factor Rev, 9*(3-4), 259-275.

Berghauser Pont, L. M., Dammers, R., Schouten, J. W., Lingsma, H. F., & Dirven, C. M. (2012). Clinical factors associated with outcome in chronic subdural hematoma: a retrospective cohort study of patients on preoperative corticosteroid therapy. *Neurosurgery, 70*(4), 873-880; discussion 880. doi:10.1227/NEU.0b013e31823672ad

Berghauser Pont, L. M., Dirven, C. M., Dippel, D. W., Verweij, B. H., & Dammers, R. (2012). The role of corticosteroids in the management of chronic subdural hematoma: a systematic review. *Eur J Neurol, 19*(11), 1397-1403. doi:10.1111/j.1468-1331.2012.03768.x

Brennan, P. M., Kolias, A. G., Joannides, A. J., Shapey, J., Marcus, H. J., Gregson, B. A., . . . Coulter, I. C. (2017). The management and outcome for patients with chronic subdural hematoma: a prospective, multicenter, observational cohort study in the United Kingdom. *J Neurosurg*, 1-8. doi:10.3171/2016.8.JNS16134.test

Brough, D., Rothwell, N. J., & Allan, S. M. (2015). Interleukin-1 as a pharmacological target in acute brain injury. *Exp Physiol, 100*(12), 1488-1494. doi:10.1113/EP085135

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., . . . Wilberger, J. E. (2006). Surgical Management of Acute Subdural Hematomas. *Neurosurgery, 58*(Supplement), S2-16-S12-24. doi:10.1227/01.Neu.0000210364.29290.C9

Burbridge, M. F., Coge, F., Galizzi, J. P., Boutin, J. A., West, D. C., & Tucker, G. C. (2002). The role of the matrix metalloproteinases during in vitro vessel formation. *Angiogenesis, 5*(3), 215-226.

Cala, L. A., Thickbroom, G. W., Black, J. L., Collins, D. W., & Mastaglia, F. L. (1981). Brain density and cerebrospinal fluid space size: CT of normal volunteers. *AJNR Am J Neuroradiol, 2*(1), 41-47.

Casault, C., Al Sultan, A. S., Banoei, M., Couillard, P., Kramer, A., & Winston, B. W. (2018). Cytokine Responses in Severe Traumatic Brain Injury: Where There Is Smoke, Is There Fire? *Neurocrit Care*. doi:10.1007/s12028-018-0522-z

Chapin, J. C., & Hajjar, K. A. (2015). Fibrinolysis and the control of blood coagulation. *Blood Rev, 29*(1), 17-24. doi:10.1016/j.blre.2014.09.003

Clausen, F., Hanell, A., Bjork, M., Hillered, L., Mir, A. K., Gram, H., & Marklund, N. (2009). Neutralization of interleukin-1beta modifies the inflammatory response and improves histological and cognitive outcome following traumatic brain injury in mice. *Eur J Neurosci, 30*(3), 385-396. doi:10.1111/j.1460-9568.2009.06820.x

Colotta, F., Re, F., Muzio, M., Bertini, R., Polentarutti, N., Sironi, M., . . . Mantovani, A. (1993). Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science, 261*(5120), 472-475.

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol, 335*(1), 2-13. doi:10.1016/j.mce.2010.04.005

Crooks, D. A. (1991). Pathogenesis and biomechanics of traumatic intracranial haemorrhages. *Virchows Arch A Pathol Anat Histopathol, 418*(6), 479-483.

Czock, D., Keller, F., Rasche, F. M., & Haussler, U. (2005). Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet, 44*(1), 61-98. doi:10.2165/00003088-200544010-00003

D'Abbondanza, J. A., & Loch Macdonald, R. (2014). Experimental models of chronic subdural hematoma. *Neurol Res, 36*(2), 176-188. doi:10.1179/1743132813Y.0000000279

D'Errico, A. P., & German, W. J. (1930). Chronic Subdural Hematoma. *Yale J Biol Med, 3*(1), 11-20.

da Silva, A. R., Fraga-Silva, R. A., Stergiopulos, N., Montecucco, F., & Mach, F. (2015). Update on the role of angiotensin in the pathophysiology of coronary atherothrombosis. *Eur J Clin Invest, 45*(3), 274-287. doi:10.1111/eci.12401

De Bonis, P., Trevisi, G., de Waure, C., Sferrazza, A., Volpe, M., Pompucci, A., . . . Mangiola, A. (2013). Antiplatelet/anticoagulant agents and chronic subdural hematoma in the elderly. *PLoS One, 8*(7), e68732. doi:10.1371/journal.pone.0068732

Delgado-Lopez, P. D., Martin-Velasco, V., Castilla-Diez, J. M., Rodriguez-Salazar, A., Galacho-Harriero, A. M., & Fernandez-Arconada, O. (2009). Dexamethasone treatment in chronic subdural haematoma. *Neurocirugia (Astur), 20*(4), 346-359.

Deshmane, S. L., Kremlev, S., Amini, S., & Sawaya, B. E. (2009). Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res, 29*(6), 313-326. doi:10.1089/jir.2008.0027

Dietrich, J., Rao, K., Pastorino, S., & Kesari, S. (2011). Corticosteroids in brain cancer patients: benefits and pitfalls. *Expert Rev Clin Pharmacol, 4*(2), 233-242. doi:10.1586/ecp.11.1

Dinarello, C. A. (2010). Anti-inflammatory Agents: Present and Future. *Cell, 140*(6), 935-950. doi:10.1016/j.cell.2010.02.043

Dran, G., Berthier, F., Fontaine, D., Rasenrarijao, D., & Paquis, P. (2007). [Effectiveness of adjuvant corticosteroid therapy for chronic subdural hematoma: a retrospective study of 198 cases]. *Neurochirurgie, 53*(6), 477-482. doi:10.1016/j.neuchi.2007.09.146

Dulak, J., Loboda, A., Jazwa, A., Zagorska, A., Dorler, J., Alber, H., . . . Jozkowicz, A. (2005). Atorvastatin affects several angiogenic mediators in human endothelial cells. *Endothelium, 12*(5-6), 233-241. doi:10.1080/10623320500476559

Edwards, P., Arango, M., Balica, L., Cottingham, R., El-Sayed, H., Farrell, B., . . . Yutthakasemsunt, S. (2005). Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months. *Lancet, 365*(9475), 1957-1959. doi:10.1016/s0140-6736(05)66552-x

electronic Medicines Compendium. Summary of product characteristics of dexamethasone. Retrieved from https://www.medicines.org.uk/emc/product/5411/smpc

Emich, S., Richling, B., McCoy, M. R., Al-Schameri, R. A., Ling, F., Sun, L., . . . Hitzl, W. (2014). The efficacy of dexamethasone on reduction in the reoperation rate of chronic subdural hematoma--the DRESH study: straightforward study protocol for a randomized controlled trial. *Trials, 15*, 6. doi:10.1186/1745-6215-15-6

Foelholm, R., & Waltimo, O. (1975). Epidemiology of chronic subdural haematoma. *Acta Neurochir (Wien), 32*(3-4), 247-250.

Fornebo, I., Sjavik, K., Alibeck, M., Kristiansson, H., Stahl, F., Forander, P., . . . Bartek, J., Jr. (2017). Role of antithrombotic therapy in the risk of hematoma recurrence and

thromboembolism after chronic subdural hematoma evacuation: a population-based consecutive cohort study. *Acta Neurochir (Wien), 159*(11), 2045-2052. doi:10.1007/s00701-017-3330-x

Forster, C., Silwedel, C., Golenhofen, N., Burek, M., Kietz, S., Mankertz, J., & Drenckhahn, D. (2005). Occludin as direct target for glucocorticoid-induced improvement of blood-brain barrier properties in a murine in vitro system. *J Physiol, 565*(Pt 2), 475-486. doi:10.1113/jphysiol.2005.084038

Fox, N. C., & Schott, J. M. (2004). Imaging cerebral atrophy: normal ageing to Alzheimer's disease. *Lancet, 363*(9406), 392-394. doi:10.1016/S0140-6736(04)15441-X

Frati, A., Salvati, M., Mainiero, F., Ippoliti, F., Rocchi, G., Raco, A., . . . Delfini, R. (2004). Inflammation markers and risk factors for recurrence in 35 patients with a posttraumatic chronic subdural hematoma: a prospective study. *J Neurosurg, 100*(1), 24-32. doi:10.3171/jns.2004.100.1.0024

French, L. A. (1966). The use of steroids in the treatment of cerebral edema. *Bull N Y Acad Med, 42*(4), 301-311.

Fujisawa, H., Nomura, S., Tsuchida, E., & Ito, H. (1998). Serum protein exudation in chronic subdural haematomas: a mechanism for haematoma enlargement? *Acta Neurochir (Wien), 140*(2), 161-165; discussion 165-166.

Gandhoke, G. S., Kaif, M., Choi, L., Williamson, R. W., & Nakaji, P. (2013). Histopathological features of the outer membrane of chronic subdural hematoma and correlation with clinical and radiological features. *J Clin Neurosci, 20*(10), 1398-1401. doi:10.1016/j.jocn.2013.01.010

Garlanda, C., Dinarello, C. A., & Mantovani, A. (2013). The interleukin-1 family: back to the future. *Immunity, 39*(6), 1003-1018. doi:10.1016/j.immuni.2013.11.010

Gelabert-Gonzalez, M., Iglesias-Pais, M., Garcia-Allut, A., & Martinez-Rumbo, R. (2005). Chronic subdural haematoma: surgical treatment and outcome in 1000 cases. *Clin Neurol Neurosurg, 107*(3), 223-229. doi:10.1016/j.clineuro.2004.09.015

Gimbrone, M. A., Jr., Obin, M. S., Brock, A. F., Luis, E. A., Hass, P. E., Hebert, C. A., . . . et al. (1989). Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. *Science, 246*(4937), 1601-1603.

Giorgio, A., Santelli, L., Tomassini, V., Bosnell, R., Smith, S., De Stefano, N., & Johansen-Berg, H. (2010). Age-related changes in grey and white matter structure throughout adulthood. *Neuroimage, 51*(3), 943-951. doi:10.1016/j.neuroimage.2010.03.004

Glover, D., & Labadie, E. L. (1976). Physiopathogenesis of subdural hematomas. Part 2: Inhibition of growth of experimental hematomas with dexamethasone. *J Neurosurg, 45*(4), 393-397. doi:10.3171/jns.1976.45.4.0393

Gong, D., Hao, M., Liu, L., Liu, C., Dong, J., Cui, Z., . . . Zhang, J. (2012). Prognostic relevance of circulating endothelial progenitor cells for severe traumatic brain injury. *Brain Inj, 26*(3), 291-297. doi:10.3109/02699052.2011.648710

Gonugunta, V., & Buxton, N. (2001). Warfarin and chronic subdural haematomas. *Br J Neurosurg, 15*(6), 514-517.

Goodell, C. L., & Mealey, J., Jr. (1963). Pathogenesis of chronic subdural hematoma. Experimental studies. *Arch Neurol, 8*, 429-437.

Goto, H., Ishikawa, O., Nomura, M., Tanaka, K., Nomura, S., & Maeda, K. (2015). Magnetic resonance imaging findings predict the recurrence of chronic subdural hematoma. *Neurol Med Chir (Tokyo), 55*(2), 173-178. doi:10.2176/nmc.oa.2013-0390

Green, J. A., Tran, C. T., Farrar, J. J., Nguyen, M. T., Nguyen, P. H., Dinh, S. X., . . . Thwaites, G. E. (2009). Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. *PLoS One, 4*(9), e7277. doi:10.1371/journal.pone.0007277

Grossetete, M., Phelps, J., Arko, L., Yonas, H., & Rosenberg, G. A. (2009). Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic brain injury. *Neurosurgery, 65*(4), 702-708. doi:10.1227/01.NEU.0000351768.11363.48

Guilfoyle, M. R., Carpenter, K. L., Helmy, A., Pickard, J. D., Menon, D. K., & Hutchinson, P. J. (2015). Matrix Metalloproteinase Expression in Contusional Traumatic Brain Injury: A Paired Microdialysis Study. *J Neurotrauma, 32*(20), 1553-1559. doi:10.1089/neu.2014.3764

Haines. (1991). On the Question of a Subdural Space. *The Anatomical Record, 230*, 3-21.

Han, S. B., Choi, S. W., Song, S. H., Youm, J. Y., Koh, H. S., Kim, S. H., & Kwon, H. J. (2014). Prediction of Chronic Subdural Hematoma in Minor Head Trauma Patients. *Korean J Neurotrauma, 10*(2), 106-111. doi:10.13004/kjnt.2014.10.2.106

Hara, M., Tamaki, M., Aoyagi, M., & Ohno, K. (2009). Possible role of cyclooxygenase-2 in developing chronic subdural hematoma. *J Med Dent Sci, 56*(3), 101-106.

Hausmann, E. H., Berman, N. E., Wang, Y. Y., Meara, J. B., Wood, G. W., & Klein, R. M. (1998). Selective chemokine mRNA expression following brain injury. *Brain Res, 788*(1-2), 49-59.

Helmy, A., De Simoni, M. G., Guilfoyle, M. R., Carpenter, K. L., & Hutchinson, P. J. (2011). Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. *Prog Neurobiol, 95*(3), 352-372. doi:10.1016/j.pneurobio.2011.09.003

Henaux, P. L., Le Reste, P. J., Laviolle, B., & Morandi, X. (2017). Steroids in chronic subdural hematomas (SUCRE trial): study protocol for a randomized controlled trial. *Trials, 18*(1), 252. doi:10.1186/s13063-017-1990-8

Heula, A. L., Ohlmeier, S., Sajanti, J., & Majamaa, K. (2013). Characterization of chronic subdural hematoma fluid proteome. *Neurosurgery, 73*(2), 317-331. doi:10.1227/01.neu.0000430323.24623.de

Heula, A. L., Sajanti, J., & Majamaa, K. (2009). Procollagen propeptides in chronic subdural hematoma reveal sustained dural collagen synthesis after head injury. *J Neurol, 256*(1), 66-71. doi:10.1007/s00415-009-0048-6

Hoeben, A., Landuyt, B., Highley, M. S., Wildiers, H., Van Oosterom, A. T., & De Bruijn, E. A. (2004). Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev, 56*(4), 549-580. doi:10.1124/pr.56.4.3

Hohenstein, A., Erber, R., Schilling, L., & Weigel, R. (2005). Increased mRNA expression of VEGF within the hematoma and imbalance of angiopoietin-1 and -2 mRNA within the neomembranes of chronic subdural hematoma. *J Neurotrauma, 22*(5), 518-528. doi:10.1089/neu.2005.22.518

Hong, H. J., Kim, Y. J., Yi, H. J., Ko, Y., Oh, S. J., & Kim, J. M. (2009). Role of angiogenic growth factors and inflammatory cytokine on recurrence of chronic subdural hematoma. *Surg Neurol, 71*(2), 161-165; discussion 165-166. doi:10.1016/j.surneu.2008.01.023

Hosoda, K., Tamaki, N., Masumura, M., Matsumoto, S., & Maeda, F. (1987). Magnetic resonance images of chronic subdural hematomas. *J Neurosurg, 67*(5), 677-683. doi:10.3171/jns.1987.67.5.0677

Hua, C., Zhao, G., Feng, Y., Yuan, H., Song, H., & Bie, L. (2016). Role of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, and Vascular Endothelial Growth Factor in the Development of Chronic Subdural Hematoma. *J Neurotrauma, 33*(1), 65-70. doi:10.1089/neu.2014.3724

Huang, Y. H., Lin, W. C., Lu, C. H., & Chen, W. F. (2014). Volume of chronic subdural haematoma: is it one of the radiographic factors related to recurrence? *Injury, 45*(9), 1327-1331. doi:10.1016/j.injury.2014.02.023

Hutchinson, P. J., O'Connell, M. T., Rothwell, N. J., Hopkins, S. J., Nortje, J., Carpenter, K. L., . . . Pickard, J. D. (2007). Inflammation in human brain injury: intracerebral concentrations of IL-1alpha, IL-1beta, and their endogenous inhibitor IL-1ra. *J Neurotrauma, 24*(10), 1545-1557. doi:10.1089/neu.2007.0295

Inglis, K. (1946). Subdural Haemorrhage, cysts and false membranes. *Brain, 69*(3).

Iorio-Morin, C., Blanchard, J., Richer, M., & Mathieu, D. (2016). Tranexamic Acid in Chronic Subdural Hematomas (TRACS): study protocol for a randomized controlled trial. *Trials, 17*(1), 235. doi:10.1186/s13063-016-1358-5

Ito, H., Komai, T., & Yamamoto, S. (1978). Fibrinolytic enzyme in the lining walls of chronic subdural hematoma. *J Neurosurg, 48*(2), 197-200. doi:10.3171/jns.1978.48.2.0197

Ito, H., Maeda, M., Uehara, T., Yamamoto, S., Tamura, M., & Takashima, T. (1984). Attenuation values of chronic subdural haematoma and subdural effusion in CT scans. *Acta Neurochir (Wien), 72*(3-4), 211-217.

Ito, H., Yamamoto, S., Komai, T., & Mizukoshi, H. (1976). Role of local hyperfibrinolysis in the etiology of chronic subdural hematoma. *J Neurosurg, 45*(1), 26-31. doi:10.3171/jns.1976.45.1.0026

Ito, H., Yamamoto, S., Saito, K., Ikeda, K., & Hisada, K. (1987). Quantitative estimation of hemorrhage in chronic subdural hematoma using the 51Cr erythrocyte labeling method. *J Neurosurg, 66*(6), 862-864. doi:10.3171/jns.1987.66.6.0862

Izumihara, A., Yamashita, K., & Murakami, T. (2013). Acute subdural hematoma requiring surgery in the subacute or chronic stage. *Neurol Med Chir (Tokyo), 53*(5), 323-328.

Jack, A., O'Kelly, C., McDougall, C., & Findlay, J. M. (2015). Predicting recurrence after chronic subdural haematoma drainage. *Can J Neurol Sci, 42*(1), 34-39. doi:10.1017/cjn.2014.122

Jafari, N., Gesner, L., Koziol, J. M., Rotoli, G., & Hubschmann, O. R. (2017). The Pathogenesis of Chronic Subdural Hematomas: A Study on the Formation of Chronic Subdural Hematomas and Analysis of Computed Tomography Findings. *World Neurosurg, 107*, 376-381. doi:10.1016/j.wneu.2017.07.108

Jeong, E. O., Choi, S. W., Lim, J. W., Kwon, H. J., Kim, S. H., Koh, H. S., . . . Song, S. H. (2016). Effectiveness of Cortical Atrophy Scale and Indirect Indices of Brain Atrophy to Predict Chronic Subdural Hematoma in Older Patients. *Korean J Neurotrauma, 12*(2), 112-117. doi:10.13004/kjnt.2016.12.2.112

Jiang, R., Zhao, S., Wang, R., Feng, H., Zhang, J., Li, X., . . . Zhang, J. (2018). Safety and Efficacy of Atorvastatin for Chronic Subdural Hematoma in Chinese Patients: A Randomized ClinicalTrial. *JAMA Neurol, 75*(11), 1338-1346. doi:10.1001/jamaneurol.2018.2030

Jones, N., Iljin, K., Dumont, D. J., & Alitalo, K. (2001). Tie receptors: new modulators of angiogenic and lymphangiogenic responses. *Nat Rev Mol Cell Biol, 2*(4), 257-267. doi:10.1038/35067005

Ju, M. W., Kim, S. H., Kwon, H. J., Choi, S. W., Koh, H. S., Youm, J. Y., & Song, S. H. (2015). Comparision between Brain Atrophy and Subdural Volume to Predict Chronic Subdural Hematoma: Volumetric CT Imaging Analysis. *Korean J Neurotrauma, 11*(2), 87-92. doi:10.13004/kjnt.2015.11.2.87

Jung, S., Moon, K. S., Jung, T. Y., Kim, I. Y., Lee, Y. H., Rhu, H. H., . . . Kang, S. S. (2006). Possible pathophysiological role of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) in metastatic brain tumor-associated intracerebral hemorrhage. *J Neurooncol, 76*(3), 257-263. doi:10.1007/s11060-005-6876-z

Kageyama, H., Toyooka, T., Tsuzuki, N., & Oka, K. (2013). Nonsurgical treatment of chronic subdural hematoma with tranexamic acid. *J Neurosurg, 119*(2), 332-337. doi:10.3171/2013.3.Jns122162

Kalamatianos, T., Stavrinou, L. C., Koutsarnakis, C., Psachoulia, C., Sakas, D. E., & Stranjalis, G. (2013). PlGF and sVEGFR-1 in chronic subdural hematoma: implications for hematoma development. *J Neurosurg, 118*(2), 353-357. doi:10.3171/2012.10.JNS12327

Katano, H., Kamiya, K., Mase, M., Tanikawa, M., & Yamada, K. (2006). Tissue plasminogen activator in chronic subdural hematomas as a predictor of recurrence. *J Neurosurg, 104*(1), 79-84. doi:10.3171/jns.2006.104.1.79

Kishimoto, T., Akira, S., & Taga, T. (1992). Interleukin-6 and its receptor: a paradigm for cytokines. *Science, 258*(5082), 593-597.

Kitazono, M., Yokota, H., Satoh, H., Onda, H., Matsumoto, G., Fuse, A., & Teramoto, A. (2012). Measurement of inflammatory cytokines and thrombomodulin in chronic subdural hematoma. *Neurol Med Chir (Tokyo), 52*(11), 810-815.

Kolias, A. G., Chari, A., Santarius, T., & Hutchinson, P. J. (2014). Chronic subdural haematoma: modern management and emerging therapies. *Nat Rev Neurol, 10*(10), 570-578. doi:10.1038/nrneurol.2014.163

Kolias, A. G., Edlmann, E., Thelin, E. P., Bulters, D., Holton, P., Suttner, N., . . . Hutchinson, P. J. (2018). Dexamethasone for adult patients with a symptomatic chronic subdural haematoma (Dex-CSDH) trial: study protocol for a randomised controlled trial. *Trials, 19*(1), 670. doi:10.1186/s13063-018-3050-4

Kristof, R. A., Grimm, J. M., & Stoffel-Wagner, B. (2008). Cerebrospinal fluid leakage into the subdural space: possible influence on the pathogenesis and recurrence frequency of chronic subdural hematoma and subdural hygroma. *J Neurosurg, 108*(2), 275-280. doi:10.3171/JNS/2008/108/2/0275

Laviv, Y., Rappaport, Z. H. (2014). Risk factors for development of significant chronic subdural hematoma following conservative treatment of acute subdural hemorrhage. *Br J Neurosurg, 28*(6), 733-738. doi:10.3109/02688697.2014.918578

Lee, J. J., Won, Y., Yang, T., Kim, S., Choi, C. S., Yang, J. (2015). Risk Factors of Chronic Subdural Hematoma Progression after Conservative Management of Cases with Initially Acute Subdural Hematoma. *Korean J Neurotrauma, 11*(2), 52-57. doi:10.13004/kjnt.2015.11.2.52

Lee, K. (1998). The pathogenesis and clinical significance of traumatic subdural hygroma. *Brain Injury, 12*(7), 595-603. doi:10.1080/026990598122359

Lee, K. (2004). Natural history of chronic subdural haematoma. *Brain Inj, 18*(4), 351-358. doi:10.1080/02699050310001645801

Lee, K. (2016). Chronic Subdural Hematoma in the Aged, Trauma or Degeneration? *J Korean Neurosurg Soc, 59*(1), 1-5. doi:10.3340/jkns.2016.59.1.1

Lee, K., Bae, W., Park, Y., & Yun, I. (1994). The pathogenesis and fate of traumatic subdural hygroma. *British Journal of Neurosurgery, 8*(5), 551-558. doi:10.3109/02688699409002947

Lee, K. B., WK; Doh, JW; Bae, HG; Yun, IG. (1998). Review: Origin of chronic subdural haematoma and relation to traumatic subdural lesions. *Brain Injury, 12*(11), 901-910. doi:10.1080/026990598121972

Li, A., Varney, M. L., Valasek, J., Godfrey, M., Dave, B. J., & Singh, R. K. (2005). Autocrine role of interleukin-8 in induction of endothelial cell proliferation, survival, migration and MMP-2 production and angiogenesis. *Angiogenesis, 8*(1), 63-71. doi:10.1007/s10456-005-5208-4

Liu, W., Bakker, N. A., & Groen, R. J. M. (2014). Chronic subdural hematoma: a systematic review and meta-analysis of surgical procedures. *Journal of Neurosurgery, 121*(3), 665-673. doi:10.3171/2014.5.Jns132715

Lucke-Wold, B., Turner, R., Josiah, D., Knotts, C., & Bhatia, S. (2016). Do Age and Anticoagulants Affect the Natural History of Acute Subdural Hematomas? *Arch Emerg Med Crit Care, 1*(2).

Manicone, A., & McGuire, J. (2008). Matrix metalloproteinases as modulators of inflammation. *Semin Cell Dev Biol, 19*(1), 34-41. doi:10.1016/j.semcdb.2007.07.003

Marcus, H. J., Carpenter, K. L., Price, S. J., & Hutchinson, P. J. (2010). In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. *J Neurooncol, 97*(1), 11-23. doi:10.1007/s11060-009-9990-5

Markwalder, T. M. (1981). Chronic subdural hematomas: a review. *J Neurosurg, 54*(5), 637-645. doi:10.3171/jns.1981.54.5.0637

Mathew, P., Oluoch-Olunya, D. L., Condon, B. R., & Bullock, R. (1993). Acute subdural haematoma in the conscious patient: outcome with initial non-operative management. *Acta Neurochir (Wien), 121*(3-4), 100-108.

Maxwell RE, L. D., French LA. . (1972). *The clinical effects of synthetic glucocorticoid used for brain edema in the practice of neurosurgery* (S. K. Reulen HJ Ed.). Berlin: Springer-Verlag.

Meikle, A. W., & Tyler, F. H. (1977). Potency and duration of action of glucocorticoids: Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *The American Journal of Medicine, 63*(2), 200-207. doi:10.1016/0002-9343(77)90233-9

Miah, I. P., Holl, D. C., Peul, W. C., Walchenbach, R., Kruyt, N., de Laat, K., . . . van der Gaag, N. A. (2018). Dexamethasone therapy versus surgery for chronic subdural haematoma (DECSA trial): study protocol for a randomised controlled trial. *Trials, 19*(1), 575. doi:10.1186/s13063-018-2945-4

Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P., & Mosmann, T. R. (1993). Interleukin-10. *Annu Rev Immunol, 11*, 165-190. doi:10.1146/annurev.iy.11.040193.001121

Moskala, M., Goscinski, I., Kaluza, J., Polak, J., Krupa, M., Adamek, D., . . . Miodonski, A. J. (2007). Morphological aspects of the traumatic chronic subdural hematoma capsule: SEM studies. *Microsc Microanal, 13*(3), 211-219. doi:10.1017/S1431927607070286

Murakami, H., Hirose, Y., Sagoh, M., Shimizu, K., Kojima, M., Gotoh, K., . . . Kawase, T. (2002). Why do chronic subdural hematomas continue to grow slowly and not

coagulate? Role of thrombomodulin in the mechanism. *J Neurosurg, 96*(5), 877-884. doi:10.3171/jns.2002.96.5.0877

Murata, K. (1993). Chronic subdural hematoma may be preceded by persistent traumatic subdural effusion. *Neurol Med Chir (Tokyo), 33*(10), 691-696.

Nakagawa, T., Kodera, T., & Kubota, T. (2000). Expression of matrix metalloproteinases in the chronic subdural haematoma membrane. *Acta Neurochir (Wien), 142*(1), 61-66.

Nakaguchi, H., Tanishima, T., & Yoshimasu, N. (2001). Factors in the natural history of chronic subdural hematomas that influence their postoperative recurrence. *J Neurosurg, 95*(2), 256-262. doi:10.3171/jns.2001.95.2.0256

Nanko, N., Tanikawa, M., Mase, M., Fujita, M., Tateyama, H., Miyati, T., & Yamada, K. (2009). Involvement of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in the mechanism of development of chronic subdural hematoma. *Neurol Med Chir (Tokyo), 49*(9), 379-385.

Nathan, S., Goodarzi, Z., Jette, N., Gallagher, C., & Holroyd-Leduc, J. (2017). Anticoagulant and antiplatelet use in seniors with chronic subdural hematoma: Systematic review. *Neurology, 88*(20), 1889-1893. doi:10.1212/wnl.0000000000003918

Neidert, M. C., Schmidt, T., Mitova, T., Fierstra, J., Bellut, D., Regli, L., . . . Bozinov, O. (2016). Preoperative angiotensin converting enzyme inhibitor usage in patients with chronic subdural hematoma: Associations with initial presentation and clinical outcome. *J Clin Neurosci, 28*, 82-86. doi:10.1016/j.jocn.2015.09.022

Neils, D. M., Singanallur, P. S., Wang, H., Tracy, P., Klopfenstein, J., Dinh, D., . . . Tsung, A. (2012). Recurrence-free chronic subdural hematomas: a retrospective analysis of the instillation of tissue plasminogen activator in addition to twist drill or burr hole drainage in the treatment of chronic subdural hematomas. *World Neurosurg, 78*(1-2), 145-149. doi:10.1016/j.wneu.2011.08.032

Nicolaides NC, P. A., Maria Alexandra MA et al. (2018). *Glucocorticoid Therapy and Adrenal Suppression*. In C. G. De Groot LJ, Dungan K, et al. (Ed.), *Endotext (internet)*

Nomura, S., Kashiwagi, S., Fujisawa, H., Ito, H., & Nakamura, K. (1994a). Characterization of local hyperfibrinolysis in chronic subdural haematomas by SDS-PAGE and immunoblot. *J Neurosurg, 81*, 910-913.

Nomura, S., Kashiwagi, S., Fujisawa, H., Ito, H., & Nakamura, K. (1994b). Characterization of local hyperfibrinolysis in chronic subdural hematomas by SDS-PAGE and immunoblot. *J Neurosurg, 81*(6), 910-913. doi:10.3171/jns.1994.81.6.0910

Nowinski, W. L., Gomolka, R. S., Qian, G., Gupta, V., Ullman, N. L., & Hanley, D. F. (2014). Characterization of intraventricular and intracerebral hematomas in non-contrast CT. *Neuroradiol J, 27*(3), 299-315. doi:10.15274/nrj-2014-10042

Ohba, S., Kinoshita, Y., Nakagawa, T., & Murakami, H. (2013). The risk factors for recurrence of chronic subdural hematoma. *Neurosurg Rev, 36*(1), 145-149; discussion 149-150. doi:10.1007/s10143-012-0396-z

Ohno, K., Suzuki, R., Masaoka, H., Matsushima, Y., Inaba, Y., & Monma, S. (1987). Chronic subdural haematoma preceded by persistent traumatic subdural fluid collection. *J Neurol Neurosurg Psychiatry, 50*(12), 1694-1697.

Olivero, W. C., Wang, H., Farahvar, A., Kim, T. A., & Wang, F. (2017). Predictive (subtle or overlooked) initial head CT findings in patients who develop delayed chronic subdural hematoma. *J Clin Neurosci, 42*, 129-133. doi:10.1016/j.jocn.2017.03.005

Ommaya, A. K., & Yarnell, P. (1969). Subdural haematoma after whiplash injury. *Lancet, 2*(7614), 237-239.

Oppenheim, J. J., Zachariae, C. O., Mukaida, N., & Matsushima, K. (1991). Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol, 9*, 617-648. doi:10.1146/annurev.iy.09.040191.003153

Ostergaard, L., Hochberg, F. H., Rabinov, J. D., Sorensen, A. G., Lev, M., Kim, L., . . . Rosen, B. R. (1999). Early changes measured by magnetic resonance imaging in cerebral blood flow, blood volume, and blood-brain barrier permeability following dexamethasone treatment in patients with brain tumors. *J Neurosurg, 90*(2), 300-305. doi:10.3171/jns.1999.90.2.0300

Osuka, K., Watanabe, Y., Usuda, N., Aoyama, M., Kawaguchi, R., Watabe, T., & Takayasu, M. (2016). Activation of Signal Transducer and Activator of Transcription 3 in Endothelial Cells of Chronic Subdural Hematoma Outer Membranes. *World Neurosurg, 91*, 376-382. doi:10.1016/j.wneu.2016.04.025

Osuka, K., Watanabe, Y., Usuda, N., Atsuzawa, K., Aoyama, M., Niwa, A., . . . Takayasu, M. (2012). Activation of Ras/MEK/ERK signaling in chronic subdural hematoma outer membranes. *Brain Res, 1489*, 98-103. doi:10.1016/j.brainres.2012.10.013

Osuka, K., Watanabe, Y., Usuda, N., Atsuzawa, K., Shima, H., Takeuchi, M., . . . Takayasu, M. (2013). Activation of JAK-STAT3 signaling pathway in chronic subdural hematoma outer membranes. *Neurosci Lett, 534*, 166-170. doi:10.1016/j.neulet.2012.11.011

Park, K. S., Park, S. H., Hwang, S. K., Kim, C., & Hwang, J. H. (2015). Correlation of the Beta-trace protein and inflammatory cytokines with magnetic resonance imaging in chronic subdural hematomas : a prospective study. *J Korean Neurosurg Soc, 57*(4), 235-241. doi:10.3340/jkns.2015.57.4.235

Park, S. H., Lee, S. H., Park, J., Hwang, J. H., Hwang, S. K., & Hamm, I. S. (2008). Chronic subdural hematoma preceded by traumatic subdural hygroma. *J Clin Neurosci, 15*(8), 868-872. doi:10.1016/j.jocn.2007.08.003

Poon, M. T. C., & Al-Shahi Salman, R. (2018). Association between antithrombotic drug use before chronic subdural haematoma and outcome after drainage: a systematic review and meta-analysis. *Neurosurg Rev, 41*(2), 439-445. doi:10.1007/s10143-017-0860-x

Poulsen, F. R., Munthe, S., Soe, M., & Halle, B. (2014). Perindopril and residual chronic subdural hematoma volumes six weeks after burr hole surgery: a randomized trial. *Clin Neurol Neurosurg, 123*, 4-8. doi:10.1016/j.clineuro.2014.05.003

Pripp, A. H., & Stanisic, M. (2014). The correlation between pro- and anti-inflammatory cytokines in chronic subdural hematoma patients assessed with factor analysis. *PLoS One, 9*(2), e90149. doi:10.1371/journal.pone.0090149

Prud'homme, M., Mathieu, F., Marcotte, N., & Cottin, S. (2016). A Pilot Placebo Controlled Randomized Trial of Dexamethasone for Chronic Subdural Hematoma. *Can J Neurol Sci, 43*(2), 284-290. doi:10.1017/cjn.2015.393

Putnam, T. J., & Cushing, H. (1925). CHRONIC SUBDURAL HEMATOMA: ITS PATHOLOGY, ITS RELATION TO PACHYMENINGITIS HEMORRHAGICA AND ITS SURGICAL TREATMENT. *Archives of Surgery, 11*(3), 329-393. doi:10.1001/archsurg.1925.01120150002001

Qian, Z., Yang, D., Sun, F., & Sun, Z. (2017). Risk factors for recurrence of chronic subdural hematoma after burr hole surgery: potential protective role of dexamethasone. *Br J Neurosurg, 31*(1), 84-88. doi:10.1080/02688697.2016.1260686

Rodney, T., Osier, N., & Gill, J. (2018). Pro- and anti-inflammatory biomarkers and traumatic brain injury outcomes: A review. *Cytokine, 110*, 248-256. doi:10.1016/j.cyto.2018.01.012

Rothwell, N. J., & Luheshi, G. N. (2000). Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci, 23*(12), 618-625.

Sajanti, J., & Majamaa, K. (2003). High concentrations of procollagen propeptides in chronic subdural haematoma and effusion. *J Neurol Neurosurg Psychiatry, 74*(4), 522-524.

Santarius, T., & Hutchinson, P. J. (2009). Chronic subdural haematoma: time to rationalize treatment? *British Journal of Neurosurgery, 18*(4), 328-332. doi:10.1080/02688690400004845

Sato, S., & Suzuki, J. (1975). Ultrastructural observations of the capsule of chronic subdural hematoma in various clinical stages. *J Neurosurg, 43*(5), 569-578. doi:10.3171/jns.1975.43.5.0569

Schall, T. J. (1993). Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. *Journal of Experimental Medicine, 177*(6), 1821-1826. doi:10.1084/jem.177.6.1821

Semple, B. D., Bye, N., Rancan, M., Ziebell, J. M., & Morganti-Kossmann, M. C. (2010). Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2-/- mice. *J Cereb Blood Flow Metab, 30*(4), 769-782. doi:10.1038/jcbfm.2009.262

Seymour, R. M., & Henderson, B. (2001). Pro-inflammatory--anti-inflammatory cytokine dynamics mediated by cytokine-receptor dynamics in monocytes. *IMA J Math Appl Med Biol, 18*(2), 159-192.

Shakur, H., Roberts, I., Bautista, R., Caballero, J., Coats, T., Dewan, Y., . . . Yutthakasemsunt, S. (2010). Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet, 376*(9734), 23-32. doi:10.1016/s0140-6736(10)60835-5

Shim, Y. S., Park, C. O., Hyun, D. K., Park, H. C., & Yoon, S. H. (2007). What are the causative factors for a slow, progressive enlargement of a chronic subdural hematoma? *Yonsei Med J, 48*(2), 210-217. doi:10.3349/ymj.2007.48.2.210

Shono, T., Inamura, T., Morioka, T., Matsumoto, K., Suzuki, S. O., Ikezaki, K., . . . Fukui, M. (2001). Vascular endothelial growth factor in chronic subdural haematomas. *J Clin Neurosci, 8*(5), 411-415. doi:10.1054/jocn.2000.0951

Siegal, T. (1995). Spinal cord compression: from laboratory to clinic. *Eur J Cancer, 31a*(11), 1748-1753.

Sims, J. E., & Smith, D. E. (2010). The IL-1 family: regulators of immunity. *Nat Rev Immunol, 10*(2), 89-102. doi:10.1038/nri2691

Stanisic, M., Aasen, A. O., Pripp, A. H., Lindegaard, K. F., Ramm-Pettersen, J., Lyngstadaas, S. P., . . . Saehle, T. (2012). Local and systemic pro-inflammatory and anti-

inflammatory cytokine patterns in patients with chronic subdural hematoma: a prospective study. *Inflamm Res, 61*(8), 845-852. doi:10.1007/s00011-012-0476-0

Stanisic, M., Lyngstadaas, S. P., Pripp, A. H., Aasen, A. O., Lindegaard, K. F., Ivanovic, J., . . . Saehle, T. (2012). Chemokines as markers of local inflammation and angiogenesis in patients with chronic subdural hematoma: a prospective study. *Acta Neurochir (Wien), 154*(1), 113-120; discussion 120. doi:10.1007/s00701-011-1203-2

Stanisic, M., & Pripp, A. H. (2017). A Reliable Grading System for Prediction of Chronic Subdural Hematoma Recurrence Requiring Reoperation After Initial Burr-Hole Surgery. *Neurosurgery, 81*(5), 752-760. doi:10.1093/neuros/nyx090

Stavrinou, P., Katsigiannis, S., Lee, J. H., Hamisch, C., Krischek, B., Mpotsaris, A., . . . Goldbrunner, R. (2017). Risk Factors for Chronic Subdural Hematoma Recurrence Identified Using Quantitative Computed Tomography Analysis of Hematoma Volume and Density. *World Neurosurg, 99*, 465-470. doi:10.1016/j.wneu.2016.12.058

Stream, J. O., & Grissom, C. K. (2008). Update on high-altitude pulmonary edema: pathogenesis, prevention, and treatment. *Wilderness Environ Med, 19*(4), 293-303. doi:10.1580/07-weme-rev-173.1

Stroobandt, G., Fransen, P., Thauvoy, C., & Menard, E. (1995). Pathogenetic factors in chronic subdural haematoma and causes of recurrence after drainage. *Acta Neurochir (Wien), 137*(1-2), 6-14.

Sun, T. F., Boet, R., & Poon, W. S. (2005). Non-surgical primary treatment of chronic subdural haematoma: Preliminary results of using dexamethasone. *Br J Neurosurg, 19*(4), 327-333. doi:10.1080/02688690500305332

Tang, R., Shi, J., Li, X., Zou, Y., Wang, L., Chen, Y., . . . Feng, H. (2018). Effects of Atorvastatin on Surgical Treatments of Chronic Subdural Hematoma. *World Neurosurg, 117*, e425-e429. doi:10.1016/j.wneu.2018.06.047

Taub, D. D., Lloyd, A. R., Conlon, K., Wang, J. M., Ortaldo, J. R., Harada, A., . . . Oppenheim, J. J. (1993). Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med, 177*(6), 1809-1814.

Thau-Zuchman, O., Shohami, E., Alexandrovich, A. G., & Leker, R. R. (2010). Vascular endothelial growth factor increases neurogenesis after traumatic brain injury. *J Cereb Blood Flow Metab, 30*(5), 1008-1016. doi:10.1038/jcbfm.2009.271

Thotakura, A. K., & Marabathina, N. R. (2015). Nonsurgical Treatment of Chronic Subdural Hematoma with Steroids. *World Neurosurg, 84*(6), 1968-1972. doi:10.1016/j.wneu.2015.08.044

Tokmak, M., Iplikcioglu, A. C., Bek, S., Gokduman, C. A., & Erdal, M. (2007). The role of exudation in chronic subdural hematomas. *J Neurosurg, 107*(2), 290-295. doi:10.3171/JNS-07/08/0290

Trotter, W. (1914). Chronic subdural hæmorrhage of traumatic origin, and its relation to pachymeningitis hæmorrhagica interna. *BJS, 2*(6), 271-291. doi:doi:10.1002/bjs.1800020608

Tsutsumi, S., Ogino, I., Miyajima, M., Nonaka, S., Ito, M., Yasumoto, Y., & Arai, H. (2017). Role of cathepsin K in the development of chronic subdural hematoma. *J Clin Neurosci, 45*, 343-347. doi:10.1016/j.jocn.2017.08.021

Vecht, C. J., Hovestadt, A., Verbiest, H. B., van Vliet, J. J., & van Putten, W. L. (1994). Dose-effect relationship of dexamethasone on Karnofsky performance in metastatic brain tumors: a randomized study of doses of 4, 8, and 16 mg per day. *Neurology, 44*(4), 675-680.

Wada, M., Yamakami, I., Higuchi, Y., Tanaka, M., Suda, S., Ono, J., & Saeki, N. (2014). Influence of antiplatelet therapy on postoperative recurrence of chronic subdural hematoma: a multicenter retrospective study in 719 patients. *Clin Neurol Neurosurg, 120*, 49-54. doi:10.1016/j.clineuro.2014.02.007

Wada, T., Kuroda, K., Yoshida, Y., Ogasawara, K., Ogawa, A., & Endo, S. (2006). Local elevation of the anti-inflammatory interleukin-10 in the pathogenesis of chronic subdural hematoma. *Neurosurg Rev, 29*(3), 242-245. doi:10.1007/s10143-006-0019-7

Wang, Y., Zhou, J., Fan, C., Wang, D., Jiao, F., Liu, B., & Zhang, Q. (2017). Influence of antithrombotic agents on the recurrence of chronic subdural hematomas and the quest about the recommencement of antithrombotic agents: A meta-analysis. *J Clin Neurosci, 38*, 79-83. doi:10.1016/j.jocn.2016.12.001

Weigel, R., Hohenstein, A., Schlickum, L., Weiss, C., & Schilling, L. (2007). Angiotensin converting enzyme inhibition for arterial hypertension reduces the risk of recurrence in patients with chronic subdural hematoma possibly by an antiangiogenic mechanism. *Neurosurgery, 61*(4), 788-792; discussion 792-783. doi:10.1227/01.Neu.0000298907.56012.E8

Weigel, R., Schilling, L., & Schmiedek, P. (2001). Specific pattern of growth factor distribution in chronic subdural hematoma (CSH): evidence for an angiogenic disease. *Acta Neurochir (Wien), 143*(8), 811-818; discussion 819.

Weir, B., & Gordon, P. (1983). Factors affecting coagulation: fibrinolysis in chronic subdural fluid collections. *J Neurosurg, 58*(2), 242-245. doi:10.3171/jns.1983.58.2.0242

Weissman, D. E., Dufer, D., Vogel, V., & Abeloff, M. D. (1987). Corticosteroid toxicity in neuro-oncology patients. *J Neurooncol, 5*(2), 125-128.

Whitehouse, K. J., Jeyaretna, D. S., Wright, A., & Whitfield, P. C. (2016). Neurosurgical Care in the Elderly: Increasing Demands Necessitate Future Healthcare Planning. *World Neurosurg, 87*, 446-454. doi:10.1016/j.wneu.2015.10.099

Yamamoto, H., Hirashima, Y., Hamada, H., Hayashi, N., Origasa, H., & Endo, S. (2003). Independent predictors of recurrence of chronic subdural hematoma: results of multivariate analysis performed using a logistic regression model. *J Neurosurg, 98*(6), 1217-1221. doi:10.3171/jns.2003.98.6.1217

Yamashima, T., & Friede, R. L. (1984). Why do bridging veins rupture into the virtual subdural space? *J Neurol Neurosurg Psychiatry, 47*(2), 121-127.

Yamashima, T., Yamamoto, S., & Friede, R. L. (1983). The role of endothelial gap junctions in the enlargement of chronic subdural hematomas. *J Neurosurg, 59*(2), 298-303. doi:10.3171/jns.1983.59.2.0298

Yan, C., Yang, M. F., & Huang, Y. W. (2018). A Reliable Nomogram Model to Predict the Recurrence of Chronic Subdural Hematoma After Burr Hole Surgery. *World Neurosurg, 118*, e356-e366. doi:10.1016/j.wneu.2018.06.191

Yang, W., & Huang, J. (2017). Chronic Subdural Hematoma: Epidemiology and Natural History. *Neurosurg Clin N Am, 28*(2), 205-210. doi:10.1016/j.nec.2016.11.002

Zhang, Z. G., Zhang, L., Tsang, W., Soltanian-Zadeh, H., Morris, D., Zhang, R., . . . Chopp, M. (2002). Correlation of VEGF and Angiopoietin Expression with Disruption of Blood–Brain Barrier and Angiogenesis after Focal Cerebral Ischemia. *Journal of Cerebral Blood Flow & Metabolism, 22*(4), 379-392. doi:10.1097/00004647-200204000-00002

Chapter 2: Two pathological origins of chronic subdural haematoma

2.1 Introduction

Many people believe that trauma, resulting in a small acute subdural haematoma (ASDH), occurs at the start of all CSDHs and is simply forgotten. However, widespread access to computed tomography (CT) imaging has provided evidence against this and anecdotal clinical experience has demonstrated that some patients with normal imaging at the time of trauma can still develop a CSDH. This led to the hypothesis that a CSDH is the end-point of two distinct pathophysiological processes defined as;

1. ***Acute transformed chronic subdural haematoma (CSDH-AT);*** patients where an ASDH precedes the formation of a CSDH, usually, but not exclusively, subsequent to trauma, as evidenced by serial post-traumatic imaging.
2. ***De-Novo chronic subdural haemtoma (CSDH-DN)***; patients who develop a CSDH without evidence of a preceding ASDH on serial post-traumatic imaging.

To prove this hypothesis, baseline imaging is required, and this is nearly always done in the context of recent trauma. It is conceivable that patients without reported trauma, and therefore no reason to have an ASDH, develop a CSDH through the "de-novo" route. Pathophysiologically, either delamination of the dural border cell layer or some kind of local shear forces are likely to be necessary to initiate the inflammatory cascade resulting in a CSDH. If acute haemorrhage is not necessarily present when this occurs, then this suggests relatively minor injury can be the trigger. This may mean that the dural border cells can also be injured by non-traumatic, seemingly innocuous stimuli, such as sudden brief changes in intra-cranial pressure (ICP) or CSF dynamics. This could include the combined effects of progressive cerebral atrophy, and movement (e.g. bending) causing strain on this important layer of cells. There are currently no studies assessing ways in which the dural border cells can be injured other than via a trauma, but this should be a focus for future research. Currently it is impossible to ascertain the initiating pathophysiology of a CSDH in non-trauma patients as they have no prior imaging for interrogation, however by evidencing that CSDH-DN exists, this alternative pathway for CSDH development can be investigated.

Understanding the exact chain of events that occurs between normal trauma imaging and the development of a CSDH-DN is also challenging and can only occur through serial imaging of such patients. As discussed in chapter one, CSF leakage and subdural hygroma have been implicated in CSDH pathophysiology, therefore careful review of "normal" baseline imaging in CSDH-DN patients is necessary. Careful analysis of baseline cerebral atrophy may also be valuable in improving understanding on the degree to which this contributes to CSDH-DN formation.

Understanding of these two pathophysiology sub-types may be important in guiding diagnosis, treatment and prediction of outcome in a heterogeneous CSDH population. This chapter will review the imaging from a sub-group of patients recruited to the Dex-CSDH study, in whom baseline imaging was available. This allows categorisation into the two CSDH sub-types and review of factors leading to this, such as atrophy and anti-coagulant exposure. It is hypothesised that as the pathophysiology differs, so might the natural course of the CSDH sub-types, thus enabling better prediction for the timing of development, recovery and even risk of recurrence from the differing sub-types.

Finally, the diagnostic CSDH imaging of the two sub-types will be compared to identify whether there are distinguishing features of each sub-type, and thus if they can be categorised without the benefit of the baseline imaging. This would enable differences between the sub-types to be investigated on a larger scale, in all patients with a CSDH who haven't had the benefit of baseline imaging.

2.2 Methods

Out of 205 patients recruited to the Dex-CSDH study in Cambridge between 2015-2017, 46 (22%) had cranial imaging prior to their CSDH diagnosis. This so called "baseline" imaging was undertaken due to minor head injury (within 10 days) or in one case, presentation with spontaneous headache. Subsequent CSDH diagnosis was within five months in all cases. The baseline imaging was not available for review in five cases therefore these patients were excluded and the remaining 41 patients comprised the study group for analysis. Demographics, baseline data and modified Rankin scale (mRS) scores dichotomised into favourable (0-3) and unfavourable (4-6) outcome at three and six months were collected for all patients in the study group. Breakdown of all mRS scores can be seen in chapter eight, this is a continuous assessment scale grading patients from asymptomatic (0) to dead (6) by increasing levels of disability. Dichotamising changes this binary categoriee, thereby making the comparison of groups easier, particularly when there are such small numbers of patients distributed among the categories. However, statistically there are also well-recognised drawbacks of dichotamising, as a great deal of information is lost and the assumption that there is a difference between the groups closet to the cut-off point (e.g. score 3 and 4) may not be correct, as it relies on there being a linear relationship between variables and outcome. It is important to consider this when looking at the final dichotamised groups.

All baseline imaging was analysed for presence of ASDH ipsilateral to subsequent CSDH development, and if present, the patient was classified as a CSDH-AT, otherwise they were classified as a CSDH-DN.

All scans containing an ASDH or a CSDH were measured using a by computer-assisted technique for total volume and mean density (see imaging chapter seven for methods). Any associated mid-line shift (MLS) was measured as the greatest distance from a central line dividing the cranium to the septum pellucidum (Figure 2.1). All CSDH scans were also classified by observation into either homogenous or mixed density (see imaging chapter seven for examples).

Figure 2.1; example of midline shift measurement (red line).

Baseline imaging for CSDH-DN patients was assessed for three measures of atrophy; bicaudate ratio (BCR), cortical atrophy scale (CAS) and subdural space depth (SSD). Further to this, each scan was classified for presence or absence of a subdural hygroma (SDG), and whether this was unilateral or bilateral.

The bicaudate ratio (BCR) is an indirect measure of cerebral atrophy, which has been shown to have excellent interobserver agreement (van Zagtan, Kessels, Boiten, & Lodder, 1999). Whilst it correlates significantly with volumetric measures of brain atrophy, it may be a stronger measure of ventricular volume than overall atrophy per se (Jeong et al., 2016). The BCR is calculated as the distance between the two caudate nuclei apices at the level they make the greatest indentation on the lateral ventricles, and dividing by the maximum width of the skull at the same level (Figure 2.2).

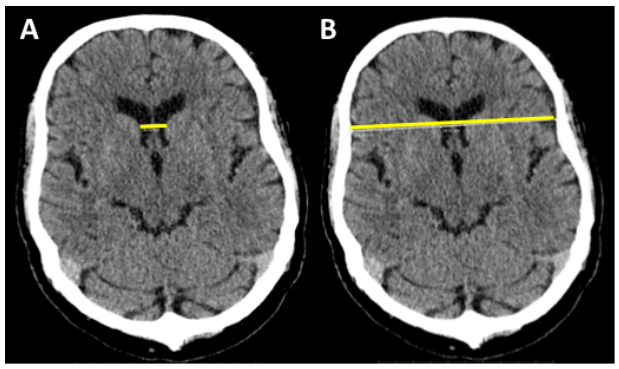

Figure 2.2; example of bicaudate ratio measurements: **(A)** yellow line = distance between two caudate nuclei, **(B)** yellow line = maximum skull width at same level.

The cortical atrophy scale (CAS) is a descriptive scale of brain atrophy, with higher scores (moderate-severe atrophy) correlated to an increased risk of CSDH following head injury (Jeong et al., 2016). Examples of scoring from A to D in patients from this study can be seen in Figure 2.3; there was only one patient classed as A and this patient has frontal hygromas.

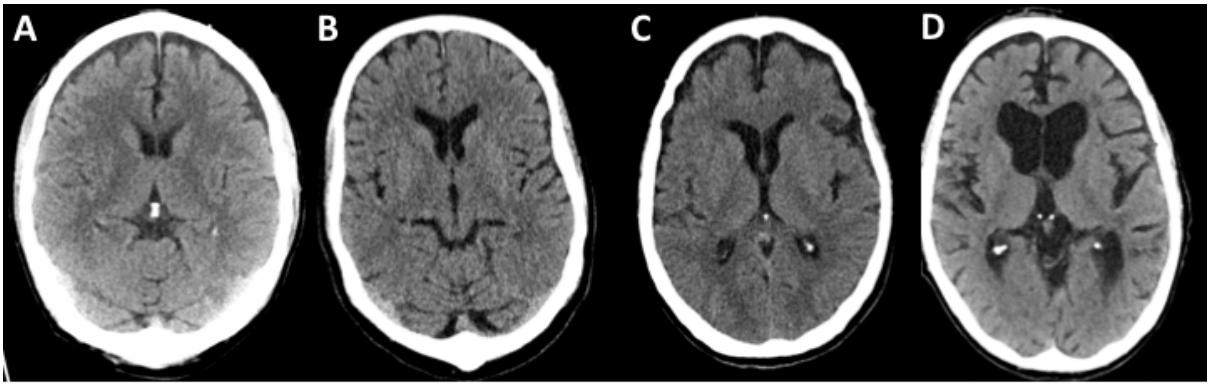

Figure 2.3; example of images assessed with cortical atrophy scale: **(A)** none (but this patient does have bilateral frontal subdural hygroma), **(B)** mild, **(C)** moderate, **(D)** severe.

An enlarged subdural space has also previously been associated with an increased risk of CSDH formation (Han et al., 2014; Ju et al., 2015). Therefore, the subdural space depth (SSD) was measured as the maximum distance from the cortex to the inner skull on any slice, as shown in Figure 2.4.

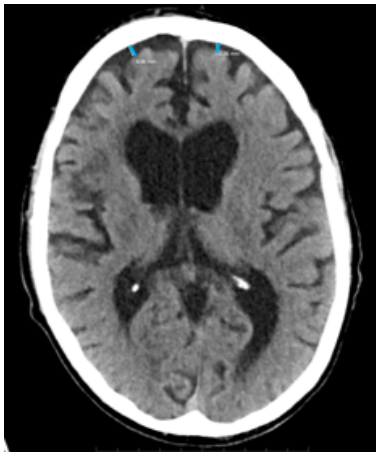

Figure 2.4; example of bilateral subdural space depth measurements (blue lines in frontal region)

Finally, the presence or absence of a SDG was made by blinded observer assessment. A SDG was considered present if the cortical surface was flattened and sulci obliterated, or if there was clear asymmetry between sides, as per Figure 2.5A (Lee, 1998).

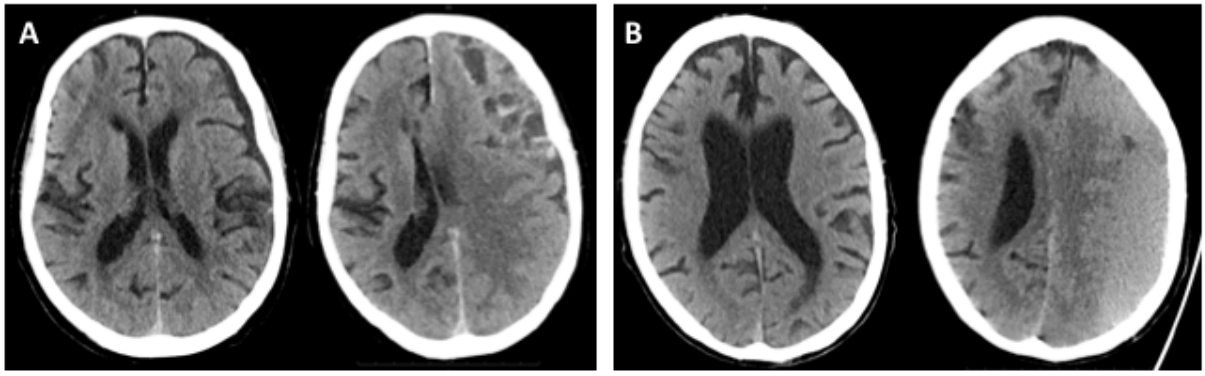

Figure 2.5; (A) example of left subdural hygroma present at baseline and left CSDH-DN on day 61, **(B)** example of no subdural hygroma at baseline and left CSDH-DN on day 45.

All linear and descriptive measurements (BCR, CAS, SSD, MLS, density) were undertaken using a Navigatium DICOM viewer by a single assessor (E Edlmann), as they have been previously validated. The volumetric analyses were undertaken using ITK-snap software (Yushkevich et al., 2006) also by a single assessor (E Edlmann), but validated by multiple assessors (results in chapter seven).

Statistical analysis

GraphPad Prism 7 was used for all statistical analysis. P values of <0.05 were considered significant. Continuous data (e.g. age) were compared using an unpaired t test if likely to results from a normal distribution and Mann-Whitney if not. Categorical data (e.g. gender) were tested with Fishers exact or Chi squared test, and Chi squared test for trend was used for multiple categories (such as GCS and mRS). Marginal significance is considered carefully, as with exploratory work such as this there is always the risk of false positives due to multiple comparisons.

2.3 Results

2.3.1 Classifying CSDH-AT and CSDH-DN

In 40/41 patients the baseline CSDH imaging was performed following head trauma. One patient experienced severe, sudden-onset headache prompting hospital attendance and baseline imaging revealed an idiopathic spontaneous ASDH.

In 26/41 patients (63%), there was no blood, acute or chronic, ipsilateral to the side of subsequent CSDH development; these patients were classified as having a CSDH-DN. In 15/41 patients (37%) there was presence of ASDH ipsilateral to the side of subsequent CSDH development; these patients were classified as having a CSDH-AT. 31/41 (76%) patients were scanned on the same day as the trauma/spontaneous ASDH, whilst the remaining 10 patients had a delayed scan within 10 days of trauma (Table 2.1). Two of the 41 patients underwent initial magnetic resonance imaging (MRI) rather than CT, both of these cases were in the CSDH-DN group.

Table 2.1: CSDH sub-types and timing of pre-CSDH imaging.

No. of days from trauma (or spontaneous ASDH):	**All patients**	**CSDH-DN**	**CSDH-AT**
All patients	41	26	15
Day 0 (same day)	31	19	12
Day 1 (next day)	2	1	1
Day 2 - 5	2	1 (D5)	1 (D2)
Day 7 - 10	6	5 (D7 x2, D10 x 3)	1

(D=day)

Several case examples of CSDH-DN and CSDH-AT can be seen in Figures 2.6 & 2.7. One case of CSDH-DN had serial imaging displaying the phases in CSDH formation from a normal baseline CT 10 days after initial trauma (Figure 2.6C), to bilateral hygromas on day 30, an isodense left subdural collection on day 51, to a symptomatic left-sided CSDH on day 76. This appears to support the theory of hygroma in the early stages, followed by isodense expansion, presumably secondary to repeated haemorrhage and finally continued expansion into a larger CSDH. This reflects the process previously described, where numerous fragile capillaries in the CSDH membrane contribute to repeated micro-haemorrhage, rather than

one single haemorrhage as seen with an ASDH (Moskala et al., 2007). It also exemplifies how although the injury may be widespread, causing bilateral hygromas, only one side progressed to CSDH, suggesting a specific process or level of dural border cell injury is required to initiate the inflammatory processes leading to CSDH membrane formation.

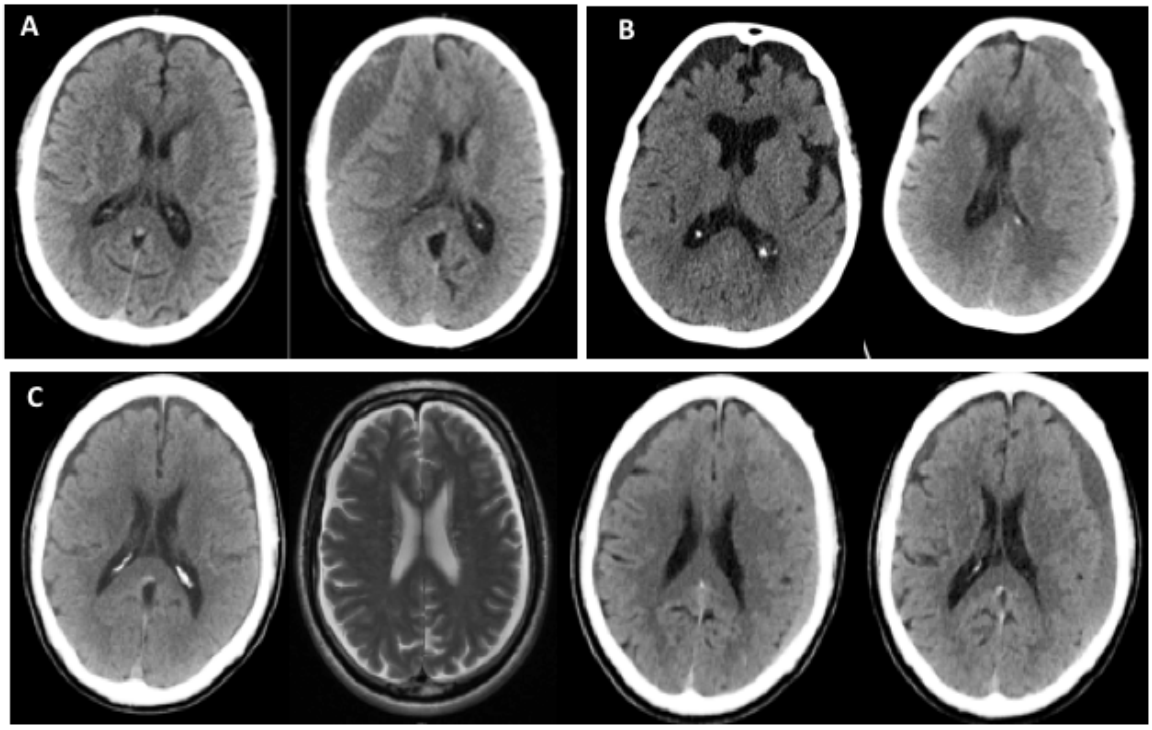

Figure 2.6; CSDH-DN case examples: **(A)** trauma scan normal day 0 and R CSDH day 112, **(B)** trauma scan bilateral frontal enlarged CSDH spaces, classified as atrophy, day 0 and L CSDH day 47, **(C)** trauma scan normal day 10, interval MRI small bilateral hygromas day 30, interval CT progressive change to CSDH day 51, final CT enlarging L CSDH day 76. (L = left, R = right).

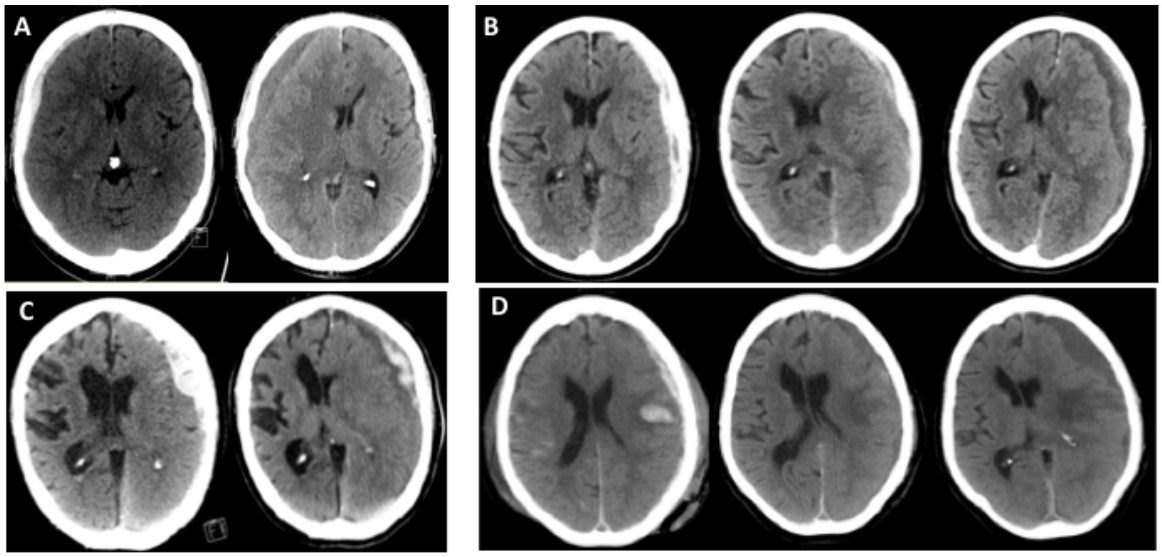

Figure 2.7; CSDH-AT case examples: **(A)** trauma scan R ASDH day 0, R CSDH day 18; **(B)** Trauma scan L ASDH day 0, interval scan day 6 and large L CSDH day 13, **(C)** trauma scan L ASDH day 0, mixed density L CSDH with increased shift day 7, (D) trauma scan L ASDH day 0, Interval scan day 14, repeat scan with larger L CSDH and increased shift day 17, (L = left, R = right).

2.3.2 Baseline data

There were no significant differences between the demographics for the CSDH sub-types, including age, gender and anti-platelet or anti-coagulant use (Table 2.2). However, the median time interval from trauma (or spontaneous ASDH, in one case) to CSDH diagnosis was significantly longer for CSDH-DN (median 57 days) than CSDH-AT (median 16 days) (Table 2.2).

Table 2.2; summary of baseline characteristics and time from trauma

	CSDH-DN	**CSDH-AT**	**p value (significance)**
Total no. of patients	26 (63%)	15 (37%)	
Mean age	78	75	p = 0.2836 (NS)
Gender	20 Male (77%) 6 Female (23%)	8 Male (53%) 7 Female (47%)	p = 0.4818 (NS)
Patients on AP or AC	14 (54%)	9 (60%)	p = 0.7021 (NS)
Median time interval to CSDH diagnosis	57 days (range 32-145 days)	16 days (range 7-45 days)	p < 0.0001 (S)

(AP = anti-platelet, AC = anti-coagulant, NS = not significant, S = significant)

The distribution of time intervals can be seen in Figure 2.8, with all CSDH-ATs occurring between one and three weeks (21 days), apart from one outlier who was diagnosed 45 days later. This outlier was also the smallest ASDH, only 20cm^3 which may go some way to explaining the prolonged time interval. The CSDH-DNs occurred up to 20 weeks (145 days) after trauma, and none before four weeks (32 days). This data is comparable to the literature regarding a CSDH transformed from an ASDH, with means of 15.3 to 23.3 days (Izumihara, Yamashita, & Murakami, 2013; Laviv, 2014). It is also logical that a CSDH-AT forms more rapidly as there is potentially greater disruption of the dural border cells layer with a volume of acute blood to both contribute to the CSDH volume and act as a constant stimulus for inflammation. Whereas in a CSDH-DN the entire collection must form from scratch and it may take longer for the inflammatory response to escalate.

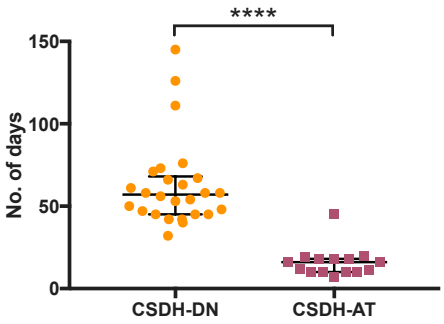

Figure 2.8; time from trauma (or spontaneous ASDH) in CSDH-DN and CSDH-AT. Line (median), bars (IQR), statistically significant differences denoted as P ≤ 0.0001 (****).

2.3.3 Baseline imaging characteristics

There were significantly more patients with bilateral CSDH-DN (9/26) than CSDH-AT (0/15) (p=0.0154, Table 2.3). However, in only five of the bilateral CSDH-DNs were both sides treated, with the other four having only the larger CSDH treated. Volumes were compared between sub-types for all treated CSDHs, excluding the untreated side of bilateral CSDHs and combining the volumes from each side if both sides were treated (see Table 2.3). This showed CSDH-DNs were significantly larger in volume than CSDH-ATs (p = 0.0111), which remained significant when only unilateral CSDHs were included (p = 0.05) (Figure 2.9).

Table 2.3: diagnostic CSDH imaging

	All CSDHs	CSDH-DN	CSDH-AT	P value (significance)
Laterality; all patients (n)	**n = 41**	**n = 26**	**n = 15**	
i. Patients with unilateral CSDH	32	17	15	p = 0.0154 (S)
ii. Patients with bilateral CSDH	9	9	0	
All treated CSDH volume (cm^3)		n = 26	n = 15	
Median	113	125.6	85.5	p = 0.0111 (S)
Min.		49.8	37	
Max.		246.5	137.7	
Unilateral CSDH volume (cm^3)		n = 17	n = 15	
Median	113	121.2	85.5	p = 0.05 (S)
Min.		49.8	37	
Max.		179.7	137.7	
Unilateral CSDH MLS (mm)		n = 17	n = 15	
Mean	10.3	9.9	10.7	p = 0.4968 (NS)
Min.		5.2	3.5	
Max.		15.8	16.5	
Mean density of all treated CSDHs (HU)	41.3	n = 26 40.5	n = 15 43.1	p = 0.3239 (NS)
No. of all CSDHs by density type				
Homogenous	23	16	7	p > 0.9999 (NS)
Mixed Density	23	15	8	

(BL - bilateral, MLS = midline shift)

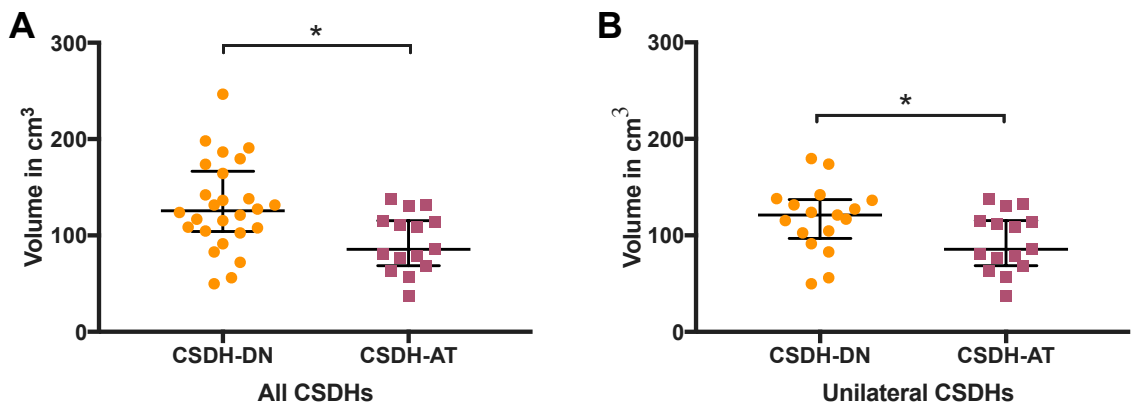

Figure 2.9; comparison of volume between CSDH-DN and CSDH-AT: (A) all CSDHs, (B) Unilateral CSDHs only. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.05$ (*).

All bilateral cases were excluded from MLS assessments due to the potential interaction between sides. There was very little difference in the mean midline shift between the CSDH sub-types (Figure 2.10). This suggests that although CSDH-DNs are significantly larger, they do not have an associated increase in MLS, either because they have more baseline atrophy to tolerate a greater CSDH volume, or because the longer time it takes to develop a CSDH-DN allows for better cerebral compensation and thus less MLS. Baseline atrophy cannot be compared between groups as it is too difficult to assess atrophy in the CSDH-AT who already have ASDH present on their baseline imaging.

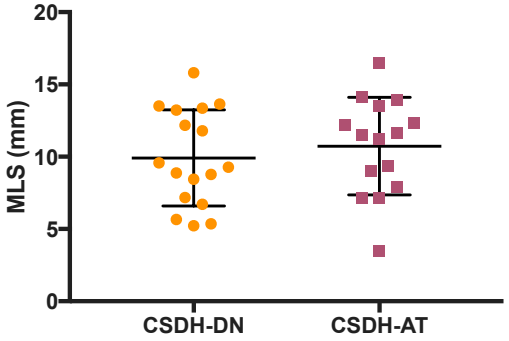

Figure 2.10; comparison of midline shift (MLS) between CSDH-DN and CSDH-AT. Line (mean), bars (S.D).

There was no significant difference in the mean density between CSDH-DN and CSDH-AT, and the grouping by density into homogenous or mixed density was almost the same for both sub-types (Table 2.3 and Figure 2.11). This suggests although they may have different pathological beginnings, both type of CSDH can similar patterns of either homogenous or mixed density by the time of diagnosis.

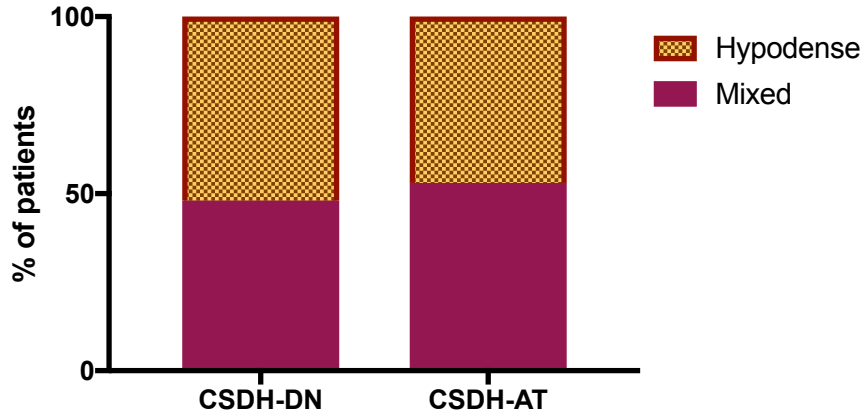

Figure 2.11; comparison of CSDH density between subtypes, as per Table 3.

ASDH imaging

The majority of patients who developed a CSDH-AT had an isolated ASDH (13/15) on the baseline imaging, which was most commonly homogenous (Table 2.4). Two patients had mixed density ASDHs, both of which had much greater volumes and MLS than all the other ASDHs. Counter-intuitively the presence of low density in the acute setting can represent hyper-acute blood, thus these mixed density ASDHs may be continuing to bleed, resulting in larger ASDHs with more associated MLS. Alternatively, the mixed-density may suggest that there was pre-existing CSDH present and there has been a large bleed within this. As all the mixed density ASDHs were following significant trauma and contained the vast majority of acute blood they continue to be classified as CSDH-AT, with the caveat that it is possible there was pre-existing blood there. The presence of underlying brain injury (contusions and traumatic subarachnoid blood, as seen in Figure 2.7D), is also associated with greater MLS, despite similar volumes. This is as expected, as the underlying parenchymal injury contributes to the MLS, independent of the volume of the ASDH.

Table 2.4; data on ASDHs from baseline imaging

	No. of patients	Mean MLS in mm	Median ASDH volume in cm^3
All ASDHs	15	5.18 (range 0 - 11.6)	60.7 (range 21.5 - 107.2)
Homogenous ASDH	11	3.78	59.6
Mixed density ASDH	2	11.5	110.1
ASDH + underlying brain injury	2	6.51	53.7

(ASDH = acute subdural haematoma, MLS = midline shift)

ASDH volume and time to CSDH-AT diagnosis had a significant negative correlation (Spearman r = -0.6811, p = 0.0174, Figure 2.12A), which remains significant, but weaker with removal of an obvious outlier (Spearman r = -0.5386, p = 0.0493, Figure 2.12B). Therefore, larger volume ASDHs have a shorter time interval to CSDH-AT development than smaller ones, which is logical. However, there was no significant correlation between ASDH volume and subsequent CSDH-AT volume (Spearman r = 0.3536, p = 0.1964), therefore larger ASDHs do not necessarily form larger CSDHs. Again, this is logical, as although they are forming more rapidly, you would expect most patients to be diagnosed with a similar volume CSDH, as this is the point at which they get cerebral decompensation and become symptomatic.

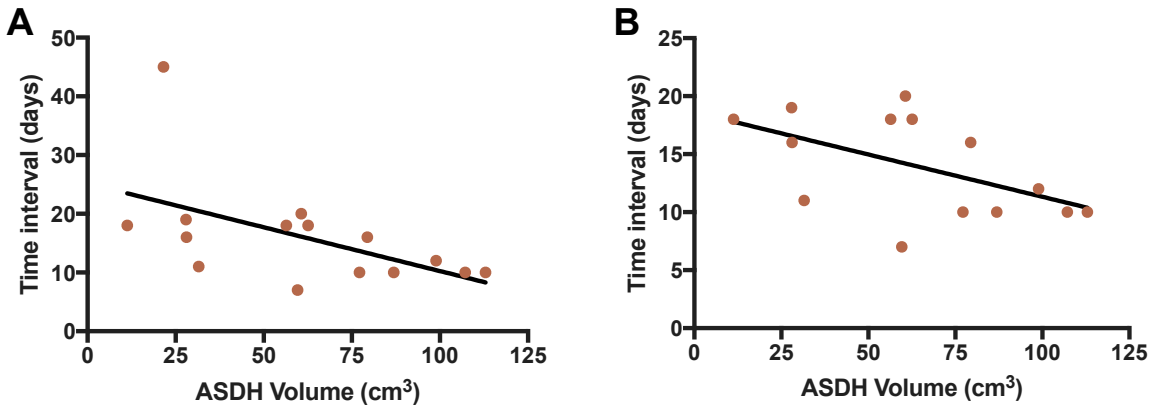

Figure 2.12; (A) correlation between ASDH volume and time interval to diagnosis, linear regression line y = -0.1492x + 25.17, **(B)** correlation between ASDH volume and time interval to diagnosis with removal of outlier, linear regression line y = -0.0729x + 18.62.

CSDH-DN and the subdural space

The baseline imaging was assessed in 25/26 CSDH-DN patients, with one patient excluded due to the abnormal gantry prohibiting BCR calculation. The BCR, CAS, SSD and presence or absence of SDG was assessed in all remaining 25 patients, with results summarised in Table 2.5.

An SDG was reported as present on baseline imaging in 14/26 (54%) CSDH-DN patients. In four patients this was unilateral, all of which correlated with the side of subsequent CSDH-DN development. The remaining 10 SDGs were bilateral; five developed only a unilateral CSDH-DN, three developed a bilateral CSDH but only one side needed treating, and two developed a bilateral CSDH-DN where both sides were treated. Therefore, counting all sides individually, two-thirds of SDGs (16/24) developed into a CSDH-DN which required treatment, making a strong case for their role in CSDH-DN pathophysiology.

Table 2.5; baseline trauma imaging measurement for CSDH-DN patients.

	All CSDH-DNs	**No SDG**	**SDG present**	**p value comparing SDG and no SDG (significance)**
No. of patients	25	11	14	
Median bicaudate ratio (range)	0.14 (0.09-0.26)	0.14 (0.09-0.26)	0.13 (0.09-0.18)	p = 0.6867 (NS)
Mean unilateral SSD (mm)	8.3	4.6	9.1	p < 0.0001 (S)
Mean time from trauma to baseline imaging (range)	2 days (0-10)	0.5 days (0-5)	3.2 days (0-10)	p = 0.0451 (S)
Median CSDH-DN volume (cm^3)*****	104.7	116.8	103.6	No (p = 0.5196)

* excludes any untreated sides of bilateral CSDH (SDG = subdural hygroma, SSD = subdural space depth).

The SSD was significantly higher on sides that had an SDG present than those that didn't (p < 0.0001) (Figure 2.13A). This suggests the SSD is a good objective measure of SDG presence. The limitation to this is the degree of pre-existing cerebral atrophy, as it is more difficult to measure an SDG in an already atrophic brain with large CSF spaces. This is

exemplified by Figure 2.13B which excludes all patients with the greatest degree of atrophy, CAS D, and shows a more obvious difference in the SSD between the SDG groups.

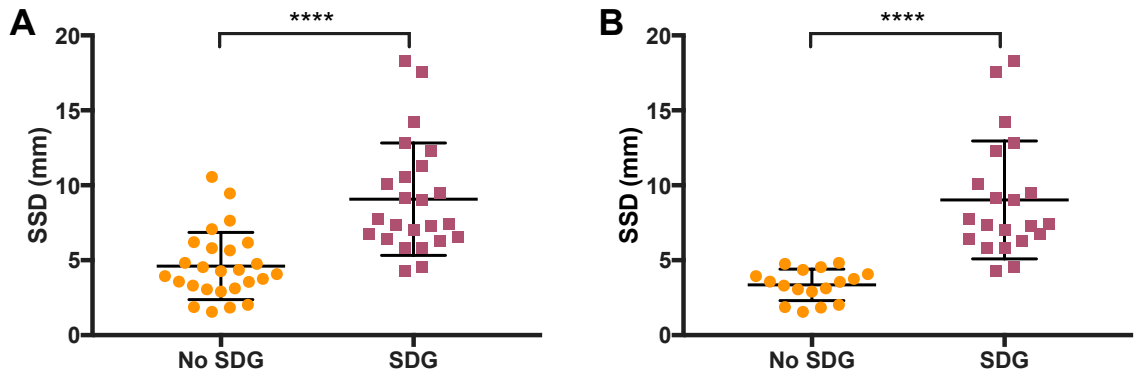

Figure 2.13; (A) Subdural space depth (SSD) in patients with and without subdural hygroma (SDG) (B) as per A, with CAS D patients excluded. Line (mean), bars (S.D.), statistically significant differences denoted as $P \leq 0.0001$ (****).

The time delay from trauma to baseline imaging was significantly longer in patients who had an SDG diagnosed (mean 3.2 days versus 0.5 days, $p = 0.0451$). This suggests that SDGs may take >24 hours to develop, and therefore immediate imaging misses them.

There was no significant difference in the CSDH-DN volume between those that had a prior SDG reported and those that did not ($p = 0.5125$). There was also no correlation between the final CSDH-DN volume and the time interval from initial trauma, Figure 2.14 ($r = -0.0835$, $p = 0.6552$), unlike with CSDH-AT.

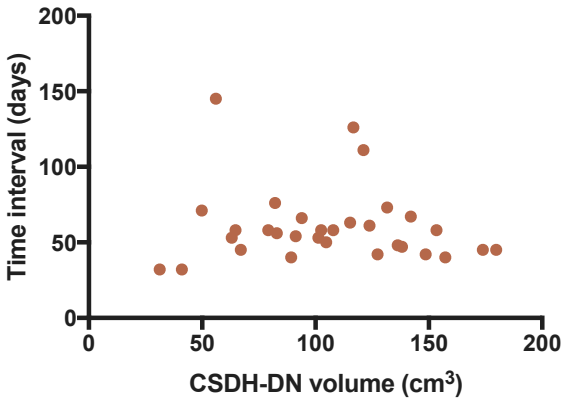

Figure 2.14; correlation between CSDH-DN volume and time interval from trauma to diagnosis.

The median BCR for all patients was 0.14 and there was no difference in BCR between patients with an SDG present or absent, suggesting increased atrophy doesn't necessarily increase the risk of an SDG (p = 0.6867). No correlation was found between the BCR and the total SSD, an alternative measure of atrophy (Spearman's r = 0.07043, p = 0.7436). However, the BCR did match well with the CAS grade, with significant differences in BCR between the four grades (One-way ANOVA, p = 0.0018) (Figure 2.15A). The BCR also showed a trend towards correlation with CSDH-DN volume, although this was weak (Spearman r = 0.3176, p 0.0931) (Figure 2.15B). This may be due to the increased space available for CSDH expansion in patients with a more atrophic brain, and hence higher BCR.

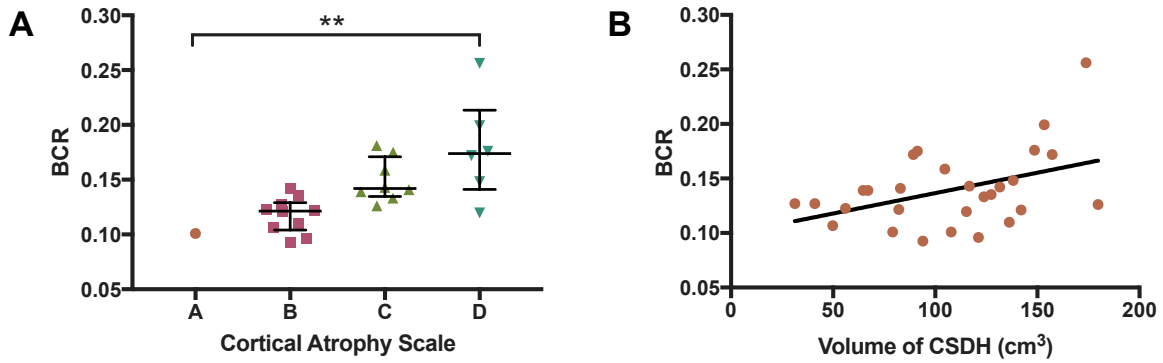

Figure 2.15; (A) comparison of bicaudate ratio (BCR) and cortical atrophy scale, line (median), bars (IQR), statistically significant differences denoted as P ≤ 0.01 (**), **(B)** correlation between BCR and CSDH-DN volume.

2.3.4 Outcome data

CSDH-AT patients had significantly lower GCS scores on their date of admission to the NSU compared with CSDH-DN patients (p = 0.0358) (Figure 2.16A). The "GCS ≤12" group included GCS's of 7, 8 and 9 in the CSDH-AT group, whereas the lowest GCS in the CSDH-DN group was 12, in only one patient. This difference in GCS had resolved by the time of discharge from NSU, with no significant difference between the sub-types, and the lowest GCS score was 13 (Figure 2.16B). Although not significant, it is interesting that despite having lower GCS scores to start with, the CSDH-AT group had better overall GCS scores at discharge. Perhaps suggesting the recovery is slower in the more long-standing CSDH-DNs.

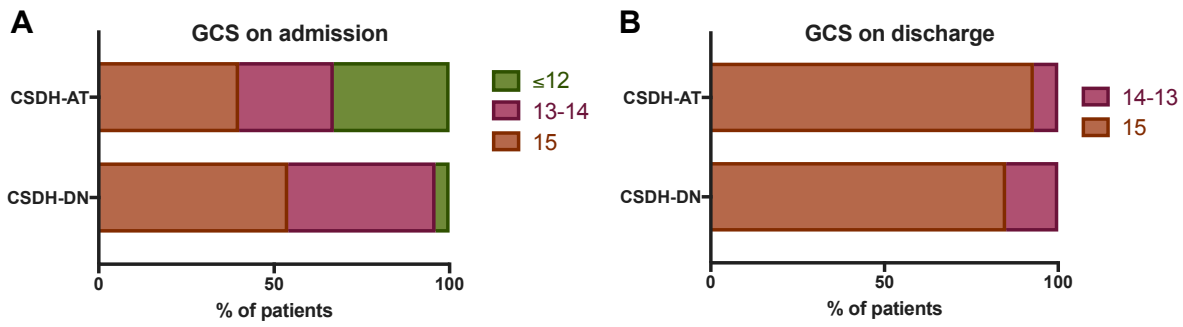

Figure 2.16; (A) GCS on admission to neurosurgical unit, **(B)** GCS on discharge from neurosurgical unit. CSDH-AT n = 15, CSDH-DN n = 26, (GCS = Glasgow coma score).

Two patients were managed conservatively with medical treatment only; both CSDH-DNs. The remaining 39 CSDHs were treated surgically, at a median of two days (range 0-12 days) from the diagnostic CSDH imaging. There were two recurrences, one was a CSDH-DN and the other CSDH-AT. There was no significant difference between the dichotomised 3-month or 6-month mRS scores for the CSDH sub-types (Figure 2.17).

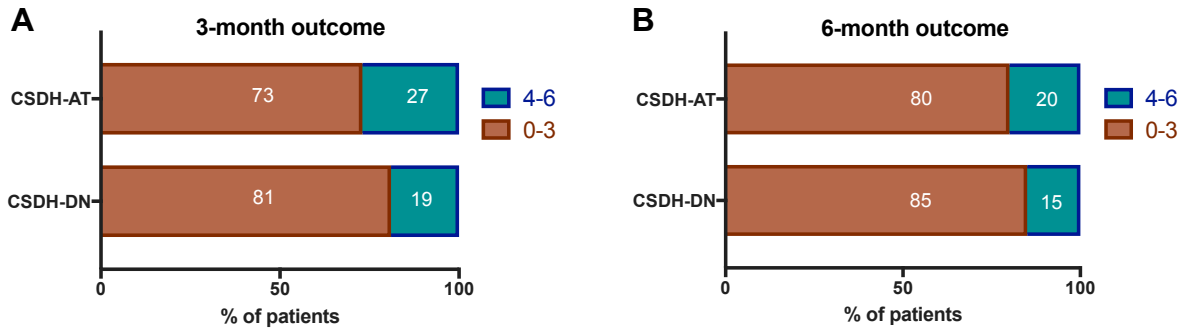

Figure 2.17; outcome with dichotamised mRS scores at: **(A)** three months, **(B)** six months. CSDH-AT n = 15, CSDH-DN n = 26, (mRS = modified Rankin scale).

2.4 Conclusions

In this study 41 CSDH patients had baseline imaging around the time of head injury or, in one case, unprovoked headache. Perhaps surprisingly, the baseline imaging was more commonly normal (63%), than showing ASDH (37%), and normal images were observed up to 10 days after traumatic injury. This evidences the hypothesis that whilst some patients undergo a transformational process from ASDH to CSDH (CSDH-AT), even more common is the de-novo development of CSDH (CSDH-DN) following trauma with no acute haemorrhage.

The main limitation is that only 46/205 patients had baseline imaging, therefore ideally large-scale studies are needed to get more numbers for statistical power. Time intervals were also calculated using the date of CSDH diagnostic imaging, rather than symptom onset, since the latter is much more difficult to define. This could potentially introduce bias, as patients with an ASDH on original imaging may be more likely to present earlier for follow-up imaging than those who have been told they have a normal original scan. However, the highly significant difference in time interval between CSDH-AT and CSDH-DN diagnosis, suggests the timeline of the pathophysiological processes is truly different.

As well as taking longer to present following trauma, CSDH-DN were also significantly larger than CSDH-AT. The volume difference was not simply a product of the longer time interval, as CSDH-DN volume was not correlated to time, and two of the largest CSDH-DNs formed in the shortest time interval. Therefore, time to develop a CSDH after trauma and volume of final CSDH appear to be independent factors, this may mean that the CSDH expansion rate is more related to variations in inflammation than time, and will be assessed in more detail in chapter six.

The size of ASDHs that become CSDHs appears to be small, with a mean volume of 61.5 cm^3 and mean MLS of 5.18 mm, compared to a mean volume of 146.1 cm^3 and MLS of 14.4mm in operated ASDHs in patients over 70 (Benedetto, Gambacciani, Montemurro, Morganti, & Perrini, 2017). Notably, Benedetto reported that 85.7% of patients with an ASDH volume over 200 cm^3 died within 10 days, thus there is a pre-selection of smaller ASDHs in those that survive long enough to have an CSDH-AT. However, there is also evidence that thicker ASDHs are more likely to develop a CSDH requiring surgical treatment (Benedetto et al.,

2017; Laviv, 2014). In our study the mean increase in MLS from ASDH to CSDH-AT was 5.52 mm, with volume increasing by over a third from a median of 60.7 cm^3 to 85.5 cm^3. This demonstrates reasonably rapid growth in a mean time-period of only 16 days. There was a significant inverse correlation between the volume of ASDH and the time interval to CSDH-AT diagnosis (Figure 2.12A). Thus, larger volume ASDHs transformed into symptomatic CSDHs more quickly, suggesting either a larger original blood load, or perhaps a more significant trauma, provides a greater stimulus for rapid conversion to CSDH. However, the CSDH-AT volume is not correlated to the original ASDH volume, therefore although forming more slowly, small ASDHs have the capacity to expand to the same extent as larger ASDHs.

Some authors believe that all CSDHs which do not form from an ASDH must involve an SDG (Lee, 2004; Nakaguchi, Tanishima, & Yoshimasu, 2001; Park et al., 2008). This study found significantly more bilateral CSDH-DN's (9/26) compared with CSDH-AT (0/15), and as SDGs are more commonly bilateral that unilateral, this adds some support to the theory of SDGs in CSDH-DN evolution (Ahn et al., 2016). Analysis of the baseline imaging specifically for SDG showed that 54% (14/26) of CSDH-DN patients had evidence of SDG, although those with SDG also had a significantly longer time interval from trauma to baseline imaging. This may mean that SDG occurs in all patients with CSDH-DN, but is only observed if the imaging is delayed following trauma, allowing time for CSF to accumulate. Only 1/7 patients with delayed imaging (at day five post-trauma) had no SDG reported, and all images performed after seven days had an SDG.

The subdural space depth (SSD) was significantly higher on the side of the cranium where an SDG was reported, compared to no SDG. Therefore, SSD could be used as a useful objective measure of the presence of SDG, which is often under-reported. This might help highlight patients who are more likely to develop a CSDH-DN following head trauma with a "normal" CT, however it is likely to be more challenging to diagnose these in patients with severe grades of atrophy. The bicaudate ratio (BCR) appeared to be a good reflection of observational assessments of atrophy, such as the cortical atrophy scale (CAS). However there was only a weak trend between BCR and CSDH-DN volume. Therefore, whilst brain atrophy is a well-recognised risk factor for CSDH, it only seems to have a small impact on the final volume of CSDH that forms. Van Gijn reported the upper limit of normal BCR in

patients as 0.21 in those under 80, therefore the measurements in these patients (mean 0.14) appear well within the normal range for a population of patients with a mean age of 78 (van Gijn, Hijdra, Wijdicks, Vermeulen, & van Crevel, 1985). To understand the role of atrophy further it would be useful to assess BCR measurements in all elderly patients with trauma to assess their predictive value in CSDH formation.

The low number of recurrences (two, with one in each CSDH sub-type) mean that no inferences can be made about CSDH pathophysiology with respect to this. It has been suggested that recurrence is higher in patients who present fewer than 60 days after trauma compared to after 60 days (Nakaguchi et al., 2001). This would indicate a higher recurrence rate for the CSDH-ATs, which tend to form more rapidly after trauma, but insufficient data is available to support this. Closer observation of patients with known CSDH-AT in the future may help elucidate whether this is a factor.

The GCS on admission to the neurosurgical unit was significantly lower in CSDH-AT patients than CSDH-DN, although this had resolved by discharge and there were no differences in three and six month mRS. The caveat to this is that the mRS was dichotomised, and is therefore only measuring the functional outcome in terms of patients that are independently mobile compared to those that are not, and will not be sensitive to more subtle differences. Further to this, with such a high good outcome rate in CSDH patients overall (80-85% at six months, see chapter 8), much larger patient numbers would be needed to see any significant differences. Longer and more detailed follow-up on these patients would be valuable to discern whether there are more subtle differences between the CSDH pathological sub-types.

There is already evidence of widespread brain tissue volume loss over a one year period in the chronic phase after moderate to severe TBI, unrelated to time since injury (Cole et al., 2018). This has not been studied in CSDH patients specifically but is likely to impact this population of patients, many of whom already have a significant degree of cerebral atrophy (6/25 CSDH-DN had grade D on CAS). Increased global cerebral atrophy may have implications for increased long-term dementia risk, as is seen in patients who develop dementia post-stroke (Leys, Henon, Mackowiak-Cordoliani, & Pasquier, 2005).

The different sub-types of CSDH may represent different severity of injury, with CSDH-AT more likely to have sustained a higher impact trauma causing ASDH, and thus potentially prone to long-term degenerative effects. Alternatively, it may be that the prolonged exposure to inflammation around the brain impacts future neurodegeneration and therefore CSDH-DN patients would be at greater risk due to the longer delay from trauma to CSDH diagnosis. Either way long-term follow-up studies on CSDH patients with a known pathophysiological process will be valuable to understand these risks and what factors contribute to possible secondary neurodegeneration.

Increased uptake of CSDH sub-categorisation such as de-novo and acute-transformed, where possible, may help increase understanding of some of the differences seen in this very heterogeneous condition. This is of particularly relevance to the assessment of new treatments such as dexamethasone, where understanding the pathophysiological sub-type or stage of inflammation may impact on how/whether the patient responds to therapy.

2.5 References

Ahn, J. H., Jun, H. S., Kim, J. H., Oh, J. K., Song, J. H., & Chang, I. B. (2016). Analysis of Risk Factor for the Development of Chronic Subdural Hematoma in Patients with Traumatic Subdural Hygroma. *J Korean Neurosurg Soc, 59*(6), 622-627. doi:10.3340/jkns.2016.59.6.622

Benedetto, N., Gambacciani, C., Montemurro, N., Morganti, R., & Perrini, P. (2017). Surgical management of acute subdural haematomas in elderly: report of a single center experience. *Br J Neurosurg, 31*(2), 244-248. doi:10.1080/02688697.2016.1244249

Cole, J. H., Jolly, A., de Simoni, S., Bourke, N., Patel, M. C., Scott, G., & Sharp, D. J. (2018). Spatial patterns of progressive brain volume loss after moderate-severe traumatic brain injury. *Brain, 141*(3), 822-836. doi:10.1093/brain/awx354

Han, S. B., Choi, S. W., Song, S. H., Youm, J. Y., Koh, H. S., Kim, S. H., & Kwon, H. J. (2014). Prediction of Chronic Subdural Hematoma in Minor Head Trauma Patients. *Korean J Neurotrauma, 10*(2), 106-111. doi:10.13004/kjnt.2014.10.2.106

Izumihara, A., Yamashita, K., & Murakami, T. (2013). Acute subdural hematoma requiring surgery in the subacute or chronic stage. *Neurol Med Chir (Tokyo), 53*(5), 323-328.

Jeong, E. O., Choi, S. W., Lim, J. W., Kwon, H. J., Kim, S. H., Koh, H. S., . . . Song, S. H. (2016). Effectiveness of Cortical Atrophy Scale and Indirect Indices of Brain Atrophy to Predict Chronic Subdural Hematoma in Older Patients. *Korean J Neurotrauma, 12*(2), 112-117. doi:10.13004/kjnt.2016.12.2.112

Ju, M. W., Kim, S. H., Kwon, H. J., Choi, S. W., Koh, H. S., Youm, J. Y., & Song, S. H. (2015). Comparision between Brain Atrophy and Subdural Volume to Predict Chronic Subdural Hematoma: Volumetric CT Imaging Analysis. *Korean J Neurotrauma, 11*(2), 87-92. doi:10.13004/kjnt.2015.11.2.87

Laviv, Y., Rappaport, Z. H. (2014). Risk factors for development of significant chronic subdural hematoma following conservative treatment of acute subdural hemorrhage. *Br J Neurosurg, 28*(6), 733-738. doi:10.3109/02688697.2014.918578

Lee, K. (1998). The pathogenesis and clinical significance of traumatic subdural hygroma. *Brain Injury, 12*(7), 595-603. doi:10.1080/026990598122359

Lee, K. (2004). Natural history of chronic subdural haematoma. *Brain Inj, 18*(4), 351-358. doi:10.1080/02699050310001645801

Leys, D., Henon, H., Mackowiak-Cordoliani, M. A., & Pasquier, F. (2005). Poststroke dementia. *Lancet Neurol, 4*(11), 752-759. doi:10.1016/s1474-4422(05)70221-0

Moskala, M., Goscinski, I., Kaluza, J., Polak, J., Krupa, M., Adamek, D., . . . Miodonski, A. J. (2007). Morphological aspects of the traumatic chronic subdural hematoma capsule: SEM studies. *Microsc Microanal, 13*(3), 211-219. doi:10.1017/S1431927607070286

Nakaguchi, H., Tanishima, T., & Yoshimasu, N. (2001). Factors in the natural history of chronic subdural hematomas that influence their postoperative recurrence. *J Neurosurg, 95*(2), 256-262. doi:10.3171/jns.2001.95.2.0256

Park, S. H., Lee, S. H., Park, J., Hwang, J. H., Hwang, S. K., & Hamm, I. S. (2008). Chronic subdural hematoma preceded by traumatic subdural hygroma. *J Clin Neurosci, 15*(8), 868-872. doi:10.1016/j.jocn.2007.08.003

van Gijn, J., Hijdra, A., Wijdicks, E. F., Vermeulen, M., & van Crevel, H. (1985). Acute hydrocephalus after aneurysmal subarachnoid hemorrhage. *J Neurosurg, 63*(3), 355-362. doi:10.3171/jns.1985.63.3.0355

van Zagtan, M., Kessels, F., Boiten, J., & Lodder, J. (1999). Interobserver agreement in the assessment of cerebral atrophy on CT using bicaudate and sylvian-fissure ratios. *Neuroradiology, 41*, 261-264.

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., & Gerig, G. (2006). User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage, 31*(3), 1116-1128. doi:10.1016/j.neuroimage.2006.01.015

Chapter 3 The fluid composition of CSDH

3.1 Introduction

Spectrophotometry is the quantitative measurement of the light transmission properties of a material, as a function of wavelength. By comparing the percentage of light that passes through a test solution (CSDH sample) in comparison to a reference solution (saline), the absorbance (also termed optical density) of the test solution can be quantified at each wavelength.

Ultraviolet-visible spectrophotometry (UV-Vis) has been used to characterise the constituents of blood, primarily determined by red blood cells (RBCs), which are approximately 99% of the cells present (Nonoyama, 2004), see Table 3.1. Haemoglobin (Hb) makes up 97% of the total protein in RBCs, and is therefore the dominant chromophore molecule which is used to estimate quantities of haemorrhage within a sample.

Table 3.1; components of blood

Blood component	Blood content	Size
55% Plasma	proteins, lipids, amino acids	<<< 1 μm
45% Cells	Red blood cells (40-45%)	6-8 μm
	White blood cells (1%)	neutrophils 12-15 μm lymphocyte 7-20 μm
	Platelets (<1%)	2- 4 μm

This study will compare the UV-Vis spectrum of whole blood with CSDH, in order to estimate the relative quantity of Hb in each. CSDH composition is similar to blood, with a cellular predominance of RBCs contained within an exudate (similar to plasma). A previous study utilising Cr-labelled RBCs showed that CSDHs contain, on average, 6.7% acute haemorrhage, and that all CSDHs contain at least some active haemorrhage (Ito, Yamamoto, Saito, Ikeda, & Hisada, 1987). RBCs have been evidenced to enter CSDH fluid by leaking through endothelial gap junctions of highly permeable CSDH outer membrane macrocapillaries, as seen in Figure 3.1 (Sato & Suzuki, 1975; Yamashima, Yamamoto, &

Friede, 1983). This bleeding is also promoted by impaired coagulation and hyperfibrinolysis, mediated by high levels of thrombin and tPA (tissue plasminogen activator) released from CSDH neomembranes (Shim, Park, Hyun, Park, & Yoon, 2007; Suzuki et al., 1998). Some of the Hb within the CSDH will also be "free" Hb, following breakdown of RBCs, and has been shown to be present at between 1-3 g/dL in CSDH fluid (Labadie & Glover, 1976; Weir & Gordon, 1983).

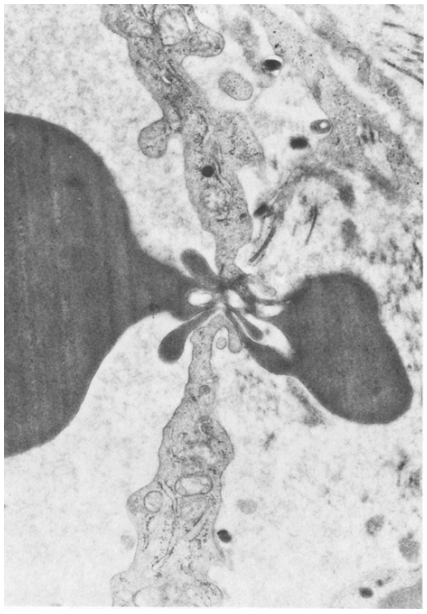

Figure 3.1; electron micrograph of two RBCs squeezing through an endothelial gap junction, out of a macrocapillary of the outer membrane of a CSDH, reproduced from (Yamashima et al., 1983).

The macrocapillary gap junctions in CSDH membranes also allow leakage of other blood components, such as the smaller platelets and plasma proteins (see Table 3.1), which forms the CSDH exudate (Fujisawa, Nomura, Tsuchida, & Ito, 1998; Tokmak, Iplikcioglu, Bek, Gokduman, & Erdal, 2007; Yamashima et al., 1983). Originally it was thought that plasma protein leakage into the subdural space would cause an oncotic gradient which would drive fluid expansion of CSDHs, however this theory was displaced by studies that clearly showed a lower oncotic and similar osmotic pressure within CSDH compared to venous blood (Labadie & Glover, 1976; Weir, 1980). The rate of protein exudation varies between CSDHs, and has been correlated with different sub-types of CSDH seen on imaging (Tokmak et al., 2007). This may reflect the different stages of membrane development and thus leakiness of capillaries, resulting in varying patterns of bleeding and fluid accumulation that appear differently on imaging. Finally, WBCs are clearly present in CSDH membranes and

important sources of inflammatory mediators, but it is less clear whether they actually enter the subdural space themselves, given their larger size (Moskala et al., 2007; Shono et al., 2001).

A complicating factor in UV-Vis spectroscopy is that particles, particularly large cells (e.g. RBCs and WBCs), cause light scattering. This means that some of the light that should reach the detector, is instead scattered by the cells, thus over-estimating the final absorption reading. To account for scattering, theoretical modelling (Mie theory) and complex mathematics can be applied, but is outside the scope of this thesis, and indeed the subject of an entire thesis already published (Nonoyama, 2004). There are also instruments which can be used to modify for scattering, such as an integrating sphere, however these have also been shown to have the adverse effect of under-estimating absorption peaks. Therefore, in this study a standard spectrophotometer was used with no re-modelling for scatter but an acceptance that the values of absorption are not exact, but the peak wavelengths and trends in absorption changes still meaningful. Another potential con-founding factor is pH, however the reported effects of pH on the UV-Vis spectra of human oxy-Hb is minimal, and therefore this was not considered to be a significant factor (Wimberley, Fogh-Andersen, Siggaard-Andersen, Lundsgaard, & Zijlstra, 1988).

Experiment Aim and hypotheses

The aim of this study is to compare the UV-Vis spectrum between paired venous whole blood and CSDH fluid to enable a relative quantification of Hb, and thus haemorrhage, within each CSDH.

Histological analysis of CSDH membranes show that they mature over time and that more mature membranes allow increased CSDH growth due to higher haemorrhage rates from macrocapillaries (Gandhoke, Kaif, Choi, Williamson, & Nakaji, 2013). Therefore, it is hypothesised that higher Hb levels measured with UV-Vis will be correlated to larger, and thus generally older, CSDHs (see chapter seven). It is also hypothesised that higher Hb will be related to CSDHs with higher imaging mean density, as higher density is considered to reflect fresh bleeding on imaging (see chapter seven).

As RBCs and their breakdown products have been previously implicated as drivers of the inflammatory response in CSDH (Labadie & Glover, 1976), it is also hypothesised that increased Hb will correlated with increased levels of inflammatory markers (see chapter five for full details on markers analysed). Further to this, it is hypothesised that this increased haemorrhage (and related inflammation), will also correlate to a higher recurrence rate.

3.2 Methods

52 patients were consented and recruited to the Dex-CSDH neurochemistry sub-study and had venous whole blood and intra-operative CSDH samples collected during their procedure. From these, 44 patients had UV-Vis spec performed, as either the primary investigator (E.Edlmann) or in some cases the laboratory post-doc (S. Giorgi-Coll), were available to process the samples immediately.

Samples were collected in 2.2 mL EDTA tubes and refrigerated immediately after collection at 5 °C until analysed. For the UV-Vis spec analysis, 5 µL of whole blood or CSDH was pipetted into 1000 µL of 0.9% saline solution in a 1.5 mL Eppendorf tube and inverted a few times for homogenous distribution of cells. The entire volume was then pipetted into a 1.5 mL quartz cuvette, with 1000 µL of 0.9% saline used as the reference sample, prior to analysis by UV-Vis at room temperature.

The CSDH samples only were then centrifuged for 10 minutes at 4,000 rpm. Following this the colour of the supernatant was categorised as either black/brown, dark-brown, dark-red, brown, light-brown, light-red or light-orange or yellow. The cell layer size was also recorded as either large, small or no cell layer (see examples in Figure 3.2). These subjective recordings of colour and cell layer size were performed by the same two people (E.Edlmann and S.Giorgi-Coll).

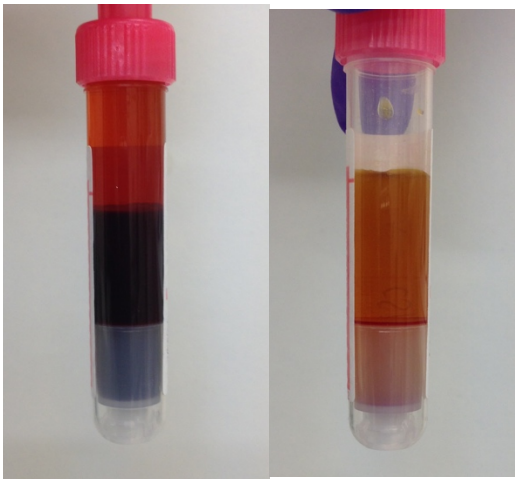

Figure 3.2; examples of cell layers in CSDH post-centrifugation; left shows a large cell layer and right shows no cell layer. Both supernatants are light red.

A Hitachi U-3900H spectrophotometer was used for UV-Vis analysis. This has a double light beam and monochromator; one beam for the reference sample and the other for test sample. There are two lamps; WI lamp (visible range) and Deuterium lamp (ultraviolet range). A spectral range of 200-700nm; a 1cm pathlength was used. The wavelength accuracy is +/- 0.1nm.

The results from the UV-Vis are displayed in spectral graphs depicting absorbance (in arbitrary units) and wavelength (nm). The software automatically fits the curve and provides values for the wavelengths of maximum absorption (i.e. the top of the peaks in the spectra), which are described as lambda-maximum (λ_{max}).

All statistical analysis was performed using Graphpad Prism 7 (GraphPad Software, La Jolla, CA, USA), with either paired/unpaired T-tests in the case of parametric data or Mann-Whitney tests for non-parametric data. All analysis assumed a significance level of $p < 0.05$.

3.3 Results

3.3.1 UV-Vis spectrophotometry of CSDH fluid and blood

One patient was excluded from the UV-vis analysis as the spectrum was flat, with no peaks. As discussed later in this chapter, it was determined that this was a subdural hygroma rather haematoma and therefore contained no RBCs and such minimal levels of any other cells or proteins that they were not detectable after the dilution method used. The UV-Vis spectra for the remaining 50 CSDH fluid samples from 43 patients (seven had bilateral CSDHs) and paired venous blood samples are shown in Figure 3.3. The pattern of peaks in the venous blood, are congruent with those previously reported and the representative constituents are seen in Table 3.2**.**

Table 3.2; UV-Vis spectra λ_{max} for blood and CSDH fluid

Peak (P)	λ_{max} **in literature** (nm)	**Blood** λ_{max} (nm)	**CSDH** λ_{max} (nm)	**Constituent of peak**
P1	210	212	210	Amide chains of proteins
P2	278	277	278	Protein peak (Tyrosine and tryptophan)
P3	344	345	351	NADH, NADPH
P4	416-7	417	410	Oxy-Haemoglobin
P5	540-7	543	542	Oxy-Haemoglobin
P6	575-8	577	577	Oxy-Haemoglobin

Data from literature as per; (Gunasekaran, 2008; Nonoyama, 2004; University of Birmingham).

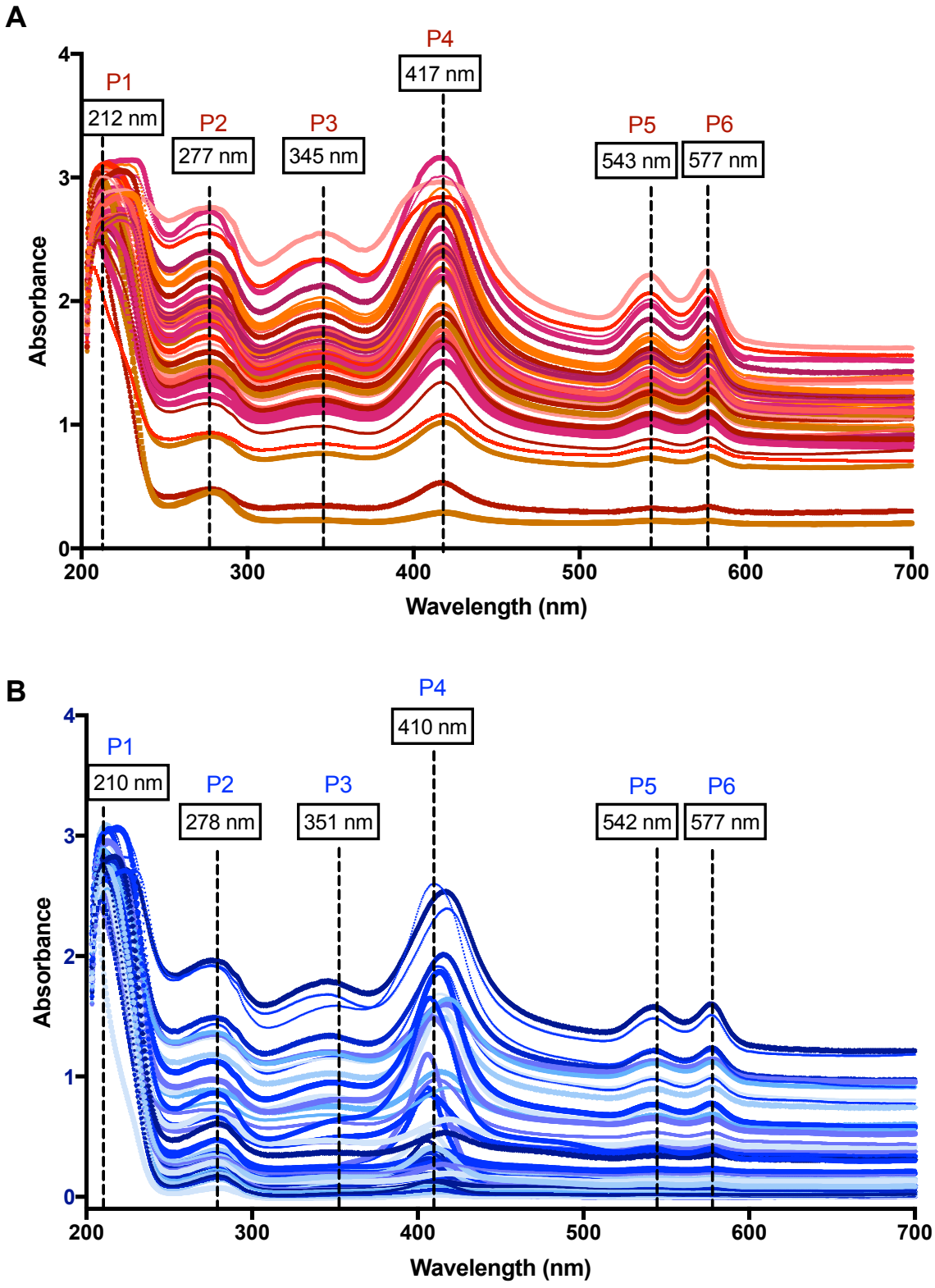

Figure 3.3; UV-Vis absorbance spectra of: **(A)** all venous whole blood samples, highlighting λ_{max} of 6 peaks, n = 43, **(B)** all CSDH fluid samples highlighting λ_{max} of 6 peaks, N = 50. (p = peak).

Peaks four to six are relevant to understanding the degree of haemorrhage within the CSDH as they relate to oxy-haemoglobin. Peak four is the only peak which shows a difference between CSDH fluid and venous blood, where a left-shift is observed from a mean wavelength of 417nm to 410nm, and is seen in more detail in individual patient number 08 in Figure 3.4. The implications of this are discussed further below.

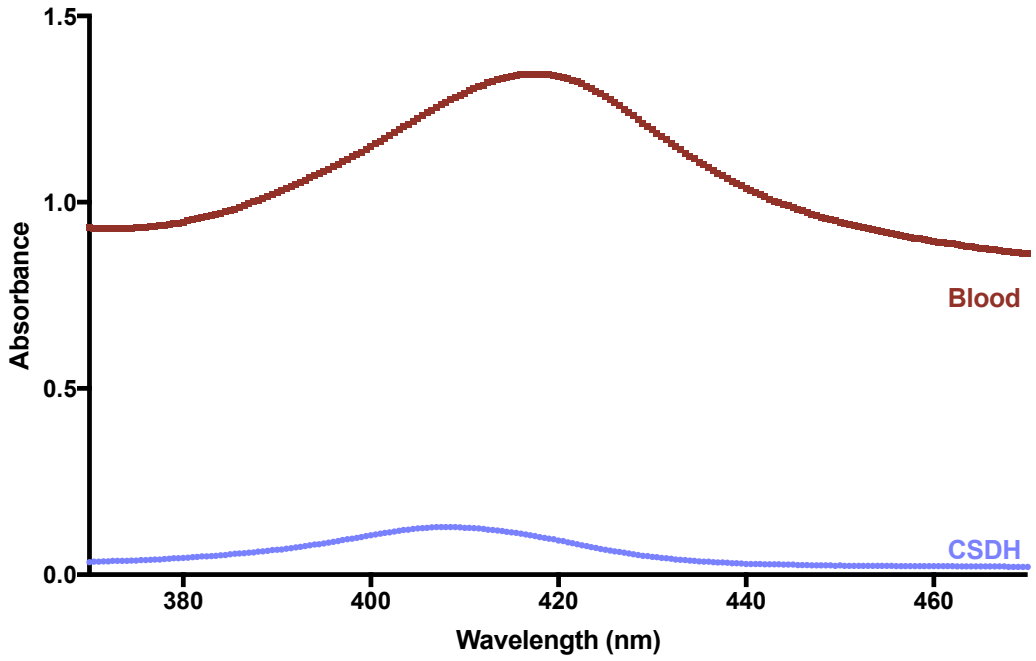

Figure 3.4; paired samples from patient 08 showing clear left shift of peak four in the CSDH fluid ($\lambda_{(max)}$= 408) compared to blood ($\lambda_{(max)}$= 417).

The mean absorbance of all the CSDH samples was approximately 25-30% of that seen in venous whole blood, as shown by the comparative absorbance maxima (Figure 3.5). This includes the main protein peak, which includes albumin (peak two), and corroborates the previous literature that there is lower protein in CSDH compared to venous blood (Labadie & Glover, 1976; Sizer & Peacock, 1947; Weir, 1980). The reduced heights of the remaining peaks suggest that all other blood components, especially RBCs, are found at lower levels in CSDH fluid compared to venous blood. This is either because they do not pass through freely, or because they are broken down once they have entered the CSDH cavity. These comparisons are only estimations as it must also be considered that the blood cell content may vary depending on the haematocrit, and could be diluted by the use of intra-operative fluids.

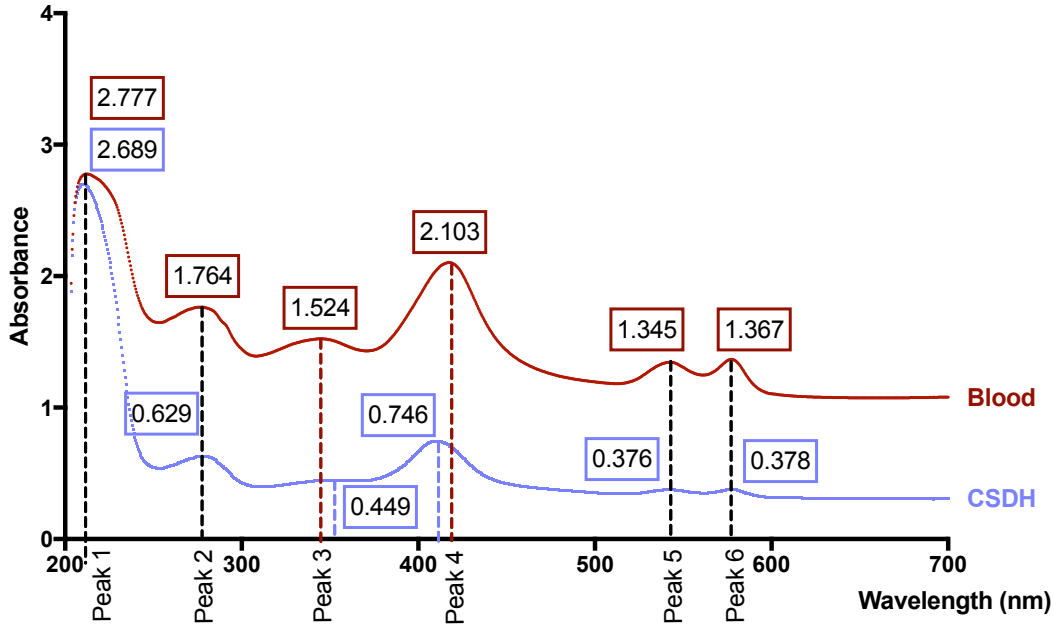

Figure 3.5; mean results of all blood and CSDH samples (n = 50), with boxes displaying mean absorbance at each peak $\lambda_{(max),}$ as per the wavelengths highlighted in Figure 3.1.

The UV-vis spectrum in Figures 3.3 and 3.5 show an elevated baseline above 600 nm despite no absorbance being expected in this range, and likely represents the increased absorbance secondary to Rayleigh light scattering by the high cell content discussed previously. To help overcome the effects of scattering the spectrum could be zeroed at the highest wavelength, although it is the λ_{max} rather than the peak height which is important. To avoid using absolute measurements, due to the confounding caused by scattering, the relative amount of RBCs in the paired CSDH and blood samples for each patient was determined. This was done by taking each CSDH fluid absorbance spectrum and converting it into a percentage of the paired venous blood sample absorbance, named the "blood fraction" (Figure 3.6). This shows a large range from just above 0% to over 100%, with higher values relating to CSDH with a larger fraction of haemorrhage within the CSDH compared to the venous blood. The highest four lines in Figure 3.6, all over 100%, are from bilateral CSDH samples in two patients (47 and 50). The abnormal spectra seen for these two patients is likely due to the fact that the CSDHs were much darker than blood, causing a large amount of scattering, and thus extremely high perceived absorbance in the CSDH samples.

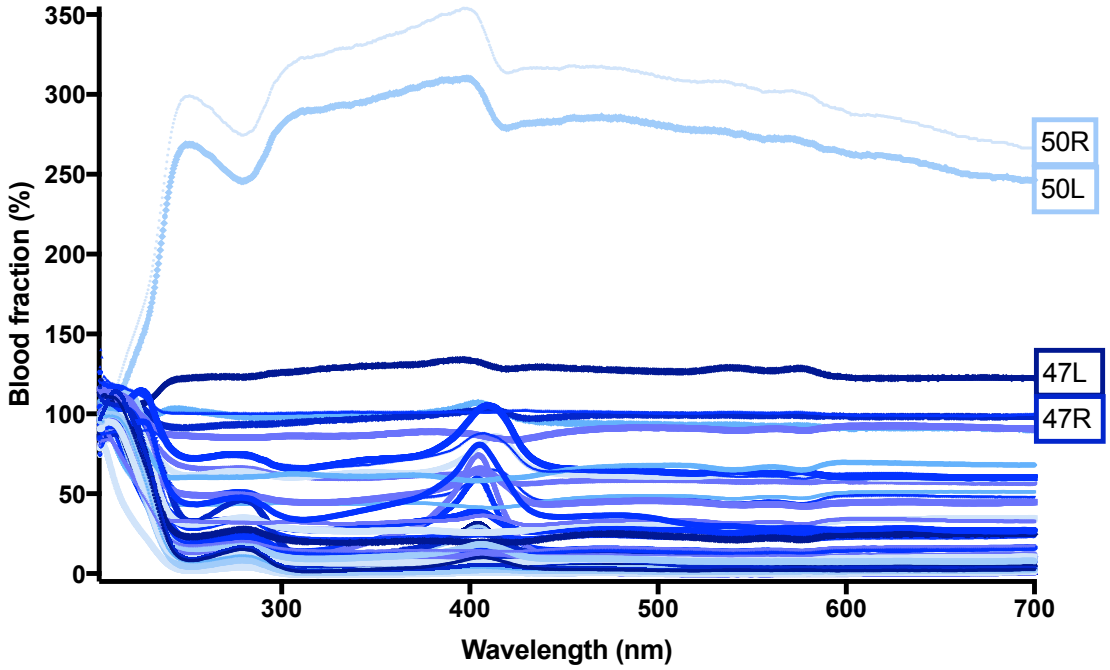

Figure 3.6; percentage difference between venous blood and CSDH fluid in UV-Vis absorbance. N = 50, patients 47 and 50 highlighted in boxes (R = right, L = left).

As highlighted previously, the main difference observed between the CSDH and venous blood samples was the wavelength of peak four. This peak is helpful in understanding the status of RBCs and type of haemoglobin, or its derivative, that is present in CSDH fluid. Kidwell et al. has shown that breakdown of intracranial haemorrhage occurs by oxidative denaturation, as the original oxy-haemoglobin becomes deoxy-haemoglobin and then methaemoglobin, which is eventually phagocytosed by macrophages (Kidwell & Wintermark, 2008). Figure 3.7 shows the formula for the dioxygenation reaction, which occurs when oxygenated Hb reacts with Nitric Oxide (NO) to form methemoglobin (FeIII) and nitrate, thus also "mopping up" any free NO (Kim-Shapiro, Lee, & Gladwin, 2011).

$$HbFe\ II - O_2 + NO \rightarrow HbFe\ III +$$

Figure 3.7; dioxygenation reaction for conversion of haemoglobin to methaemoglobin.

Whilst oxyhaemoglobin (oxyHb) has a characteristic peak at 417nm, methaemoglobin (metHb) has been shown to be left-shifted with a peak at 400nm and loss of peaks five and six (Nonoyama, 2004). Previous analysis of subarachnoid haemorrhage in cerebrospinal fluid (CSF) showed that if this peak is below 410nm, it is unequivocally suggestive of the presence

of metHb (Beetham, Fahie-Wilson, & Park, 1998)., Formation of metHb occurs more rapidly when Hb is free (i.e. following haemolysis of red blood cells), thus it was hypothesised that higher proportions of metHb, as seen with left shift of the peak to around 410nm, would represent greater RBC breakdown, and possibly an older CSDH.

MetHb has also been correlated with imaging findings, causing hyperintensity on T1 MRI (Senturk et al., 2010)**.** It has been suggested that this hyperintensity is reduced by absorption or degradation of metHb (Fobben et al., 1989). Therefore, persistent hyperintensity probably represents repeated haemorrhage and transformation to metHb, and has been reported in 67 - 79% of CSDHs (Hosoda, Tamaki, Masumura, Matsumoto, & Maeda, 1987; Senturk et al., 2010; Tsutsumi et al., 1997). The imaging collected in this study was all CT, therefore hyperintensity cannot be assessed, however the mean density, with a higher density suggesting more acute blood, is compared to the UV-Vis findings in section 3.3.5 below.

The mean wavelength at peak four across all samples was significantly lower in the CSDH fluid compared to venous blood (Mann-Whitney, $p < 0.0001$) (Figure 3.8A). This highlights the left-shift (or blue-shift) in the CSDH samples, where the lower wavelengths are at the bluer end of the spectrum and are consistent with a higher metHb/oxyHb ratio. There was also a wider spread of $\lambda_{(max)}$ at peak four in the CSDH samples (405.5 - 420 nm, see Figure 3.8B) compared to venous blood (415-418.5 nm). This highlights the variation in the balance between oxy and met-Hb in CSDH fluid, unlike blood which only contains oxyHb.

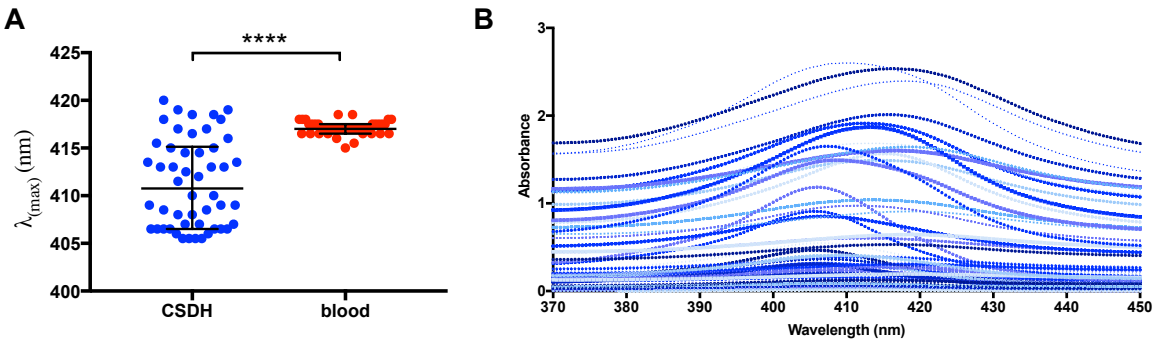

Figure 3.8; (A) peak four $\lambda_{(max)}$ in all CSDH and blood samples, CSDH n = 50, blood n = 43**,** line (mean) and bars (S.D), statistically significant differences denoted as p <0.0001 = ****. **(B)** variation in peak four in all CSDH samples, n = 50.

The shoulder of peak four was also analysed closely, as bilirubin can appear in this location, with a peak around 450-460nm (University of Birmingham). Bilirubin is another derivative of Hb formed by a rate-limited enzymatic reaction, and is often used as a diagnostic marker of subarachnoid haemorrhage (SAH) if found in the cerebrospinal fluid (CSF) (Tallur, Belton, Stephen, & Minns, 2005). There are two reports confirming the presence of bilirubin, alongside methaemoglobin, in subdural collections (Tallur et al., 2005; Wahlgren & Lindquist, 1988). However, Wahlgren et al. suggest that when the Hb concentration is low it is primarily converted in bilirubin, whilst higher Hb concentrations result in formation of methaemoglobin, due to the limited capacity of the haem oxygenase system required for bilirubin formation. Our analysis found no clearly identifiable separate bilirubin peaks, which may suggest that the CSDHs contain a large amount of Hb which exceeds the capacity of the oxygenase system to form bilirubin. Indeed, Tallur et al. report that bilirubin is correlated to bleeding in the last 24-72 hours, and therefore is less relevant to CSDH as it is to the more acute SAH (Tallur et al., 2005). However, it is likely that there is at least some bilirubin present, which may be hiding within the shoulder of peak 4, as many of the shoulders are broad and extend into the region of a bilirubin peak.

3.3.2 Methaemoglobin and CSDH fluid observations

Samples with a large "cell level" observed in the CSDH fluid had a significantly higher blood-fraction, than those with no or a small cell level (Unpaired T test, $p > 0.0001$) (Figure 3.9A). Due to the extremely high blood fraction in patient 50's bilateral samples (Figure 3.6), a further analysis was done excluding this patient, as it appeared as if it may be skewing the results. However, the difference remained at the same significance (Figure 3.9B). The high blood fraction in the CSDHs with a large cell level suggests that there is a larger volume of RBCs, and thus recent haemorrhage in these patients, as expected.

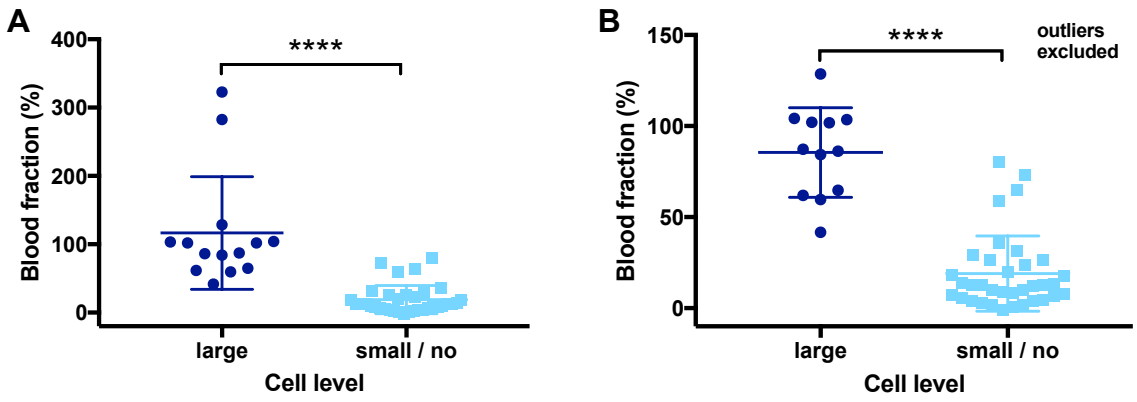

Figure 3.9; peak four blood fraction in relation to observed cell level in fluid: **(A)** all patients n = 49, **(B)** outliers excluded, n = 47. Line (mean), bars (S.D.), statistically significant differences denoted as p <0.01 = **.

When observing the colour spectrum of CSDH samples, those with the largest blood fraction (and hence also high cell level), were usually black-brown, followed by dark red-brown (Figure 3.10). The lower blood fraction correlated to dark brown and light red-brown or orange, suggesting these colours form when the RBCs have broken down and there are fewer cells left. When the colours were grouped into darker (black-brown and dark red-brown) and lighter colours (brown, light red/orange), there was a significant difference in blood fraction (unpaired T test, p = 0.0065). This gives the operative surgeon a good indication of the recent bleeding rate just by observing the CDSH fluid colour and cell level after centrifugation, or can also be seen if the sample is left undisturbed for a few hours in a test tube. The one patient with no peaks on the UV-Vis spectrum (who was excluded from all other analysis), was observed to be translucent fluid with slight yellow colouring and no cell level following centrifugation. This is in-keeping with a subdural hygroma, which are usually found to contain no Hb (Weir & Gordon, 1983).

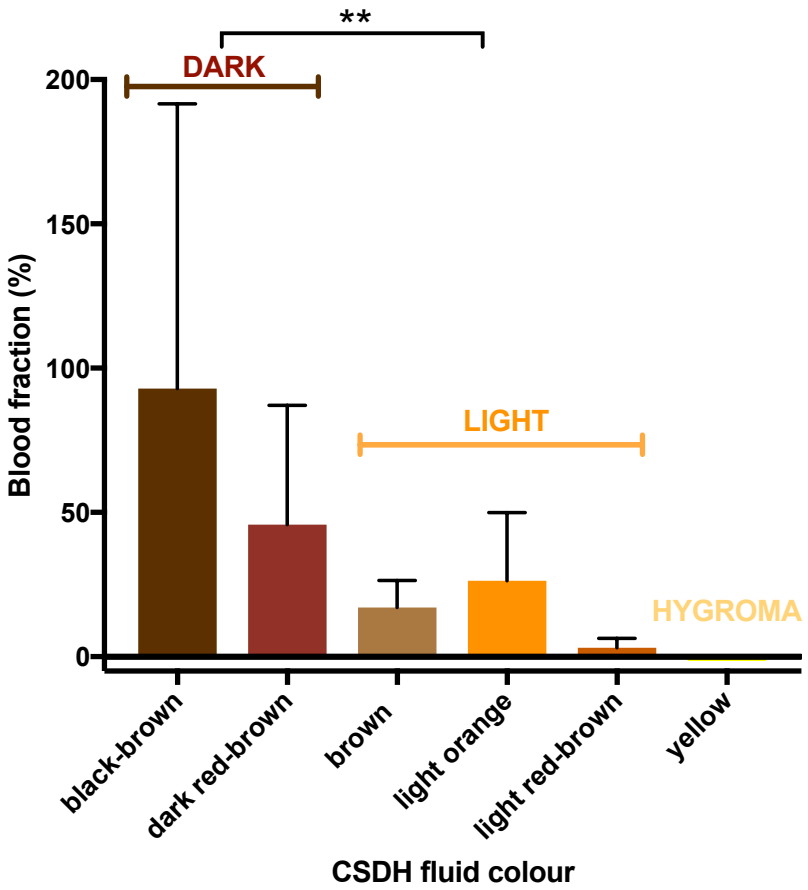

Figure 3.10; peak four blood fraction in relation to CSDH fluid colour, n = 51, bars (mean), lines (S.D.) statistically significant differences denoted as p <0.01 = **.

CSDHs with a large cell level also had a significantly higher $\lambda_{(max)}$ with a median of 414 nm compared to 408 nm in CSDHs with small/no cell level. This supports the theory that the large cell level indicates more recent bleeding, as a higher wavelength is essentially a red-shift (i.e. more red than blue) on the UV-Vis spectra, and is similar to the wavelength of the Hb peak in venous blood (417nm). CSDHs with "small" or "no" cell level appear to contain older haemorrhage, which has formed metHb following RBC breakdown, and shifted the wavelength lower (more towards the blue (UV) end of the spectrum) (Figure 3.11).

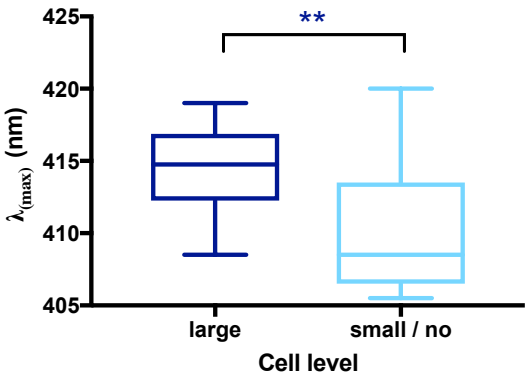

Figure 3.11; CSDH fluid peak four $\lambda_{(max)}$ in relation to observed cell level, n = 49 (one patient had no cell level recorded), line (median), box (IQR), bar (range), statistically significant differences denoted as $p \leq 0.005$ = **.

There was no significant difference in the peak four $\lambda_{(max)}$ on each side of a bilateral CSDH (paired T test, $p > 0.999$), with 4/7 cases showing very similar results on each side, whilst the other three did show variation (Figure 3.12A). There was a significant difference between the $\lambda_{(max)}$ of peak 4 between sides (paired T test, $p = 0.0245$), suggesting that there isn't always the same rate of haemorrhage or blood breakdown in co-existent, bilateral CSDHs.

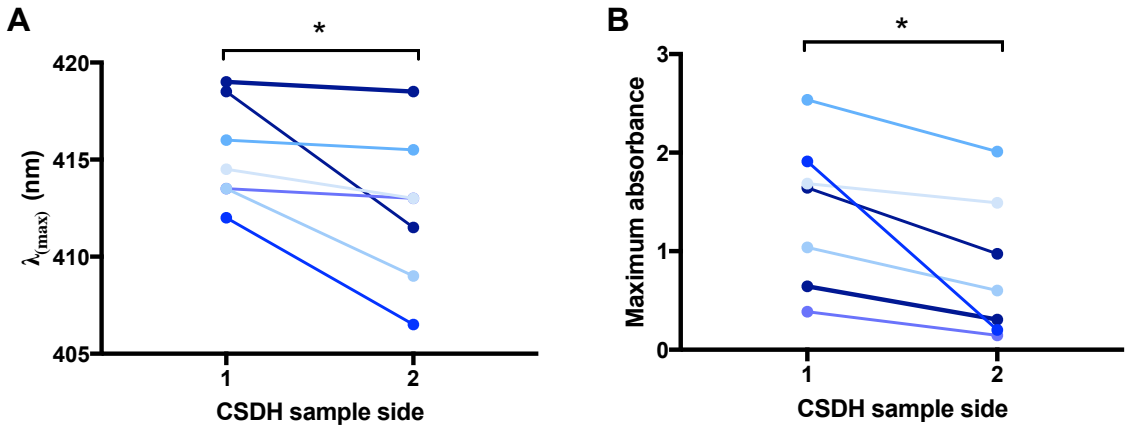

Figure 3.12; bilateral paired samples compared for: **(A)** peak four $\lambda_{(max)}$, **(B)** peak four maximum absorbance, n = 7, statistically significant differences denoted as $p \leq 0.05$ = *.

3.3.3 Methaemoglobin and inflammatory markers

It was hypothesised that an increase in Hb breakdown could act as a stimulus for inflammation, thus increasing the inflammatory markers in patients with higher proportions of metHb. The inflammatory profiles for a selection of markers (see details in chapter five) were compared to the $\lambda_{(max)}$ of peak four.

There were no correlations between peak four height, or blood fraction, and the concentration of any of the inflammatory markers. The only significant correlation found between peak four $\lambda_{(max)}$ and any of the inflammatory markers was a weak negative correlation with; MIP-1α (Spearman r = -0.484, p = 0.0004) and IL-1α (Spearman r = -0.3577, p = 0.0108) (Figure 3.13). This provides limited support for the hypothesis of higher levels of inflammation (with only two significant markers) in samples with lower Hb $\lambda_{(max)}$, and hence more metHb.

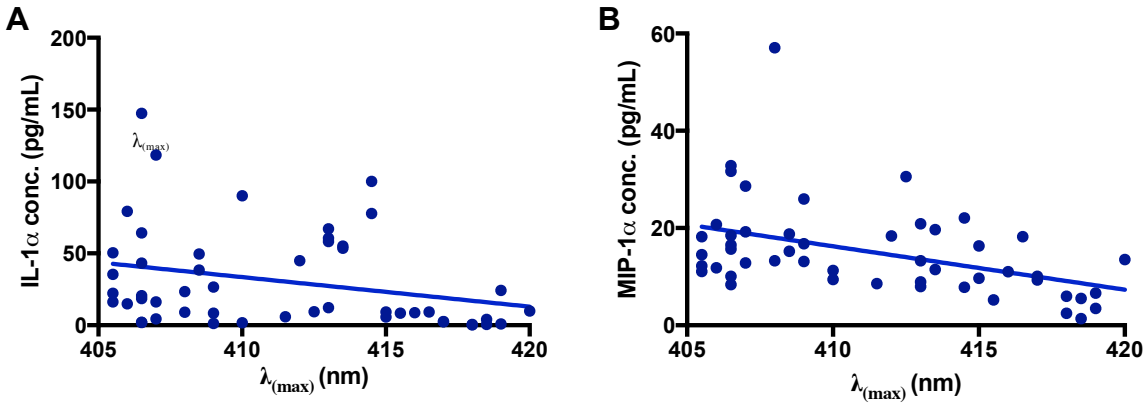

Figure 3.13; $\lambda_{(max)}$ correlated with**: (A)** IL-1α, **(B)** MIP-1α, n = 50.

A further analysis was performed by grouping all samples into those with a $\lambda_{(max)}$ equal to or below 410 nm, and those with a $\lambda_{(max)}$ above 410 nm; as the median $\lambda_{(max)}$ across all CSDH samples was approximately 410 nm. Again, only MIP-1α was significant increased in CSDHs with a low $\lambda_{(max)}$, and hence more metHb (p = 0.0028) (Figure 3.14).

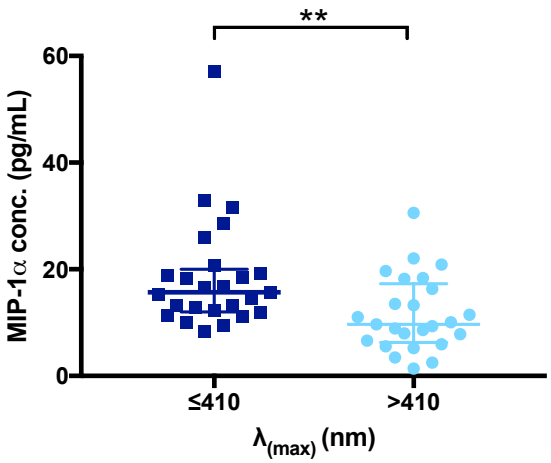

Figure 3.14; MIP-1α grouped by wavelength. ≤ 410 n = 25, >410 n = 25, statistically significant difference denoted as p ≤ 0.005 = **, line (median) and bars (IQR).

Overall, there is limited evidence to suggest that the inflammatory profile is related to the ratio of metHb or indeed overall amount of fresh blood (blood fraction) in CSDH fluid. Therefore, on the basis of this evidence, the hypothesis of bleeding rate and metHb content being correlated to the inflammatory response appears not to be true in established CSDHs. However, an alternative hypothesis is that Hb and/or metHb are important in the early stages of establishing CSDH but are less significant by the time of operation.

3.3.4 Methaemoglobin and imaging findings

49 CSDHs had both imaging and UV-vis data available for comparison, five of which were recurrent CSDHs.

It has already been established above that the cell level appears to reflect the amount of recent haemorrhage within the CSDH, therefore it is not surprising that there is a significant difference in the mean density on CT imaging between CSDHs with no (n=22), small (n=13) or large (n=13) cell levels (One-way ANOVA, p = 0.0007) (Figure 3.15A). Thus, those CSDHs with higher mean densities show more recent haemorrhage (which is hyperdense), as expected. When assessing CSDH volume, there was a trend for CDSHs with no evident cell level after centrifugation to be larger than those with small or large cell volumes as a group (Mann-Whitney, p = 0.0879) (Figure 3.15B), suggesting the largest CSDHs tend to contain less recent bleeding.

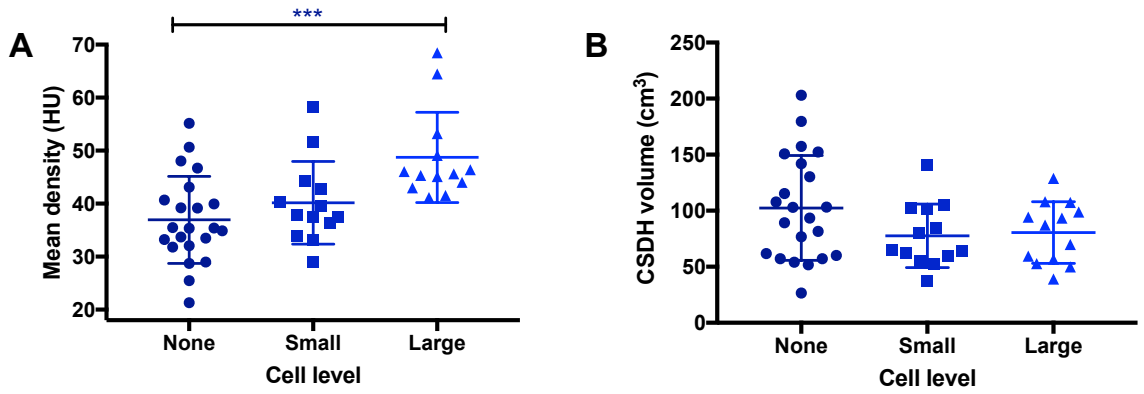

Figure 3.15; cell level by: (**A**) mean density, line (mean), bars (S.D.), (**B**) CSDH volume**,** line (median), bars (IQR), statistically significant differences denoted as p<0.05 = *, p<0.001 = ***.

The mean density was also assessed in CSDHs with high or low levels of metHb, as measured by peak four $\lambda_{(max)} \leq 410$ and > 410 nm respectively. This correlated well with the data in Figures 3.11 and 3.15A, as CSDHs with a higher mean density (and therefore a larger cell level), were also significantly more likely to have a wavelength >410 nm, corresponding to more recent haemorrhage (Unpaired T test, $p = 0.0026$) (Figure 3.16A). The CSDHs with a $\lambda_{(max)} \leq 410$ nm had lower mean densities, and thus CSDHs with more metHb, and less recent haemorrhage, are more likely to appear hypodense on imaging. This can also be seen with a significant correlation between peak four $\lambda_{(max)}$ and mean density ($p = 0.0021$, $r = 0.4332$) (Figure 3.16B).

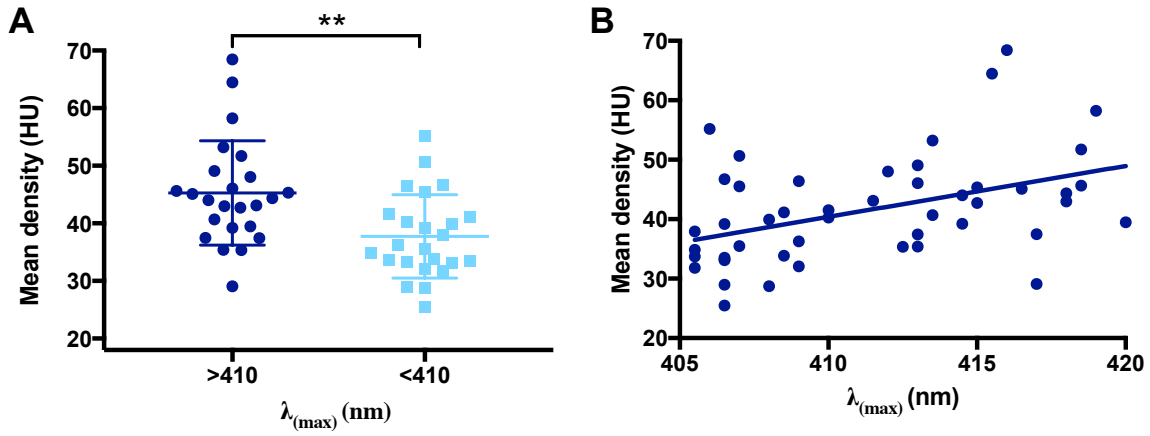

Figure 3.16; (A) CSDH mean density on CT in relation to peak 4 $\lambda_{(max)}$, line (mean), bars (S.D.). Statistical significance shown as $p<0.005$ = **, **(B)** correlation between peak 4 $\lambda_{(max)}$ and mean density on CT.

3.3.6 Methaemoglobin and recurrence risk

When comparing primary CSDHs samples from those that went on to recur (n = 5) with those with no recurrence (n = 37), there was a significantly higher peak four $\lambda_{(max)}$ in the recurrent group (Mann Whitney $p = 0.0237$) (Figure 3.17). Although the numbers of recurrences are small, there is a clear difference in wavelengths in all but one of the CSDHs in the recurrent group, suggesting that CSDHs that go on to recur have more recent acute haemorrhage at the time of primary surgery than those without this complication. This may explain part of the driving force of recurrence, as the CSDH is already in a cycle of active bleeding, and thus if this continues post-operatively, it is more likely to lead to recurrence than a more "dormant" CSDH at the time of evacuation.

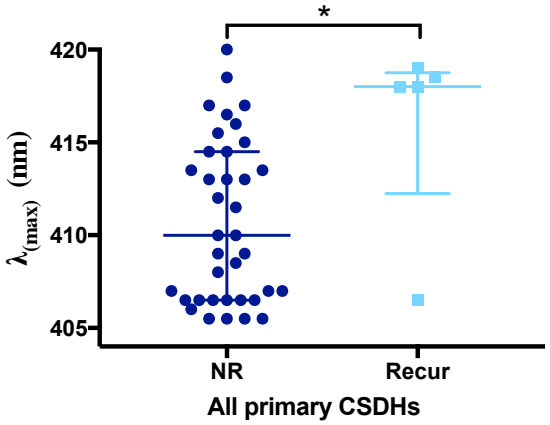

Figure 3.17; peak four $\lambda_{(max)}$ in primary CSDHs with no recurrence (NR) or recurrence (Recur), line (median), bars (IQR), statistically significant differences denoted as $p < 0.05$ = *.

When comparing the primary CSDH samples (n = 42) with the recurrent samples (n = 5), there was no significant difference in peak four $\lambda_{(max)}$ (Figure 3.18). Suggesting at the time of operation for recurrence the CSDH is no longer more likely to have recent acute haemorrhage.

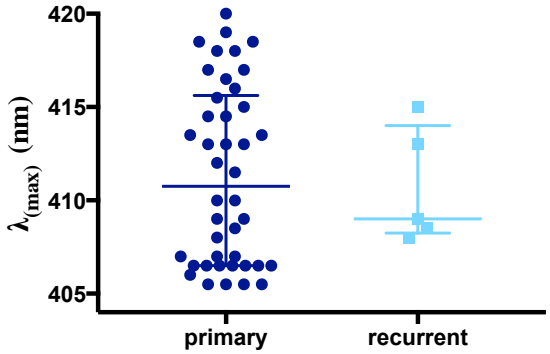

Figure 3.18; peak four wavelength in primary and recurrent (recur) CSDHs, line (median), bars (IQR).

3.4 Conclusions

In conclusion, the spectroscopy findings support the hypotheses that both protein exudation and haemorrhage contribute to the growth of CSDH. Whilst proteins appears to cross into the CSDH, they accumulates at lower levels than in venous blood, supporting the notion that oncotic gradient is not the source of CSDH expansion.

Overall, the RBC content in all 50 CSDHs is approximately 25-30% that of venous blood, but ranges from just over 0% to nearer to 100% of the RBC content within the paired blood sample. However, the latter samples are likely to have exaggerated levels due to the artifact of scattering, which increases exponentially with the number of cells within a sample, thus still highlighting that there is a very high RBC cell count within these samples.

The median $\lambda_{(max}$ of peak four is 410 nm in CSDH fluid, compared to 417 nm in venous blood, representing the left (or blue-shift) seen with presence of metHb, a breakdown product of Hb which has previously been recognised to accumulate in old cerebral haemorrhage. Therefore, the this peak informs on the ratio of old to new haemorrhage within CSDHs and a higher $\lambda_{(max)}$, associated with more acute haemorrhage, is also more likely to appear dark in colour (black/dark red-brown) and have a large cell level. The latter of which also correlates with a higher mean density on CT (as occurs with acute bleeding) and a lower volume. Conversely older CSDHs, with lower $\lambda_{(max),}$ representing more metHb, tend to be more low density and larger in volume on CT.

Perhaps the most significant finding is that CSDHs that go on to recur have significantly higher $\lambda_{(max)}$ at their original operation, and thus more recent acute haemorrhage than those that don't recur. Therefore, recent haemorrhage may be a driver of recurrence, and patients at high risk can be identified by high density on CT or simply observing a high cell-level in the drained CSDH fluid. Surprisingly, patients on anti-coagulants and anti-platelets do not have higher mean density on CT (see chapter 7), therefore the bleeding rate in CSDHs at diagnosis does not seem to be directly related to these drugs. However, there is still a perception that these medications increase the overall risk of CSDH, this is discussed further in chapter 8.

There is limited evidence that metHb or bleeding is a stimulus for inflammation, therefore they may be independent processes involved in CSDH evolution.

3.5 References

Beetham, R., Fahie-Wilson, M. N., & Park, D. (1998). What is the role of CSF spectrophotometry in the diagnosis of subarachnoid haemorrhage? *Ann Clin Biochem, 35 (Pt 1)*, 1-4. doi:10.1177/000456329803500101

Fobben, E. S., Grossman, R. I., Atlas, S. W., Hackney, D. B., Goldberg, H. I., Zimmerman, R. A., & Bilaniuk, L. T. (1989). MR characteristics of subdural hematomas and hygromas at 1.5 T. *AJR Am J Roentgenol, 153*(3), 589-595. doi:10.2214/ajr.153.3.589

Fujisawa, H., Nomura, S., Tsuchida, E., & Ito, H. (1998). Serum protein exudation in chronic subdural haematomas: a mechanism for haematoma enlargement? *Acta Neurochir (Wien), 140*(2), 161-165; discussion 165-166.

Gandhoke, G. S., Kaif, M., Choi, L., Williamson, R. W., & Nakaji, P. (2013). Histopathological features of the outer membrane of chronic subdural hematoma and correlation with clinical and radiological features. *J Clin Neurosci, 20*(10), 1398-1401. doi:10.1016/j.jocn.2013.01.010

Gunasekaran. (2008). UV-Vis approach and absorption model for discrimination diseased blood. *Asian J Chem, 20*(1).

Hosoda, K., Tamaki, N., Masumura, M., Matsumoto, S., & Maeda, F. (1987). Magnetic resonance images of chronic subdural hematomas. *J Neurosurg, 67*(5), 677-683. doi:10.3171/jns.1987.67.5.0677

Ito, H., Yamamoto, S., Saito, K., Ikeda, K., & Hisada, K. (1987). Quantitative estimation of hemorrhage in chronic subdural hematoma using the 51Cr erythrocyte labeling method. *J Neurosurg, 66*(6), 862-864. doi:10.3171/jns.1987.66.6.0862

Kidwell, C. S., & Wintermark, M. (2008). Imaging of intracranial haemorrhage. *The Lancet Neurology, 7*(3), 256-267. doi:10.1016/s1474-4422(08)70041-3

Kim-Shapiro, D. B., Lee, J., & Gladwin, M. T. (2011). Storage lesion: role of red blood cell breakdown. *Transfusion, 51*(4), 844-851. doi:10.1111/j.1537-2995.2011.03100.x

Labadie, E. L., & Glover, D. (1976). Physiopathogenesis of subdural hematomas. Part 1: Histological and biochemical comparisons of subcutaneous hematoma in rats with subdural hematoma in man. *J Neurosurg, 45*(4), 382-392. doi:10.3171/jns.1976.45.4.0382

Moskala, M., Goscinski, I., Kaluza, J., Polak, J., Krupa, M., Adamek, D., . . . Miodonski, A. J. (2007). Morphological aspects of the traumatic chronic subdural hematoma capsule: SEM studies. *Microsc Microanal, 13*(3), 211-219. doi:10.1017/S1431927607070286

Nonoyama, A. (2004). Using multiwavelength UV-visible spectroscopy for the characterization of red blood cells: An investigation of hypochromism. *Graduate Thesis and Dissertation, Scholar Commons University of Florida*.

Sato, S., & Suzuki, J. (1975). Ultrastructural observations of the capsule of chronic subdural hematoma in various clinical stages. *J Neurosurg, 43*(5), 569-578. doi:10.3171/jns.1975.43.5.0569

Senturk, S., Guzel, A., Bilici, A., Takmaz, I., Guzel, E., Aluclu, M. U., & Ceviz, A. (2010). CT and MR imaging of chronic subdural hematomas: a comparative study. *Swiss Med Wkly, 140*(23-24), 335-340. doi:smw-12867

Shim, Y. S., Park, C. O., Hyun, D. K., Park, H. C., & Yoon, S. H. (2007). What are the causative factors for a slow, progressive enlargement of a chronic subdural hematoma? *Yonsei Med J, 48*(2), 210-217. doi:10.3349/ymj.2007.48.2.210

Shono, T., Inamura, T., Morioka, T., Matsumoto, K., Suzuki, S. O., Ikezaki, K., . . . Fukui, M. (2001). Vascular endothelial growth factor in chronic subdural haematomas. *J Clin Neurosci, 8*(5), 411-415. doi:10.1054/jocn.2000.0951

Sizer, I. W., & Peacock, A. C. (1947). The ultraviolet absorption of serum albumin and of its constituent amino acids as a function of pH. *J Biol Chem, 171*(2), 767-777.

Suzuki, M., Kudo, A., Kitakami, A., Doi, M., Kubo, N., Kuroda, K., & Ogawa, A. (1998). Local hypercoagulative activity precedes hyperfibrinolytic activity in the subdural space during development of chronic subdural haematoma from subdural effusion. *Acta Neurochir (Wien), 140*(3), 261-265; discussion 265-266.

Tallur, K. K., Belton, N. R., Stephen, R., & Minns, R. A. (2005). Is timing of haemorrhage by spectrophotometry similar for haemorrhages in the subdural and subarachnoid space? *Arch Dis Child, 90*(11), 1203. doi:10.1136/adc.2003.036061

Tokmak, M., Iplikcioglu, A. C., Bek, S., Gokduman, C. A., & Erdal, M. (2007). The role of exudation in chronic subdural hematomas. *J Neurosurg, 107*(2), 290-295. doi:10.3171/JNS-07/08/0290

Tsutsumi, K., Maeda, K., Iijima, A., Usui, M., Okada, Y., & Kirino, T. (1997). The relationship of preoperative magnetic resonance imaging findings and closed system drainage in the recurrence of chronic subdural hematoma. *J Neurosurg, 87*(6), 870-875. doi:10.3171/jns.1997.87.6.0870

University of Birmingham. Spectrophotometry Of Cerebrospinal Fluid (CSF). Retrieved from https://www.birmingham.ac.uk/facilities/clinical-immunology-services/neuroimmunology/other-services/xanthochroma.aspx.

Wahlgren, N. G., & Lindquist, C. (1988). Haem derivatives in subdural haematomas. *Acta Neurochir (Wien), 95*(3-4), 109-113.

Weir, B. (1980). Oncotic pressure of subdural fluids. *J Neurosurg, 53*(4), 512-515. doi:10.3171/jns.1980.53.4.0512

Weir, B., & Gordon, P. (1983). Factors affecting coagulation: fibrinolysis in chronic subdural fluid collections. *J Neurosurg, 58*(2), 242-245. doi:10.3171/jns.1983.58.2.0242

Wimberley, P. D., Fogh-Andersen, N., Siggaard-Andersen, O., Lundsgaard, F. C., & Zijlstra, W. G. (1988). Effect of pH on the absorption spectrum of human oxyhemoglobin: a potential source of error in measuring the oxygen saturation of hemoglobin. *Clin Chem, 34*(4), 750-754.

Yamashima, T., Yamamoto, S., & Friede, R. L. (1983). The role of endothelial gap junctions in the enlargement of chronic subdural hematomas. *J Neurosurg, 59*(2), 298-303. doi:10.3171/jns.1983.59.2.0298

Chapter 4: Dexamethasone penetration in CSDH fluid

4.1 Introduction

Dexamethasone is a synthetic version of naturally-occurring corticosteroid hormone (Figure 4.1). This hormone is made by the adrenal glands in response to adrenocorticotropic hormone (ACTH) from the adenohypophysis, and has a primary role in regulating the metabolism of carbohydrates, proteins and fats (Woodbury, 1958). Chemical manipulation of this hormone in 1958 led to the first production of dexamethasone, known chemically as 9α-fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione (Arth et al., 1958; Buavari. S., 1996; Hart, 1960).

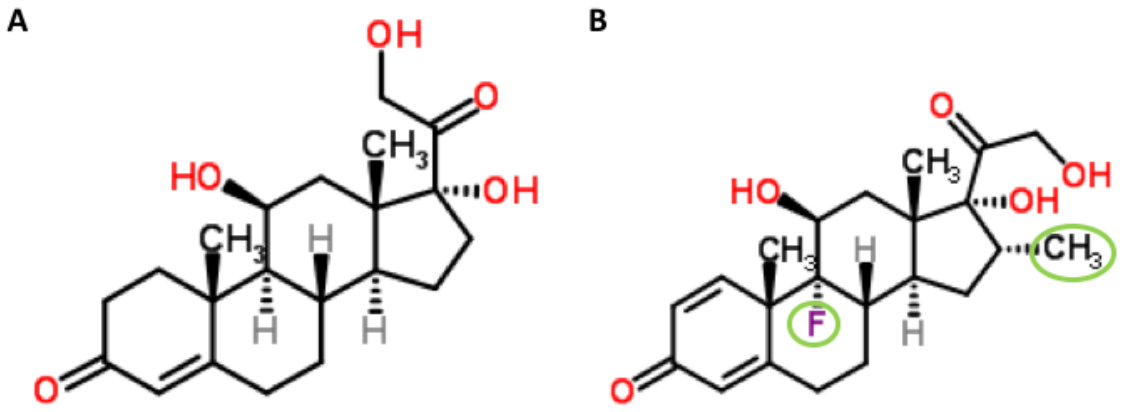

Figure 4.1; (A) naturally occurring hormone cortisol, **(B)** synthetic drug dexamethasone with additional fluoride (F) and methyl group (CH_3) indicated with green circles**.**

Dexamethasone has a potent anti-inflammatory effect without the salt and water retaining properties seen with other corticosteroid hormones (Arth et al., 1958). It therefore became a popular therapy for conditions such as rheumatoid arthritis, asthma, pemphigus and ulcerative colitis (Boland, 1958; Hart, 1960). In 1961 Galicich and French published the first work on the use of dexamethasone in humans to treat cerebral oedema (Maxwell RE, 1972). They reported dramatic relief of the signs and symptoms of raised ICP secondary to brain-tumour associated cerebral oedema. This was supported by histological analysis, which showed that dexamethasone was able to effectively reverse all the changes observed in oedematous tissue, short of necrosis (French, 1966). Following this, dexamethasone became widely used for a variety of neurosurgical conditions, including tumours, head injury, abscesses and

intracranial haemorrhage (French, 1966; Maxwell RE, 1972). The first report of its application in CSDH came in 1974, when it was proposed as an effective treatment by reducing cerebral oedema related to the haematoma (Bender, 1974). More recent theories suggest that it is the anti-inflammatory properties of dexamethasone that may be of benefit in promoting resolution of CSDH, although this is yet to be proven.

4.1.1 Understanding the anti-inflammatory role of dexamethasone role in CNS

Glucocorticoids, such as dexamethasone, have been shown to have extensive anti-inflammatory actions through up-regulating the gene transcription of anti-inflammatory cytokines such as IL-10, and down-regulating expression of inflammatory cytokines, chemokines, adhesion molecules and nitric oxide (Barnes, 1998; Cosio, Torrego, & Adcock, 2005; Czock, Keller, Rasche, & Haussler, 2005).

In relation to the central nervous system (CNS) specifically, the anti-inflammatory effects of dexamethasone have been interrogated in only a few cases, and mostly in animal models. A good example is Lyme disease of the CNS, where an inflammatory leptomeningitis occurs and treatment with oral dexamethasone results in significantly lower CSF white cell counts, IL-6, IL-8, MCP-1 and B-lymphocyte chemoattractant concentrations in macaques (Ramesh et al., 2015). Further to this, when post-mortem macaque frontal cortex tissue was exposed to live Lyme disease infection, pre-incubation with dexamethasone led to significantly lower levels of IL-6, IL-8, MCP-1, IL-1β, IL-18, TNF-α, VEGF and granulocyte-colony stimulating factor (Ramesh, Martinez, Martin, & Philipp, 2017). This is important as it shows the potential mechanism for dexamethasone to down-regulate molecules such as VEGF and ILs, which are suspected to be highly relevant in CSDH pathophysiology (Hara, Tamaki, Aoyagi, & Ohno, 2009; Hohenstein, Erber, Schilling, & Weigel, 2005; Hong et al., 2009; Hua et al., 2016; Kalamatianos et al., 2013; Nanko et al., 2009; Shono et al., 2001; Weigel, Schilling, & Schmiedek, 2001).

Astrocyte cell cultures from rat brains incubated with mycoplasma, to induce an exaggerated inflammatory response, showed that pre-incubation with dexamethasone almost completely suppressed any TNF-α production and markedly reduced that of prostaglandin E (Brenner,

Yamin, Abramsky, & Gallily, 1993). The latter is also implicated in CSDH pathophysiology and so another potential target for dexamethasone in this context (Hara et al., 2009).

The beneficial effects of dexamethasone in reducing morbidity and mortality in meningitis are well recognised, but despite this there is still significant debate regarding the mechanism by which this occurs (Fitch & van de Beek, 2008). Human cases of tuberculous meningitis treated with intra-venous dexamethasone have shown reduced early CSF concentrations of MMP-9, which was suggested to be a product of altered neutrophil function (Green et al., 2009). This mechanism may also apply to CSDH, where MMP-9 has been highlight as a potential marker of interest in aiding infiltration of inflammatory cells and increasing vascular permeability (Hua et al., 2016; Manicone & McGuire, 2008; Nakagawa, Kodera, & Kubota, 2000).

Finally, the role of dexamethasone in reducing cerebral oedema related to brain tumours may also involve inflammatory molecules, such as the down-regulation of VEGF and up-regulation of Angiopoetin-1 transcription resulting in decreased BBB permeability (Kim et al., 2008; Lewis, Harford-Wright, Vink, & Ghabriel, 2012). Increases in anti-inflammatory molecules IL-1ra and tissue inhibitor of metalloproteinases (TIMP-1) have also been shown in the cerebral extra-cellular fluid of brain tumour patients following dexamethasone treatment (Marcus, Carpenter, Price, & Hutchinson, 2010).

4.1.2 Dexamethasone pharmacokinetics and dynamics

Given its widespread use in neurological and neurosurgical conditions, there is surprisingly limited data available on the pharmacokinetics and dynamics of dexamethasone in relation to the CNS. It is also unknown whether dexamethasone penetrates the subdural space.

The oral bioavailability of dexamethasone is very high, around 78% (Duggan, Yeh, Matalia, Ditzler, & McMahon, 1975). It also reaches its peak plasma concentration very quickly within 1-1.5 hrs, and has a short terminal half-life of less than 4 hrs (Czock et al., 2005; Meikle & Tyler, 1977; Queckenberg et al., 2011). Although the biological half-life, and hence time period it is clinically effective, is much longer, at around 36-54 hrs (electronic Medicines Compendium). In 1987, Balis reported a study comparing intravenous (IV) and intra-thecal (IT) dexamethasone and prednisolone administration in monkeys (Balis, Lester,

Chrousos, Heideman, & Poplack, 1987). The high protein-binding of both steroids (around 70%), is the main limitation to its movement, but Balis showed that unbound or "free" steroid diffused rapidly into the intrathecal (IT) space following intra-venous (IV) administration. The peak IT concentrations were the same for both steroids, but dexamethasone had a longer half-life in the CSF, thus explaining why it is the preferred steroid for use in neurological conditions. There are no equivalent studies in humans, however the high level of protein binding of dexamethasone (75-77%) is well accepted and therefore a potential barrier to its ability to penetrate other spaces (Cummings, Larijani, Conner, Ferguson, & Rocci, 1990; electronic Medicines Compendium).

4.1.3 Hypothesis and aims

Part of understanding why dexamethasone may work as a treatment in CSDH is to understand the site and mechanism of action. Later chapters (five and six) will investigate the molecules involved in inflammation in CSDH and how these are altered by dexamethasone. The aim of this chapter is to assess whether dexamethasone penetrates the subdural space, working directly on the locally inflamed fluid, or rather whether it remains only in the peripheral blood and has downstream effects on inflammation.

There are two potential routes for dexamethasone to pass from the peripheral circulation into the CSDH cavity. The first route is via the internal surface of the CSDH, which is separated from CSF by the inner CSDH membrane and the arachnoid mater. The inner membrane rarely contains any blood vessels, therefore for dexamethasone to enter the subdural space by this route, it would need to pass from the circulation into the CSF and then through the basement membrane of the arachnoid mater and inner CSDH membrane (Haines, 1991; Moskala et al., 2007). This is a rather circuitous route, therefore the alternative, of entering via the outer CSDH membrane, appears more likely. The outer membrane is recognised to be well-vascularised by macrocapillaries (Yamashima, Yamamoto, & Friede, 1983), and therefore has its own peripheral blood supply through which dexamethasone could enter directly. As the blood vessels within the outer membrane are also known to be highly permeable, it is hypothesised that dexamethasone will be able to pass freely into the subdural cavity. Once inside the cavity it will be incorporated into the expanded mass of haematoma, fluid and inflammatory cells; where it can have a direct anti-inflammatory effect. Once it has

entered the subdural space, dexamethasone will not be eliminated as it would from the peripheral circulation, and therefore may accumulate within this space, enabling a prolonged anti-inflammatory action. This is necessary for the many weeks it would take for a CSDH to be absorbed.

The hypothesis that dexamethasone can enter and accumulate within the subdural space will be tested by analysing plasma and subdural fluid in patients treated with either dexamethasone or placebo as part of the Dex-CSDH trial. This chapter describes the development and validation of an appropriate technique for doing this in addition to the findings of this analysis.

4.2 Materials and methods

Previous reports analysing dexamethasone presence in human plasma, urine and saliva have used the technique High Performance Liquid Chromatography (HPLC) (Difrancesco et al., 2007; Djedovic & Rainbow, 2011; Frerichs & Tornatore, 2004; Kovarik et al., 1998; Kumar, Mostafa, Kayo, Goldberg, & Derendorf, 2006; Lamiable et al., 1986; Lariya, 2015; McWhinney, Ward, & Hickman, 1996; Song, Kim, & Kim, 2004; Tsuei, Moore, Ashley, & McBride, 1979). HPLC is a technique which enables separation and quantifications of components from a liquid sample. The HPLC instrument continuously pumps a solvent (called the mobile phase) through a column (stationary phase) at relatively high speed (hence high performance). A tiny volume of the sample being investigated is added to the mobile phase and pumped through the column, where its component parts interact differently with the porous media inside the column so that some flow through more quickly than others and they become separated from one another (Fornstedt, 2015).

In early HPLC methods the stationary phase would be a hydrophilic surface, used with a hydrophobic mobile phase (Kazakevich, 2006). However more recently reverse-phase (RP)-HPLC is more common, where the stationary phase is hydrophobic (usually porous silica with the addition of hydrocarbons) and the mobile phase is hydrophilic (Kazakevich, 2006). This RP-HPLC relies on dispersive forces to partition the sample between the mobile phase and the stationary phase (or column) and is able to discriminate very closely between related compounds. As constituents are separated and eluted from the column they need to be detected and quantified, this is usually done by an ultra-violet-visible (UV-Vis) absorption detector (Dong, 2006). The UV-Vis detector passes light through the fluid path and detects how much is absorbed by each compound eluted. This is transmitted as an output signal proportional to absorbance which is represented as a chromatographic “peak” for that molecule (Dong, 2006). How long it takes these peaks to elute from the column after injection is called the *retention time;* this time should be consistent for each molecular species (Riley, 1996). The area under the curve is proportional to the concentration of the compound in the sample (Riley, 1996). Therefore, once a calibration curve for a specific drug has been created the concentration of drug in any given sample can be determined. The UV-Visible wavelength at which the detector is measuring can also be adapted to best suit the absorption properties of the compounds chromophore, enabling optimal detection.

CSDH fluid contains similar components to blood but also includes blood breakdown products (as seen in chapter three) and other cellular debris due to its chronic nature. It also shares some similarities with CSF, which some consider to be the main fluid source for a CSDH (Kristof, Grimm, & Stoffel-Wagner, 2008). Whilst there are many studies using HPLC on blood, there is only one report of a HPLC method for dexamethasone in CSF, which was performed on rabbits (Lamiable, Vistelle, Nguyen-Khac, & Millart, 1988). Other substances within the brain, such as glycine, histamine and brain neurotransmitters, have also been studied with HPLC, but only using animal CSF or brain extracellular fluid (Bergh, Bogen, Lundanes, & Oiestad, 2016; Gonzalez, Fernandez, Vidal, Frenich, & Perez, 2011; Voehringer, Fuertig, & Ferger, 2013; Wang, Wu, Wu, & Bao, 2013). There is one study in the HPLC literature reporting a method for human CSDH fluid, but this was applied to identifying a type of bile acid (Nagata et al., 1992). Therefore, a novel method for dexamethasone analysis in human plasma and CSDH fluid using RP-HPLC with UV-Vis absorbance detection was designed for this study.

4.2.1 HPLC conditions

Chemicals and reagents

All chemicals used were of HPLC grade and purchase from Sigma Aldrich (Gillingham, UK) unless specified. Ultrapure water of HPLC-grade was used throughout (18.2 $M\Omega.cm^{-1}$, Millipore Direct Q5 UV water purification system with LC-Pak polisher).

Dexamethasone standard (Cerilliant reference standard; 1.0 mg/mL in methanol) and Flumethasone pivalate standard (European Pharmacopoeia (EP) Reference Standard, 100mg neat) were purchase from Sigma-Aldrich (Gillingham, UK). A 1:100 dilution of the dexamethasone standard in methanol was prepared to create a working solution of 100 μg/mL, and this was stored at -20 °C. The internal standard (Flumethasone), was diluted in methanol to a working solution of 150 μg/mL and stored at -20 °C. The mobile phase was made using Ammonium acetate buffer (5mM, pH 3.5). The buffer was prepared by dissolving ammonium acetate in ultrapure water to 5mM, and adjusting with acetic acid to pH 3.5. Mobile phase A was 70:30 ammonium acetate buffer to methanol and mobile phase B 30:70 ammonium acetate buffer to methanol. Mobiles phases were vacuum filtered through Whatman 0.45 μm membrane filters (Sigma-Aldrich; Gillingham, UK) before use.

Chromatographic conditions

An Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) comprising of a binary pump, refrigerated autosampler (set to 5 °C) and a UV-Visible (UV-Vis) variable wavelength detector was used with a ChemStation data system. A C-18 Phenomenex Luna ODS2 (100 x 2 mm, particle size 3 μm, pore size 100 A; Phenomenex, Torrance, CA, USA) column was used and fitted with a Phenomenex SecurityGuard ODS guard cartridge. The UV-Vis detector was set at 238 nm for optimal dexamethasone detection (Buavari. S., 1996). The mobile phase was continuously vacuum-degassed (with a Cambridge Scientific Instruments vacuum degasser) and the column temperature maintained at 40 °C (with a Jones Chromatography column heater/chiller) (Figure 4.2).

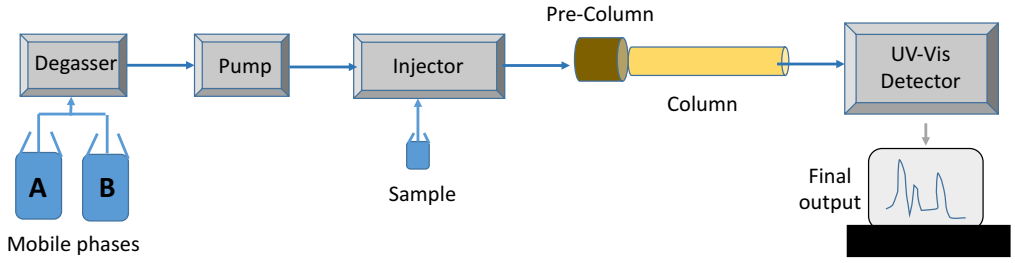

Figure 4.2; example set-up for HPLC

Prior to sample analysis, a wash-programme was run at the beginning of the day using 100% methanol for 30 min followed by 50:50 of 100% methanol to 50% methanol/50% water for 10 min, then back to 100% methanol for 10 min. Following this mobile phases A and B were run 50:50 for 30 min, then finally 100% mobile phase A for 10 min. This allowed the HPLC machine to be run through with both mobile phases and led to serial elution of any substances still residing on the column. Four blank samples were also run prior to sample analysis to ensure that no new contaminants were present.

A standard sample injection volume of 10 μL was used and the flow rate was maintained at 0.4 mL/min. A gradient method was used starting with 2 min of 100% mobile phase A, transitioning to 100% mobile phase B over 15 min and remaining stable for 7 min. Finally, the gradient returned to 100% mobile phase A over 1 min which continued for 5 min to completion of cycle. Therefore, each sample processing took a total of 29 min.

Extraction procedure;

150 µL of each sample was pipetted into a 1.5 mL Eppendorf tube and spiked with 10 µL of I.S (150 μg/mL in methanol). In the case of assay validation, the appropriate volume of dexamethasone working solution was also added to the sample. The extraction was then undertaken by adding 0.5 mL of ethyl acetate and vortex-mixing for 30 s followed by centrifugation at 10,000 rpm for 10 min. The supernatant was then transferred to a glass vial for drying on a heated block at 40 °C under a gentle stream of nitrogen gas for 10 min. The dry samples were reconstituted in 150 μL of mobile phase A, vortex-mixed and centrifuged for 5 min at 1000 rpm before transferring to an autosampler vial with inset for HPLC analysis.

4.2.2 HPLC method development

Table 4.1 summarises previous HPLC methods used to aid the method development. As several papers reported success with isocratic methods, this method was tested first (McWhinney et al., 1996). However, despite using four different mobile phase constituents, no adequate elution of the dexamethasone was achieved with an isocratic method and therefore a gradient method was used instead.

Optimisation of sample extraction was also reviewed and a solid phase-based extraction process, referenced in the literature, was trialed on our blank plasma samples spiked with dexamethasone (Difrancesco et al., 2007). However, we found this method to be inferior to the liquid-liquid extraction method and therefore continued to use this for the validation and test samples.

Table 4.1: previous dexamethasone HPLC methods used to guide method development

Author, year	**HPLC condition** (flow rate)	**Mobile Phase**	**Stationary phase and *internal standard***	**Detection, LOD/LOQ** (ng/mL)	**Test matrix**
Razzaq 2017	**Isocratic** (1.5 ml/min)	methanol & 20 mM phosphate buffer pH 2.8 (61.5:38.5)	C8 *None reported*	UV 254nm LOD = 61 LOQ = 192	Eye drops
Lariya 2014	**Isocratic** (1.5 ml/min)	70% MeOH & 30% orthophosphoric acid (1.67%)	C18 *Ibuprofen*	UV 254nm LOD = 0.3 LOQ = 0.9	MP only
Djedovic 2011	**Gradient**: 1.5min 30% B, over 3 mins to 90% B, 0.3mins to 30% B (0.5 mL/min)	MPA (water) and B (MeOH), both with 2 mmol/L ammonium acetate and 0.1% formic acid (v:v).	C8 *9,11,12,12-d4-cortisol*	TMS Not reported for Dex.	Human plasma
Kumar 2006	Isocratic (1.2 mL/min)	acetonitrile: triple distilled water (28 : 72), pH adjusted to 2.3 with phosphoric acid	C18 *Triamcinolone acetonide*	UV 254nm LOQ = 250	Human plasma
DiFrancesco 2007	**Gradient**: 70:30 MPA:B to 10:90 MPA:B over 4 min. (0.4 mL/min)	5mM ammonium acetate buffer (pH 3.5) : MeOH MPA = 95:5 MPB = 5:95	C18 *Flumethasone*	TMS LOQ = 4.8	Human plasma
Frerichs 2004	**Gradient**: 70% to 10% buffer over 4.1mins (0.4 mL/min)	MeOH and 5mM acetate buffer, pH 3.25	C18 *Flumethasone*	TMS LOD = 0.225 LOQ = 10.7	Human plasma
Song 2004	**Isocratic** (1 ml/min)	acetonitrile & 10mM sodium phosphate dibasic buffer (32:72, pH 7.0)	C18 *Desoxymethasone*	UV 240nm LOQ = 10	Human plasma
Kovarik 1998	**Isocratic** (1 ml/min)	acetonitrile-water-triethylamine (25-75-0.02)	C18 *Methylprednisolone*	UV 254nm LOQ = 5	Human plasma
McWhinney 1996	**Isocratic** (1 ml/min)	MeOH, tetrahydrofuran + water (3:25:72)	C18 *Fludrocortisone*	UV 254nm No LOQ/D	Serum and urine
Lamiable 1986	**Isocratic** (1 ml/min)	Acetate buffer-acetonitrile (58:42)	C18 *Equilenine*	UV 246 LOD = 0.5	Human plasma
Tsuei 1979	**Isocratic** (1.08 ml/min)	dichloromethane with 5% 1-butanol and 0.3% water	Silica (Si-5) *Prednisolone*	UV 240nm No LOQ/D	Human plasma

(MeOH = methanol, MPA = mobile phase A, MPB = mobile phase B, LOD = limit of detection, LOQ = limit of quantification)

4.2.3 HPLC method validation

Specificity

Human plasma samples were spiked first with the internal standard only, which displayed a retention time (RT) of 13.64 min (Figure 4.3A). Then the plasma samples were spiked with a standard stock solution of dexamethasone (concentrations ranging from 0.375 – 6 μg/mL), and a mean RT of 14.3 min was determined (Figure 4.3B, Table 4.3). There was no interference at the dexamethasone RT in the plasma spiked with only the internal standard.

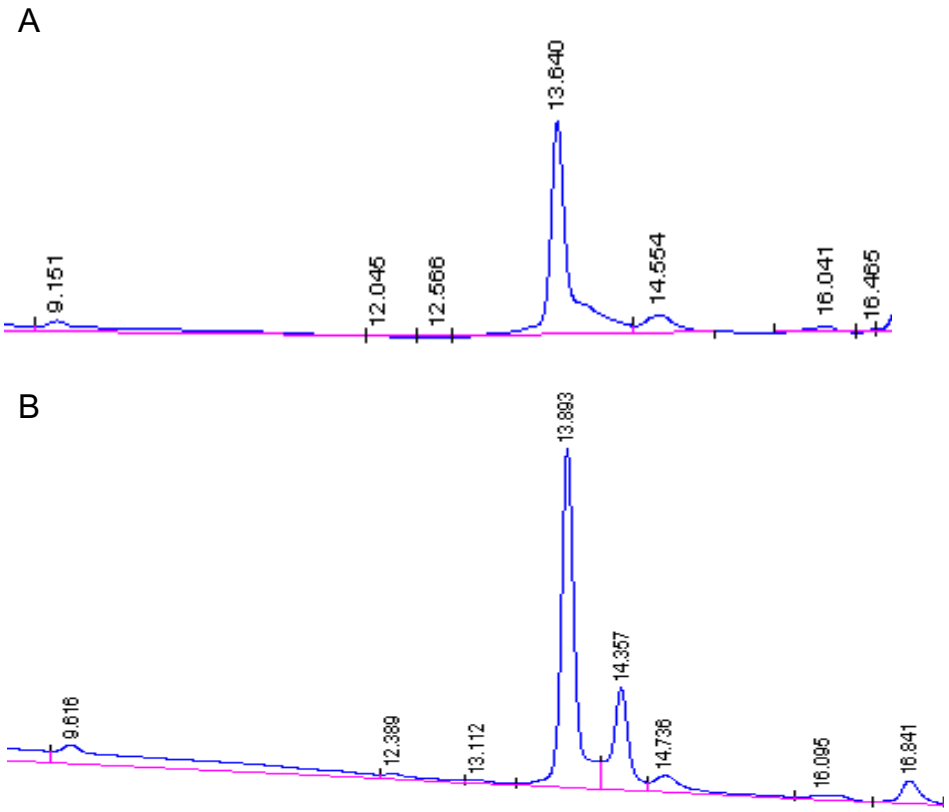

Figure 4.3; chromatogram of a dexamethasone quality control sample: **(A)** blank plasma spiked with internal standard only (RT 13.6), **(B)** blank plasma spiked with internal standard (RT 13.89) and 6 μg/mL dexamethasone (RT 14.36). *Axes are not visible, as images cut and enlarged to focus on relevant peaks*; *X-axis = time (min) with retention time (RT) written above each peak, y-axis = scale in arbitrary units (AU) measuring peak height.*

Linearity

Linearity was tested using multiple dilutions of standard stock dexamethasone. A target concentration 3 μg/mL dexamethasone was used and a range of 12.5% - 200% tested (Table 4.2). Repeats were performed over 3 different days.

Table 4.2; linearity test of dexamethasone concentrations tested.

Concentration (μg/mL)	**Concentration as % of 3 μg/mL**	**Mean Dex peak area**	**CV for peak area (%)**
0.375 (n=3)	12.5%	62.273	10.37
0.75 (n=3)	25%	83.67	6.78
1.5 (n=3)	50%	120.11	5.80
3 (n=3)	100%	182.80	8.47
6 (n=3)	200%	294.59	3.21
		Mean CV:	**6.926**

(CV = coefficient of variation, dex=dexamethasone).

The regression coefficient (r^2) was 0.997, the y-intercept 53.83 and the slope was 40.8 (Figure 4.4).

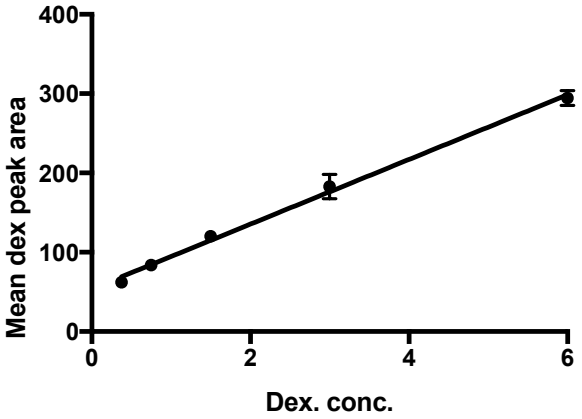

Figure 4.4; linear regression with the ratio of dexamethasone mean peak area to dexamethasone concentration. Correlation co-efficient: r^2 = 0.997; equation for regression line: **y = 40.8x + 53.83.** Error bars represent the standard deviation.

Precision

Precision, described as the coefficient of variation (CV*)* was assessed by repeatability (inta-assay precision) and intermediate precision by analysing three repeats of three different

dexamethasone concentrations in human plasma. The CV was within the acceptable limit of <15% for all concentrations (Table 4.3).

Table 4.3; repeatability tests

Concentration	**Mean Peak height (CV%)**	**Mean Peak Area (CV%)**	**Mean RT (CV%)**
0.375 (n=3)	5.30 (8.01)	10.37	14.27 (1.09)
0.75 (n=3)	6.92 (6.76)	6.78	14.28 (1.03)
1.5 (n=3)	10.55 (5.17)	5.80	14.28 (1.02)
3 (n=3)	17.67 (3.63)	8.47	14.28 (1.03)
6 (n=3)	30.49 (0.45)	3.21	14.28 (0.99)
Mean CV	**4.80**	**6.926**	**1.03**

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was calculated as 3.3 times the standard deviation (S.D.) of the y-intercept of regression line / slope of calibration line, and the LOQ as 10 times the S.D, as shown below (Shrivastava & Gupta, 2011).

$$\text{LOD} = 3.3 \times 4.049 / 40.61 = \mathbf{0.329}$$
$$\text{LOQ} = 10 \times 4.049 / 40.61 = \mathbf{0.997}$$

Absolute recovery

The absolute recovery of dexamethasone was determined by comparing the dexamethasone peak area in a non-extracted saline sample (blank), which is assumed to represent 100% recovery, with an extracted plasma sample (matrix), both spiked with 6 μg/mL dexamethasone. The dexamethasone peak area was 300.21 in the blank and 294.59 in the matrix (see Table 4.2), therefore suggesting an absolute recovery of 98% in our matrix samples.

4.2.4 Patient sampling procedure

Paired blood and CSDH samples were collected from 34 patients and analysed with HPLC. The mean age of patients sampled was 74.5 years (range 34-93 years) and there were 11

females and 23 males. This reflects the normal age and gender distribution of patients affected by CSDH.

Blood and CSDH samples were collected from patients in 2.2 mL EDTA tubes and centrifuged for 10 min at 2,500 rpm at 4 °C. The blood plasma and CSDH supernatant were transferred to 2 ml Eppendorf tubes and pipetted into 0.4 mL aliquots for freezing at - 75 °C until ready for batch extraction and immediate HPLC analysis.

All samples analysed only underwent a maximum of one freeze/thaw cycle. There is evidence in the literature that several freeze-thaw cycles can occur without degradation of dexamethasone (Ray, Kushnir, Rockwood, & Meikle, 2011).

4.3 Results

There were 28 placebo patients, with no dexamethasone exposure within at least 1 month of sampling, which were used as controls. Oral dexamethasone was given to six patients within 24 hrs of sampling. The mean time from the last dexamethasone administration to sampling was 6.2 hrs (range 2.7 - 15.5 hrs), which is outside the normal half-life of dexamethasone. Only the patient with the shortest time-window from last dexamethasone dosing to sampling (2.7 hrs) had a very small dexamethasone peak identified in plasma (Figure 4.5). This was below the LOQ but still detectable and therefore corresponds to a concentration of dexamethasone in the range 0.3 - 1 μg/mL. There was no peak in the intra-operative CSDH fluid in this patient.

No dexamethasone peaks were identified in plasma, intra-operative or post-operative CSDH drain fluid for any other treatment or control patients. Internal standard peaks were seen in all samples as expected.

Table 4.4; dexamethasone dosing and sampling time in all patients

	No. of patients (M:F)	**Mean total dex dose pre-op*** (range)	**Mean time from dex to sampling** (range)	**Dex HPLC peaks**
Control group	28 (7:21)	N/A	N/A	None
Dex Treatment group	6 (4:2)	40 mg (8 - 72 mg)	6.2 hrs (2.7- 15.5 hrs)	1 in plasma

**= cumulative dex dose given over several days, with a maximum of 16 mg per day, (F = female, HPLC = high performance liquid chromatography, M = male)*

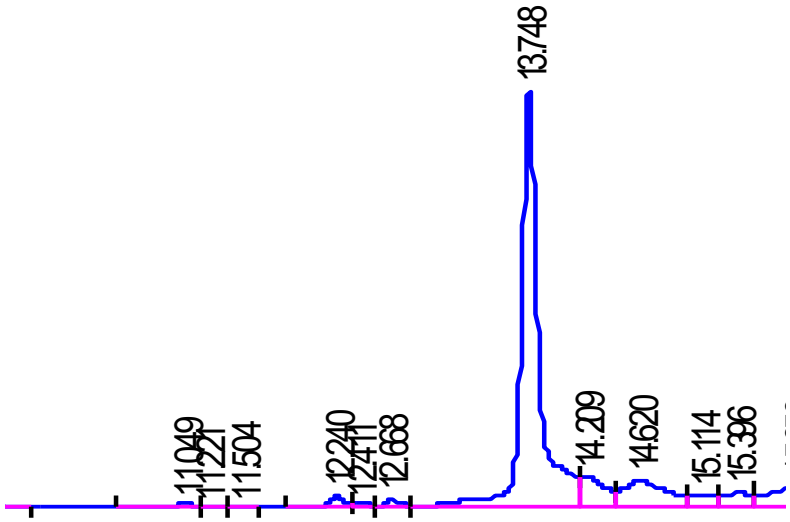

Figure 4.5; HPLC trace of plasma in patient sampled 2.7 hrs after dexamethasone dosing. Internal standard retention time (RT) = 13.748, dexamethasone RT = 14.209. *X-axis = time (min) with retention time (RT) written above each peak, y-axis = scale in arbitrary units (AU) measuring peak height*.

4.4 Conclusions

This HPLC method was designed to determine the presence of dexamethasone in CSDH fluid samples compared with serum. The method proved successful at identifying dexamethasone in both spiked plasma and CSDH fluid samples. Due to the nature of the trial, despite obtaining samples for 34 patients, only 6 patients had received the active treatment, dexamethasone, prior to sampling. One small dexamethasone peak was identified in the plasma of one patient, and none in the CSDH samples.

Dexamethasone is known to have a short terminal half-life of around 2.5-3.5 hrs when assessed in both controls and neurosurgical patients (McCafferty et al., 1981; Tsuei et al., 1979). The limit of detection and quantification is also higher with HPLC methods compared to tandem mass spectrometry (Difrancesco et al., 2007). These factors limit the ability to detect dexamethasone in clinical samples. The short terminal half-life is a particular problem in this patient cohort where the majority of CSDH operations take place in the evening, at the end of operative lists, whereas dexamethasone administration is normally with breakfast and lunch (to avoid the side-effect of insomnia). This led to a mean time from medication dosing to sampling of 7.2 hrs, with only two samples taken within 3.5 hrs and dexamethasone only detectable in that taken in the shortest time of 2.7 hrs. As the biological half-life of dexamethasone has been reported as being around 36-54 hrs, it is much easier to measure the clinical effects than the absolute values in any given biological sample (electronic Medicines Compendium).

The patient with detectable dexamethasone in the plasma had no detectable dexamethasone in the CSDH fluid. This suggests that either does not immediately cross-over into the CSDH fluid or if it does so then it is at reduced concentration compared to plasma. Patients received regular dexamethasone dosing, with a maximum cumulative dose of 72 mg given pre-operatively. Despite this, no dexamethasone was detected in any of the CSDH fluid samples, where it might be expected to accumulate should it to pass freely into the subdural space and remain there. However, as dexamethasone is lipophilic and has high levels of protein binding (over 70%) the volume of distribution is very large and therefore there is a very limited amount of free dexamethasone able to pass into the subdural space (Cummings et al., 1990; Meikle & Tyler, 1977). Furthermore, it is well established that dexamethasone diffuses easily

into cells where it binds to its cytoplasmic receptor to induce alterations in transcription of inflammatory molecules and related proteins (Barnes, 1998; Dietrich, Rao, Pastorino, & Kesari, 2011; Nicolaides, Galata, Kino, Chrousos, & Charmandari, 2010). Thus, it is likely that rather than infiltrating the CSDH fluid, its effector cells are located outside the fluid, for example within the subdural membranes.

Experimental studies have shown glucocorticoids can induce morphological changes in endothelial cells and expression of tight junctions, such that they reduce the permeability of a membrane (Forster et al., 2005). This relates to its clinical use in brain tumour patients, where it has been shown to reduce the permeability of the endothelium in blood vessels supplying the tumour (Heiss et al., 1996; Ostergaard et al., 1999). Therefore, it is possible that dexamethasone does not need to enter the subdural space per se as its primary action may be on the endothelium of the CSDH membrane blood vessels themselves. As these vessels are the source of bleeding resulting in CSDH expansion, modification of the vessel wall to prevent further fluid, blood and inflammatory cells infiltration into the subdural space could be important in treating CSDH. The on-going process of forming new blood vessels within the membrane, angiogenesis, may also be targeted by dexamethasone, as other glucocorticoids have been shown to inhibit tumour angiogenesis in animal models (Tamargo, Leong, & Brem, 1990). This theory could be further investigated with histological analysis of CSDH membranes in patients treated with dexamethasone.

In conclusion, a suitable sampling, extraction and RP-HPLC method has been determined for dexamethasone in CSDH samples. However, the hypothesis that dexamethasone would accumulate within the subdural space over serial dosing has not been shown, with the caveat that the very short half-life is a severe limitation in clinical studies such as this. Surrogate markers of the biological action of dexamethasone, as will be explored in chapters five and six, may be more suitable targets for analysis. Furthermore, analysis of CSDH membranes could also provide further information on the potential targets and location for dexamethasone action.

4.5 References

Arth, G. E., Johnston, D. B. R., Fried, J., Spooncer, W. W., Hoff, D. R., & Sarett, L. H. (1958). 16-Methylated steriods. I. 16 α -methylated analogs of cortisone, a new group of anti-inflammatory steroids. *Journal of the American Chemical Society, 80*(12), 3160-3161. doi:10.1021/ja01545a061

Balis, F. M., Lester, C. M., Chrousos, G. P., Heideman, R. L., & Poplack, D. G. (1987). Differences in cerebrospinal fluid penetration of corticosteroids: possible relationship to the prevention of meningeal leukemia. *J Clin Oncol, 5*(2), 202-207. doi:10.1200/jco.1987.5.2.202

Barnes, P. J. (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond), 94*(6), 557-572.

Bender. (1974). Nonsurgical Treatment of Subdural Hematomas. *Arch Neurol, 31*.

Bergh, M. S., Bogen, I. L., Lundanes, E., & Oiestad, A. M. L. (2016). Validated methods for determination of neurotransmitters and metabolites in rodent brain tissue and extracellular fluid by reversed phase UHPLC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci, 1028*, 120-129. doi:10.1016/j.jchromb.2016.06.011

Boland, E. W. (1958). Clinical observations with 16 alpha-methyl corticosteroid compounds; preliminary therapeutic trials with dexamethasone (16 alpha-methyl 9 alpha-fluoroprednisolone) in patients with rheumatoid arthritis. *Ann Rheum Dis, 17*(4), 376-382.

Brenner, T., Yamin, A., Abramsky, O., & Gallily, R. (1993). Stimulation of tumor necrosis factor- α production by mycoplasmas and inhibition by dexamethasone in cultured astrocytes. *Brain Research, 608*(2), 273-279. doi:https://doi.org/10.1016/0006-8993(93)91468-8

Buavari. S., O. N., M., Smith, A., Hecklman, P., Obenchain, J. . (1996). *The Merck Index* (12th ed.). New York: Meck & Co Inc.

Cosio, B. G., Torrego, A., & Adcock, I. M. (2005). [Molecular mechanisms of glucocorticoids]. *Arch Bronconeumol, 41*(1), 34-41.

Cummings, D. M., Larijani, G. E., Conner, D. P., Ferguson, R. K., & Rocci, M. L., Jr. (1990). Characterization of dexamethasone binding in normal and uremic human serum. *Dicp, 24*(3), 229-231.

Czock, D., Keller, F., Rasche, F. M., & Haussler, U. (2005). Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet, 44*(1), 61-98. doi:10.2165/00003088-200544010-00003

Dietrich, J., Rao, K., Pastorino, S., & Kesari, S. (2011). Corticosteroids in brain cancer patients: benefits and pitfalls. *Expert Rev Clin Pharmacol, 4*(2), 233-242. doi:10.1586/ecp.11.1

Difrancesco, R., Frerichs, V., Donnelly, J., Hagler, C., Hochreiter, J., & Tornatore, K. M. (2007). Simultaneous determination of cortisol, dexamethasone, methylprednisolone, prednisone, prednisolone, mycophenolic acid and mycophenolic acid glucuronide in human plasma utilizing liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci, 859*(1), 42-51. doi:10.1016/j.jchromb.2007.09.003

Djedovic, N. K., & Rainbow, S. J. (2011). Detection of synthetic glucocorticoids by liquid chromatography-tandem mass spectrometry in patients being investigated for Cushing's syndrome. *Ann Clin Biochem, 48*(Pt 6), 542-549. doi:10.1258/acb.2011.010250

Dong, M. W. (2006). *Modern HPLC for practicing scientists*. New Jersey: John Wiley & Sons Inc. .

Duggan, D. E., Yeh, K. C., Matalia, N., Ditzler, C. A., & McMahon, F. G. (1975). Bioavailability of oral dexamethasone. *Clin Pharmacol Ther, 18*(2), 205-209.

electronic Medicines Compendium. Summary of product characteristics of dexamethasone. Retrieved from https://www.medicines.org.uk/emc/product/5411/smpc

Fitch, M. T., & van de Beek, D. (2008). Drug Insight: steroids in CNS infectious diseases—new indications for an old therapy. *Nature Clinical Practice Neurology, 4*, 97. doi:10.1038/ncpneuro0713

Fornstedt, T., Forssen, P. and Westerlaund, D.,. (2015). Basic HPLC Theory and Definitions: Retention, Thermodynamics, Selectivity, Zone Spreading, Kinetics, and Resolution *Analytical Separation Science*.

Forster, C., Silwedel, C., Golenhofen, N., Burek, M., Kietz, S., Mankertz, J., & Drenckhahn, D. (2005). Occludin as direct target for glucocorticoid-induced improvement of blood-brain barrier properties in a murine in vitro system. *J Physiol, 565*(Pt 2), 475-486. doi:10.1113/jphysiol.2005.084038

French, L. A. (1966). The use of steroids in the treatment of cerebral edema. *Bull N Y Acad Med, 42*(4), 301-311.

Frerichs, V. A., & Tornatore, K. M. (2004). Determination of the glucocorticoids prednisone, prednisolone, dexamethasone, and cortisol in human serum using liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci, 802*(2), 329-338. doi:10.1016/j.jchromb.2003.12.015

Gonzalez, R. R., Fernandez, R. F., Vidal, J. L., Frenich, A. G., & Perez, M. L. (2011). Development and validation of an ultra-high performance liquid chromatography-tandem mass-spectrometry (UHPLC-MS/MS) method for the simultaneous determination of neurotransmitters in rat brain samples. *J Neurosci Methods, 198*(2), 187-194. doi:10.1016/j.jneumeth.2011.03.023

Green, J. A., Tran, C. T., Farrar, J. J., Nguyen, M. T., Nguyen, P. H., Dinh, S. X., . . . Thwaites, G. E. (2009). Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. *PLoS One, 4*(9), e7277. doi:10.1371/journal.pone.0007277

Haines. (1991). On the Question of a Subdural Space. *The Anatomical Record, 230*, 3-21.

Hara, M., Tamaki, M., Aoyagi, M., & Ohno, K. (2009). Possible role of cyclooxygenase-2 in developing chronic subdural hematoma. *J Med Dent Sci, 56*(3), 101-106.

Hart, F. D. (1960). Dexamethasone. *Postgrad Med J, 36*, 26-27.

Heiss, J. D., Papavassiliou, E., Merrill, M. J., Nieman, L., Knightly, J. J., Walbridge, S., . . . Oldfield, E. H. (1996). Mechanism of dexamethasone suppression of brain tumor-associated vascular permeability in rats. Involvement of the glucocorticoid receptor and vascular permeability factor. *J Clin Invest, 98*(6), 1400-1408. doi:10.1172/jci118927

Hohenstein, A., Erber, R., Schilling, L., & Weigel, R. (2005). Increased mRNA expression of VEGF within the hematoma and imbalance of angiopoietin-1 and -2 mRNA within the neomembranes of chronic subdural hematoma. *J Neurotrauma, 22*(5), 518-528. doi:10.1089/neu.2005.22.518

Hong, H. J., Kim, Y. J., Yi, H. J., Ko, Y., Oh, S. J., & Kim, J. M. (2009). Role of angiogenic growth factors and inflammatory cytokine on recurrence of chronic subdural hematoma. *Surg Neurol, 71*(2), 161-165; discussion 165-166. doi:10.1016/j.surneu.2008.01.023

Hua, C., Zhao, G., Feng, Y., Yuan, H., Song, H., & Bie, L. (2016). Role of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, and Vascular Endothelial Growth Factor in the Development of Chronic Subdural Hematoma. *J Neurotrauma, 33*(1), 65-70. doi:10.1089/neu.2014.3724

Kalamatianos, T., Stavrinou, L. C., Koutsarnakis, C., Psachoulia, C., Sakas, D. E., & Stranjalis, G. (2013). PlGF and sVEGFR-1 in chronic subdural hematoma: implications for hematoma development. *J Neurosurg, 118*(2), 353-357. doi:10.3171/2012.10.JNS12327

Kazakevich, Y., LoBrutto, R. (2006). *Part 1 HPLC theory and practice; Introduction* Y. Kazakevich, LoBrutto, R. (Ed.) *HPLC for Pharmaceutical Scientists*

Kim, H., Lee, J. M., Park, J. S., Jo, S. A., Kim, Y. O., Kim, C. W., & Jo, I. (2008). Dexamethasone coordinately regulates angiopoietin-1 and VEGF: a mechanism of glucocorticoid-induced stabilization of blood-brain barrier. *Biochem Biophys Res Commun, 372*(1), 243-248. doi:10.1016/j.bbrc.2008.05.025

Kovarik, J. M., Purba, H. S., Pongowski, M., Gerbeau, C., Humbert, H., & Mueller, E. A. (1998). Pharmacokinetics of dexamethasone and valspodar, a P-glycoprotein (mdr1) modulator: implications for coadministration. *Pharmacotherapy, 18*(6), 1230-1236.

Kristof, R. A., Grimm, J. M., & Stoffel-Wagner, B. (2008). Cerebrospinal fluid leakage into the subdural space: possible influence on the pathogenesis and recurrence frequency of chronic subdural hematoma and subdural hygroma. *J Neurosurg, 108*(2), 275-280. doi:10.3171/JNS/2008/108/2/0275

Kumar, V., Mostafa, S., Kayo, M. W., Goldberg, E. P., & Derendorf, H. (2006). HPLC determination of dexamethasone in human plasma and its application to an in vitro release study from endovascular stents. *Pharmazie, 61*(11), 908-911.

Lamiable, D., Vistelle, R., Millart, H., Sulmont, V., Fay, R., Caron, J., & Choisy, H. (1986). High-performance liquid chromatographic determination of dexamethasone in human plasma. *J Chromatogr, 378*(2), 486-491.

Lamiable, D., Vistelle, R., Nguyen-Khac, M., & Millart, H. (1988). High-performance liquid chromatographic determination of dexamethasone in cerebrospinal fluid and plasma in the rabbit. *J Chromatogr, 434*(1), 315-319.

Lariya, N. K., Agrawal, G.P.,. (2015). Development and validation of RP-HPLC method for simultaneous determination of methotrexate, dexamethasone and indomethacin. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*(3), 443-446.

Lewis, K. M., Harford-Wright, E., Vink, R., & Ghabriel, M. N. (2012). Targeting classical but not neurogenic inflammation reduces peritumoral oedema in secondary brain tumours. *J Neuroimmunol, 250*(1-2), 59-65. doi:10.1016/j.jneuroim.2012.06.001

Manicone, A., & McGuire, J. (2008). Matrix metalloproteinases as modulators of inflammation. *Semin Cell Dev Biol, 19*(1), 34-41. doi:10.1016/j.semcdb.2007.07.003

Marcus, H. J., Carpenter, K. L., Price, S. J., & Hutchinson, P. J. (2010). In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. *J Neurooncol, 97*(1), 11-23. doi:10.1007/s11060-009-9990-5

Maxwell RE, L. D., French LA. . (1972). *The clinical effects of synthetic glucocorticoid used for brain edema in the practice of neurosurgery* (S. K. Reulen HJ Ed.). Berlin: Springer-Verlag.

McCafferty, J., Brophy, T. R., Yelland, J. D., Cham, B. E., Bochner, F., & Eadie, M. J. (1981). Intraoperative pharmacokinetics of dexamethasone. *Br J Clin Pharmacol, 12*(3), 434-436.

McWhinney, B. C., Ward, G., & Hickman, P. E. (1996). Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine. *Clin Chem, 42*(6), 979-981.

Meikle, A. W., & Tyler, F. H. (1977). Potency and duration of action of glucocorticoids: Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *The American Journal of Medicine, 63*(2), 200-207. doi:10.1016/0002-9343(77)90233-9

Moskala, M., Goscinski, I., Kaluza, J., Polak, J., Krupa, M., Adamek, D., . . . Miodonski, A. J. (2007). Morphological aspects of the traumatic chronic subdural hematoma capsule: SEM studies. *Microsc Microanal, 13*(3), 211-219. doi:10.1017/S1431927607070286

Nagata, K., Takakura, K., Asano, T., Seyama, Y., Hirota, H., Shigematsu, N., . . . Shimizu, T. (1992). Identification of 7 alpha-hydroxy-3-oxo-4-cholestenoic acid in chronic subdural hematoma. *Biochim Biophys Acta, 1126*(2), 229-236.

Nakagawa, T., Kodera, T., & Kubota, T. (2000). Expression of matrix metalloproteinases in the chronic subdural haematoma membrane. *Acta Neurochir (Wien), 142*(1), 61-66.

Nanko, N., Tanikawa, M., Mase, M., Fujita, M., Tateyama, H., Miyati, T., & Yamada, K. (2009). Involvement of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in the mechanism of development of chronic subdural hematoma. *Neurol Med Chir (Tokyo), 49*(9), 379-385.

Nicolaides, N. C., Galata, Z., Kino, T., Chrousos, G. P., & Charmandari, E. (2010). The human glucocorticoid receptor: molecular basis of biologic function. *Steroids, 75*(1), 1-12. doi:10.1016/j.steroids.2009.09.002

Ostergaard, L., Hochberg, F. H., Rabinov, J. D., Sorensen, A. G., Lev, M., Kim, L., . . . Rosen, B. R. (1999). Early changes measured by magnetic resonance imaging in cerebral blood flow, blood volume, and blood-brain barrier permeability following dexamethasone treatment in patients with brain tumors. *J Neurosurg, 90*(2), 300-305. doi:10.3171/jns.1999.90.2.0300

Queckenberg, C., Wachall, B., Erlinghagen, V., Di Gion, P., Tomalik-Scharte, D., Tawab, M., . . . Fuhr, U. (2011). Pharmacokinetics, pharmacodynamics, and comparative bioavailability of single, oral 2-mg doses of dexamethasone liquid and tablet formulations: a randomized, controlled, crossover study in healthy adult volunteers. *Clin Ther, 33*(11), 1831-1841. doi:10.1016/j.clinthera.2011.10.006

Ramesh, G., Didier, P. J., England, J. D., Santana-Gould, L., Doyle-Meyers, L. A., Martin, D. S., . . . Philipp, M. T. (2015). Inflammation in the pathogenesis of lyme neuroborreliosis. *Am J Pathol, 185*(5), 1344-1360. doi:10.1016/j.ajpath.2015.01.024

Ramesh, G., Martinez, A. N., Martin, D. S., & Philipp, M. T. (2017). Effects of dexamethasone and meloxicam on Borrelia burgdorferi-induced inflammation in glial and neuronal cells of the central nervous system. *J Neuroinflammation, 14*(1), 28. doi:10.1186/s12974-017-0806-9

Ray, J. A., Kushnir, M. M., Rockwood, A. L., & Meikle, A. W. (2011). Analysis of cortisol, cortisone and dexamethasone in human serum using liquid chromatography tandem mass spectrometry and assessment of cortisol: cortisone ratios in patients with impaired kidney function. *Clin Chim Acta, 412*(13-14), 1221-1228. doi:10.1016/j.cca.2011.03.016

Riley, C. M. (1996). *Efficiency, retention, selectivity and resolution in chromatography*. London: Blackie Academic and Professional.

Shono, T., Inamura, T., Morioka, T., Matsumoto, K., Suzuki, S. O., Ikezaki, K., . . . Fukui, M. (2001). Vascular endothelial growth factor in chronic subdural haematomas. *J Clin Neurosci, 8*(5), 411-415. doi:10.1054/jocn.2000.0951

Shrivastava, A., & Gupta, V. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists, 2*(1), 21-25. doi:10.4103/2229-5186.79345

Song, Y. K. P., Jeong-Sook;, Kim, J. K., & Kim, C. K. (2004). HPLC Determination of Dexamethasone in Human Plasma *Journal of Liquid Chromatography & Related Technologies, 27*(14), 2293-2306. doi:10.1081/JLC-200025726

Tamargo, R. J., Leong, K. W., & Brem, H. (1990). Growth inhibition of the 9L glioma using polymers to release heparin and cortisone acetate. *J Neurooncol, 9*(2), 131-138.

Tsuei, S. E., Moore, R. G., Ashley, J. J., & McBride, W. G. (1979). Disposition of synethetic glucocorticoids. I. Pharmacokinetics of dexamethasone in healthy adults. *J Pharmacokinet Biopharm, 7*(3), 249-264.

Voehringer, P., Fuertig, R., & Ferger, B. (2013). A novel liquid chromatography/tandem mass spectrometry method for the quantification of glycine as biomarker in brain microdialysis and cerebrospinal fluid samples within 5min. *J Chromatogr B Analyt Technol Biomed Life Sci, 939*, 92-97. doi:10.1016/j.jchromb.2013.09.011

Wang, Z., Wu, J., Wu, S., & Bao, A. (2013). High-performance liquid chromatographic determination of histamine in biological samples: the cerebrospinal fluid challenge--a review. *Anal Chim Acta, 774*, 1-10. doi:10.1016/j.aca.2012.12.041

Weigel, R., Schilling, L., & Schmiedek, P. (2001). Specific pattern of growth factor distribution in chronic subdural hematoma (CSH): evidence for an angiogenic disease. *Acta Neurochir (Wien), 143*(8), 811-818; discussion 819.

Woodbury, D. M. (1958). Relation between the adrenal cortex and the central nervous system. *Pharmacol Rev, 10*(2), 275-357.

Yamashima, T., Yamamoto, S., & Friede, R. L. (1983). The role of endothelial gap junctions in the enlargement of chronic subdural hematomas. *J Neurosurg, 59*(2), 298-303. doi:10.3171/jns.1983.59.2.0298

Chapter 5: The inflammatory profile of CSDH fluid

5.1 Introduction

Previous studies have indicated a role of for inflammatory pathway modulation in CSDH pathophysiology, but the key factors involved in growth and recurrence of a CSDH remain unknown. The purpose of this chapter is to increase the understanding of the inflammatory composition of CSDH fluid both intra-operatively and post-operatively.

A Dex-CSDH neurochemistry sub-study was designed as part of the Dex-CSDH trial, with ethical approval and a separate consent form. All patients recruited into the sub-study underwent surgical drainage of their CSDH. Paired intra-operative blood and CSDH fluid samples were collected for the sub-study, but otherwise the surgery was performed in line with standard clinical practice. In the case of bilateral CSDH, separate samples were collected from each side, if possible. Patients who had a routine post-operative subdural drain left in-situ had CSDH drainage samples collected after surgery. These drains are normally removed after approximately 48 hrs, in some cases they were left up to 72 hrs. All patients included in this study were randomised to dexamethasone or placebo as part of the Dex-CSDH trial and the effect of dexamethasone and other clinical factors on the inflammatory markers are discussed in the chapter six.

Hypothesis and aims

Many of the inflammatory markers assessed in this study have been previously highlighted as relevant in CSDH, but the literature is limited and in some cases conflicting. This study aims to clarify the inflammatory marker patterns, highlighting those most relevant to CSDH pathophysiology, and include analysis of some novel markers. The first hypothesis is that VEGF will be the primary marker in driving the inflammatory response in CSDH, as shown in previous studies, and that this will correlate with raised levels of other inflammatory markers.

There is very limited data on whether concentrations of markers can be used to predict recurrence or poor outcome in relation to CSDH, and there are no published studies assessing what happens to the inflammatory marker profiles post-operatively. The second hypothesis is

that recurrent CSDH samples will contain significantly higher concentrations of VEGF, and possibly other inflammatory markers, due to escalating inflammation acting as a stimulus for the recurrence. Finally, it is hypothesised that post-operative CSDH samples will contain reduced levels of all inflammatory markers, as these have been "washed out" during the surgery. The exception to this may be primary CSDHs that develop subsequent recurrent CSDH, where higher residual levels of inflammatory markers are expected in the post-operative samples, and may aid prediction of recurrence.

5.2 Neurochemistry methods

52 patients were consented and recruited to the neurochemistry sub-study. The mean age was 76 years and 14 (27%) were women; these reflect the normal demographics of CSDH.

Sampling protocol

Blood and CSDH samples were collected intra-operatively. Blood was sampled by the anaesthetist either during cannulation, or from an arterial line once the patient was anaesthetised. CSDH fluid was collected by the operating surgeon once the subdural space was accessed, and prior to any irrigation being used. A 10 ml syringe was used to collect CSDH fluid, with care taken not to aspirate any blood from the surrounding surgical wound. All samples were then immediately dispensed into pre-labelled ethylenediamine tetra-acetic acid (EDTA) tubes (approximately 5 mL) to prevent blood clotting. Samples were either transferred immediately to the neurochemistry lab following collection, or stored at 4 °C in the theatre fridge prior to transfer.

Post-operative drain samples were collected daily from the subdural drainage bag, between 8 and 72 hrs post-operatively. Following each sampling, the drainage bag was completely emptied, so that subsequent sampling would include freshly drained fluid only. The time of sampling and total volume of fluid emptied were recorded on the biochemistry log. All blood and CSDH samples were transferred to the neurochemistry lab within 72 hrs of collection and registered with a unique lab identifier. Some samples were analysed by UV-Vis spectroscopy (see chapter three) prior to centrifugation (10,000 rpm, 10 min, at room temperature) and discarding of cells. The plasma or CSDH supernatant was divided into 0.4 mL aliquots in 1.5 mL Eppendorf tubes, and stored at -75 °C (+/- 10 °C) prior to analysis.

Sample analysis

After thawing, all samples were analysed using a magnetic bead-based immunoassay on a Luminex 200 analyser (*Luminex Corporation, Austin, TX, USA*). Analyses were performed using custom 12-plex ProcartaPlex human cytokine and chemokine magnetic bead-based assay kits (*Affymetrix eBioscience, Thermo Fisher Scientific; Paisley, UK)* for the selected panel of inflammatory markers (Table 5.1), following the manufacturers' instructions. This involved quantifying all 12 markers simultaneously, in the same assay well. Analyses were done in batches, once there were sufficient samples collected to fill a 96-well assay plate. All

standards and samples were analysed in duplicate. The micro-beads in each well were covered with the mix of all 12 marker antibodies and a detection antibody before adding the samples. This was then incubated overnight at 5 °C for optimised binding. Any unbound substance was removed by washing, and streptavidin-phycoerythrin conjugate was added to each well to bind to the detection antibody, before washing and re-suspending the microbeads in a buffer for analysis. The concentrations of each marker were obtained from the standard curve using Luminex eXPONENT software.

Table 5.1; panel of inflammatory molecules analysed.

Cytokines	Interleukins; IL-1α, IL-1β, IL- 6, IL- 8, IL-10 tumour necrosis factor; TNF-α
Chemokines	macrophage inflammatory proteins; MIP-1α, MIP-1β monocyte chemoattractant protein; MCP-1 interferon gamma-induced protein; IP-10
Other inflammatory molecules	vascular endothelial growth factor; VEGF matrix metalloprotease; MMP-9

Statistical analysis

Statistical analysis was performed using Graphpad Prism 7 (GraphPad Software, La Jolla, CA, USA). As the inflammatory profiles all followed a non-parametric pattern, the differences between paired samples (e.g. plasma and CSDH) were assessed using the Wilcoxon signed rank test. Unpaired samples (e.g. all primary CSDHs versus all recurrent CSDHs) were assessed using the Mann-Whitney Test. Spearman's rank correlation coefficient (Spearman's rho) was used to display the relationship between any two analytes. Any other statistical tests have been described throughout the results. All statistical analysis assumed a significance level of $p < 0.05$.

5.3 Intra-operative analyte concentrations in primary and recurrent CSDH

Paired samples were collected from 57 operations in 52 patients. A single primary CSDH operation was sampled in 47 patients, whilst five patients had recurrent operations sampled. Of the five recurrence patients, one patient had only the recurrent CSDH sampled (with no sample from the primary operation), three patients had the primary and recurrent CSDH sampled and one patient had a primary CSDH and two recurrences sampled.

Of the 57 operations, 68 paired plasma and CSDH samples were collected. A single CSDH sample was collected in 46 CSDHs, whilst 11 patients had dual-sampling from the same operation; 10 from bilateral CSDHs and one with both the anterior and posterior parts of a unilateral CSDH sampled. Of the 68 samples, 61 were primary CSDHs and seven from the five patients with recurrent CSDH (one bilateral and one patient with two recurrences). Figure 5.1 shows the overall number of samples comparing unilateral, bilateral and recurrences.

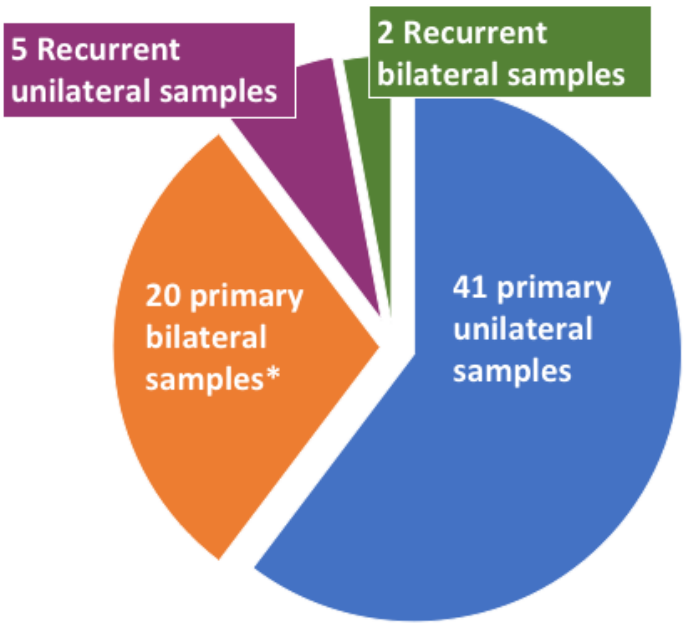

Figure 5.1; distribution of all 68 intra-operative paired CSDH samples in 52 patients. *one primary bilateral sample represents anterior and posterior parts of a unilateral CSDH.

The concentrations of inflammatory markers in all intra-operative plasma and CSDH samples are summarised in Figure 5.2. Vascular Endothelial Growth Factor (VEGF) was identified as the marker with the highest median concentration in the CSDHs at 9594 pg/mL, and the

largest difference in comparison to plasma (approximately 168 times higher). The next highest median concentrations of inflammatory markers seen in CSDH fluid were IL-6 (1600 pg/mL), MCP-1 (1500 pg/mL), IL-8 (433 pg/mL) and IP-10 (254 pg/mL). In plasma, the highest median concentration of any inflammatory marker was VEGF (57 pg/mL).

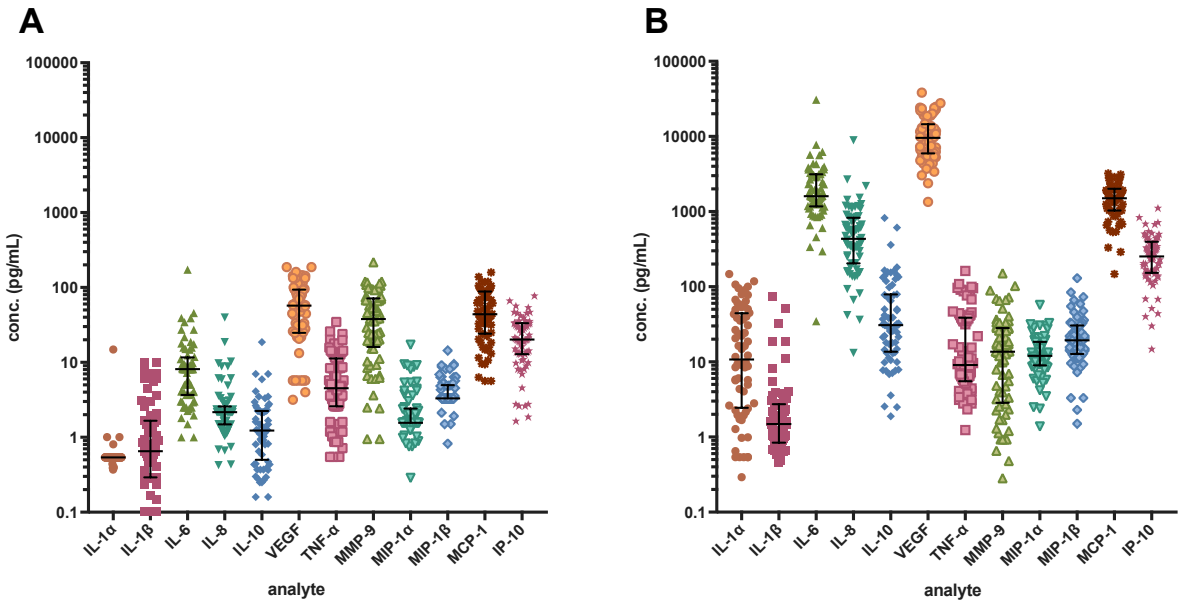

Figure 5.2; intra-operative inflammatory marker concentrations detected in: **(A)** plasma, **(B)** CSDH. All values are the mean of two replicates, n = 68 from 52 patients (as per Figure 5.1). Line (median), bars (IQR), for detailed results and statistics see Figure 5.3.

The significant differences found between the concentration of each analyte in plasma and CSDH can be seen more completely in Figure 5.3. MMP-9 was the only analyte with a median plasma concentration that was significantly higher than CSDH (Figure 5.3H). The plasma and CSDH concentrations of all analytes were compared with the literature on healthy controls and previous CSDH patients (Table 5.2). The challenge to the healthy control literature is that the collection, analytical processes and even statistical analysis are varied and poorly reported, thus there is a wide range of what can be considered "normal". This is exemplified by one meta-analysis, where the healthy controls from each study had a mean IL-8 concentration ranging from 0.91 pg/mL to 60 pg/mL (Dowlati et al., 2010). Other studies in the literature have reported median IL-8 values of 4.22 and 29.3 in healthy controls (Dahl et al., 2014; Kleiner, Marcuzzi, Zanin, Monasta, & Zauli, 2013). Thus, the overall values from the normal literature can only be used as a vague guideline, with the comparative plasma and CSDH concentrations within the CSDH population more relevant. It should also be

considered that many studies report mean values, whereas median values are more appropriate for non-normal data as is seen with the inflammatory markers assessed in this study. With these caveats, the cytokine plasma levels were comparable or lower than the control literature for all analytes apart from MMP-9, IL-6 and to a lesser extent VEGF, where there was a possible trend to higher plasma concentrations in CSDH patients.

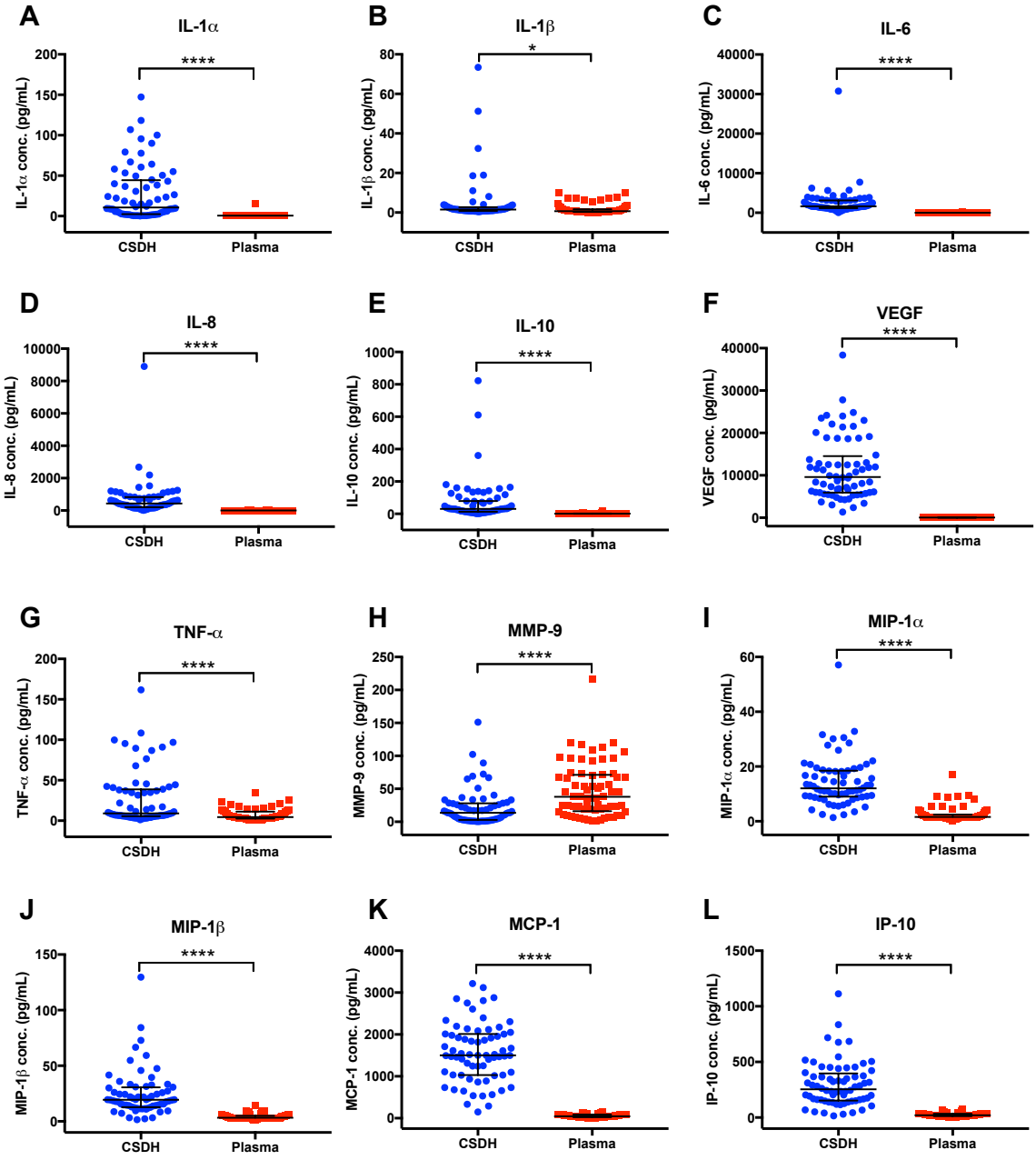

Figure 5.3; comparison between intra-operative CSDH and plasma concentrations of all 12 analytes. Values are the mean of two replicates, n = 68 from 52 patients, as per Figure 5.1. Line (median), bars (IQR), statistically significant differences denoted as; $P \leq 0.05$ (*), $P \leq 0.0001$ (****).

Table 5.2; plasma and CSDH analyte concentrations in control literature and previous CSDH studies. All values are in pg/mL unless stated as ng/mL (ng), means unless stated as median (Md) and plasma unless stated as serum.

Analyte	Md conc. in study (IQR)	Conc. in literature (pg/mL)	Study (Author, year)	Study details (normal controls or CSDH)
IL-1α Plasma	0.5 (0.5 - 0.5)	<3.2 Md	Kleiner, 2013	35 controls; serum
IL-1α CSDH	10.8 (2.5 - 44.5)	N/A	N/A	Not previously tested.
IL-1β Plasma	0.7 (0.3 - 1.7)	0.3 0.4 Md 7.3 25 183	Di Iorio, 2003 Dahl, 2014 Dowlati, 2010 Pripp', 2014 Stanisic, 2012	907 controls (*R 0.1-0.8* in 1292 controls). 34 controls. *(IQR 0.28-0.95)* 246 controls *(R 0.67- 17.68)* 51 CSDH patients. 41 CSDH patients.
IL-1β CSDH	1.5 (0.8 - 2.7)	20 607	Pripp', 2014 Stanisic, 2012	43 CSDHs 41 CSDH patients
IL-6 Plasma	8.2 (3.7 - 11.6)	1.9 10 Md 2.4 Md 2.1 12.6	Salvi, 2000 Kleiner, 2013 Dahl, 2014 Dowlati, 2010 Pripp', 2014	67 controls. 35 controls; serum. 34 controls. *(IQR 1.28-6.28)* 400 controls *(R 0.74- 5.35)* 41 CSDH patients
IL-6 CSDH	1600 (1174 - 3136)	2095 2071 1115 6309	Frati, 2004 Hong, 2009 Wada, 2006 Pripp', 2014	35 CSDHs; *1870 in NR, 3450 in recur* 64 CSDHs; *1980 in 52 NR, 2411 in 14 recur* 34 CSDH patients 41 CSDH patients
IL-8 Plasma	2.2 (1.5 - 2.6)	29.3 Md 4.2 7.7 7.9 15	Kleiner, 2013 Dahl, 2014 Dowlati, 2010 Pripp', 2014 Stanisic, 2012a	35 controls; serum *(IQR 24.4 - 35.9)* 34 controls. *(IQR 2.95-7.04)* 77 controls *(R 0.91-60)*. 52 CSDH patients. 73 CSDH patients.
IL-8 CSDH	433.4 (205 - 826.4)	1996 1384 579 8623	Pripp', 2014 Frati, 2004 Wada, 2006 Stanisic, 2012a	56 CSDH patients 35 CSDH patients; *1170 NR, 2674 in recur* 34 CSDH patients 73 CSDH patients
IL-10 Plasma	1.2 (0.5 - 2.3)	12.6 Md 2.5 Md 63.7 10 43	Kleiner, 2013 Dahl, 2014 Dowlati, 2010 Pripp', 2014 Stanisic, 2012a	35 controls; serum *(IQR 8.5-16.7)* 34 controls *(IQR 0.97-6.03)*. 200 controls *(R0.7 - 542.32)*. 57 CSDH patients 56 CSDH patients
IL-10 CSDH	30.8 (13.6 - 79)	25 57.8 37	Pripp', 2014 Wada, 2006 Stanisic, 2012a	56 CSDH patients 34 CSDH patients 56 CSDH patients
VEGF Plasma	57.2 (24.8 - 93.4)	42 50 Md 52 Md 61.6 Md 77	Kut, 2007 Lip, 2000 Shoab, 1998 Kleiner, 2013 H'stein, 2005	825 controls 16 controls *(IQR 16-113)* 25 controls *(IQR 35-71)* 35 controls; serum *(IQR 32-118.9)* 11 CSDH patients *(R 7-453)*.
VEGF CSDH	9594.5 (5942.9 - 14528.3)	23933 10277 8142 11739	H'stein, 2005 Shono, 2001 Weigel, 2001 Nanko, 2009	11 CSDH patients *(R 5,400 - 59,300)* 20 CSDH patients 19 CSDH patients *(R 1,255- 29,908)* 76 CSDH patients

		8343	Hong, 2009	66 CSDH patients; 8262 in NR, 8646 in recur
TNF-α Plasma	4.5 (2.6 - 11.3)	30 Md 9.6 Md 8.9 640 10	Kleiner, 2013 Dahl, 2014 Dowlati, 2010 Pripp*, 2014 Stanisic, 2012	35 controls; serum 34 controls *(IQR 4.92-22.65)* 360 controls *(R 0.37 -36.04)* 41 CSDH patients. 56 CSDH patients.
TNF-α CSDH	9.1 (5.5 - 38.7)	3.2 3	Pripp*, 2014 Stanisic, 2012	38 CSDH patients. 56 CSDH patients.
MMP-9 Plasma	37.9 (16.1 - 71.3)	229 (ng) 48 (ng) 132 (ng)	Li, 2018 Jonsson, 2016 Hua, 2016	88 controls; serum 65 controls 37 CSDH patients.
MMP-9 CSDH	13.6 (2.9 - 28.1)	3075 (ng)	Hua, 2016	37 CSDH patients.
MIP-1α Plasma	1.6 (1.6 - 2.4)	7.1 Md 2.6 Md 9 Md	Kleiner, 2013 Dahl, 2014 van B'man 2007	35 controls; serum (IQR 6.2-9). 34 controls (IQR 1.85-40.3). 39 controls.
MIP-1α CSDH	12.0 (9.0 - 18.5)	N/A	N/A	Not previously tested
MIP-1β Plasma	3.3 (3.3 - 5.0)	17 Md	van B'man 2007	39 controls
MIP-1β CSDH	19.39 (12.7 - 30.6)	N/A	N/A	Not previously tested
MCP-1 Plasma	44.0 (24.2 - 88.3)	41.5 Md 199 344	Kleiner, 2013 Pripp*, 2014 Stanisic, 2012a	35 controls; serum *(IQR 20.1 - 78.9)*. 56 CSDH patients. 76 CSDH patients.
MCP-1 CSDH	1500.2 (1032 - 2010)	3162 7082	Pripp*, 2014 Stanisic, 2012a	53 CSDH patients. 76 CSDH patients.
IP-10 Plasma	20.2 (12.9 - 33.4)	576.2 40 62	Kleiner, 2013 Pripp*, 2014 Stanisic, 2012a	35 controls; serum *(IQR 368.5 - 808.5)*. 57 CSDH patients 73 CSDH patients
IP-10 CSDH	253.6 (153 - 1113)	316 640	Pripp*, 2014 Stanisic, 2012a	51 CSDH patients 73 CSDH patients

* derived values from reverse log calculation of the results reported (Conc = concentration, IQR = interquartile range, Md = median, NR = non-recurrent CSDH, Recur = recurrent CSDH, R = range).

References; (Dahl et al., 2014; Di Iorio, 2003; Dowlati et al., 2010; Frati et al., 2004; Hohenstein, Erber, Schilling, & Weigel, 2005; Hong et al., 2009; Hua et al., 2016; Jonsson, Hjalmarsson, Falk, & Ivarsson, 2016; Kleiner et al., 2013; Kut, Mac Gabhann, & Popel, 2007; Li et al., 2018; Lip et al., 2000; Nanko et al., 2009; Pripp & Stanisic, 2014; Salvi et al., 2000; Shoab, Scurr, & Coleridge-Smith, 1998; Shono et al., 2001; Stanisic, Aasen, et al., 2012; Stanisic, Lyngstadaas, et al., 2012a; van Breemen et al., 2007; Wada et al., 2006; Weigel, Schilling, & Schmiedek, 2001).

5.3.1 Vascular Endothelial Growth Factor

Significantly raised blood VEGF levels have been previously linked to diabetic vascular insufficiency (Kusumanto, Meijer, Dam, Mulder, & Hospers, 2007) and chronic venous disease (Shoab et al., 1998). VEGF has also been implicated in the neovascularization that occurs in diabetic retinopathy, which is characterised by a pre-retinal neovascular membrane, not dissimilar to the CSDH neomembranes, with a network of highly permeable vessels surrounded by fibroblasts and macrophages (Malecaze et al., 1994). Patients with such proliferative retinopathy have higher circulating plasma VEGF which have shown trends of decline following laser treatment and resolution of neovascularization (Lip et al., 2000). This data supports the role of VEGF in other similar neovascular-related diseases and even has potential as monitoring for disease activity and response to treatment.

VEGF was the marker with the highest difference in concentration between CSDH and plasma, with a median concentration in CSDH 168 times that of plasma. This suggests it plays a critical role in CSDH pathophysiology, as has been highlighted by several other studies (Hohenstein et al., 2005; Hong et al., 2009; Nanko et al., 2009; Shono et al., 2001; Weigel et al., 2001). Nanko et al. have previously reported no difference in VEGF concentrations between 65 non-recurrent and 11 recurrent CSDH samples, with almost identical mean levels (Nanko et al., 2009). However, this study shows contrasting data, with significantly higher VEGF concentrations in the seven recurrent CSDH samples (median 11987 pg/mL) compared to the 61 primary CSDH samples (median 8443 pg/mL) ($p= 0.05$) (Figure 5.4). This may suggest a role for VEGF in driving CSDH recurrence, however low numbers of recurrent samples make assessment of significance difficult, and the VEGF concentrations were highly varied amongst these seven patients. To analyse this further, only the paired primary and recurrent CSDH samples were assessed, excluding the patient where the primary CSDH was not sampled (also the highest outlier). The remaining six paired samples (one patient had two recurrences, each paired to the primary CSDH) showed no significant difference but a trend to higher VEGF concentration in the recurrent samples ($p = 0.0938$) (Figure 5.5A). Perhaps surprisingly the plasma VEGF showed the opposite trend, with lower concentrations in plasma at recurrence, compared to time of primary surgery ($p = 0.0625$) (Figure 5.5B). As the plasma VEGF at primary surgery is at the higher end of the range in normal literature (Table 5.2), this may indicate there is a mild peripheral inflammatory response in CSDH, which is lower following primary treatment, even in the

case of recurrence. To validate this theory, it would be helpful to assess follow-up plasma samples in all CSDH patients, not just the recurrent cases, to look for systemic reductions in VEGF after surgical treatment of CSDH. No such data has been published, and therefore this should be considered in future studies.

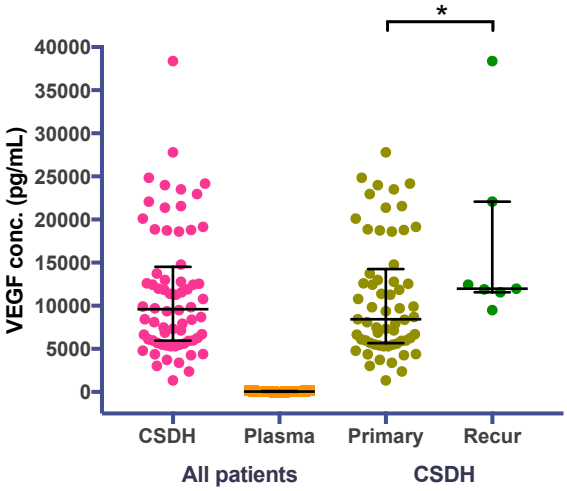

Figure 5.4; VEGF concentration in all CSDH and plasma samples (n = 68), primary CSDH (n = 61) and recurrent CSDH (n = 7) samples. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.05$ (*), (Recur = recurrence).

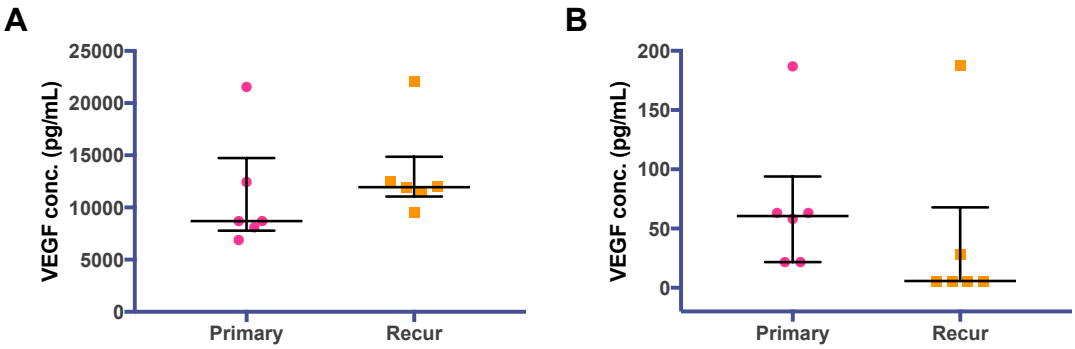

Figure 5.5; VEGF concentration in patients with recurrent CSDH in: **(A)** CSDH, **(B)** plasma. N=6, line (median), bars (IQR), (Recur = recurrence).

To help elucidate whether CSDH recurrence could be predicted from the primary surgical samples, comparison was made between patients who went on to have a recurrence and those who did not. Six primary CSDH samples were available in patients who went on to have a recurrence, with little difference in the VEGF concentration compared to the remaining 55 primary samples ($p = 0.3117$, Figure 5.6). The same was seen with plasma VEGF concentrations ($p = 0.4442$, data not shown).

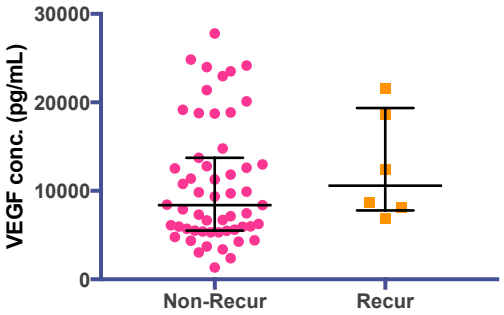

Figure 5.6; VEGF concentration in primary CSDH samples for non-recurrent (non-recur) and recurrent (recur) cases in CSDH. Non-Recur n = 55, Recur n = 6, line (median), bars (IQR).

CSDH concentrations of VEGF in all 68 samples showed a moderate positive correlation with IL-6 (Spearman r = 0.5846, $p < 0.0001$) and MIP-1β (Spearman r = 0.5575, $p < 0.0001$), and a weaker correlation with IL-8 (Spearman r = 0.4178, p = 0.0004) and MMP-9 (Spearman r = 0.3425, p = 0.0043) (Figure 5.7). This is supportive of synergistic action between some of the inflammatory markers.

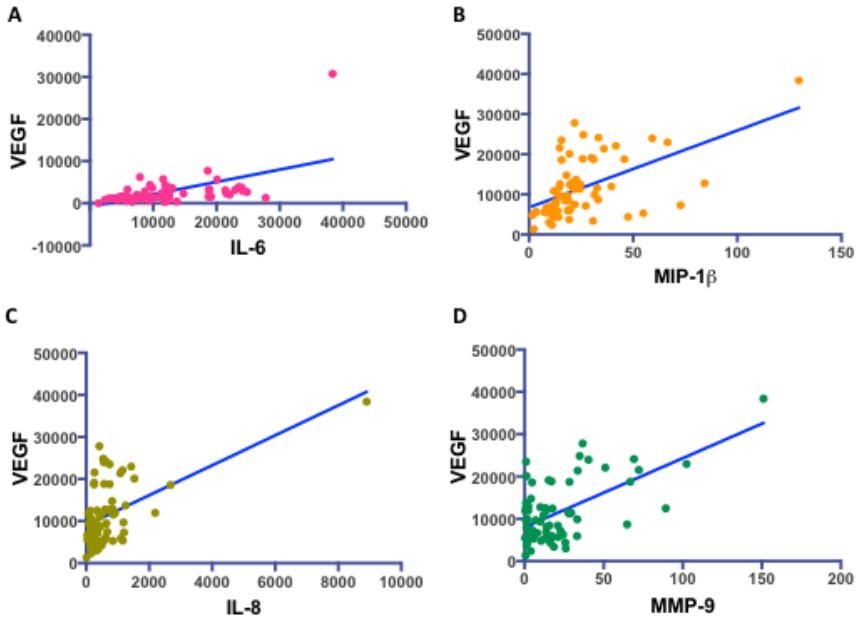

Figure 5.7; significant correlations between VEGF and other markers: **(A)** IL-6, linear regression line y = 0.2929x - 788.6, $r^2 = 0.3242$, **(B)** MIP-1β, linear regression line y = 191.7x + 6760.6, $r^2 = 0.2928$, **(C)** IL-8, linear regression line y = 3.561x - 2536, $r^2 = 0.2972$, **(D)** MMP-9, linear regression line y = 163.1x + 8022, $r^2 = 0.3652$. All axes' represent concentrations of analyte in pg/mL, n = 68.

5.3.2 IL-6, IL-8 and IL-10

IL-6 and IL-8, both considered pro-inflammatory cytokines, had the second and fourth highest concentrations in the CSDH samples. IL-10 was the sixth highest, but is conversely considered an anti-inflammatory cytokine, although previous literature has shown that patients with high IL-10 also have higher levels of IL-6 and IL-8 (Wada et al., 2006). It is suggested that this shows there is some balance of pro- and anti-inflammatory cytokine activation, attempting to modulate the inflammatory response. However, no correlation between the three markers was found in this study, and IL-6, IL-8 and IL-10 were significantly different from one another (Friedman test, $p<0.0001$) (Figure 5.8).

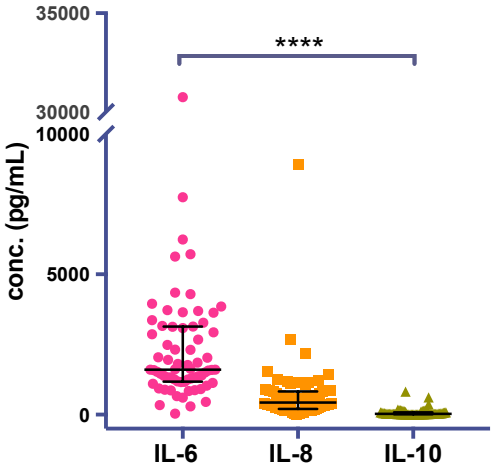

Figure 5.8; concentrations of IL-6, IL-8 and IL-10 in CSDH. N = 68. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.0001$ (****).

Co-ordinated production of IL-6 and IL-8 is often observed in inflammatory conditions (Kishimoto, Akira, & Taga, 1992), and a significant moderate correlation was observed in this study (Spearman correlation $r = 0.6314$, $p<0.0001$) (Figure 5.9A). The correlation was repeated with the largest value excluded, as it appeared as if this may have a large influence on the correlation, but there was little change (Spearman correlation $r = 0.6147$, $p<0.0001$) (Figure 5. 9B).

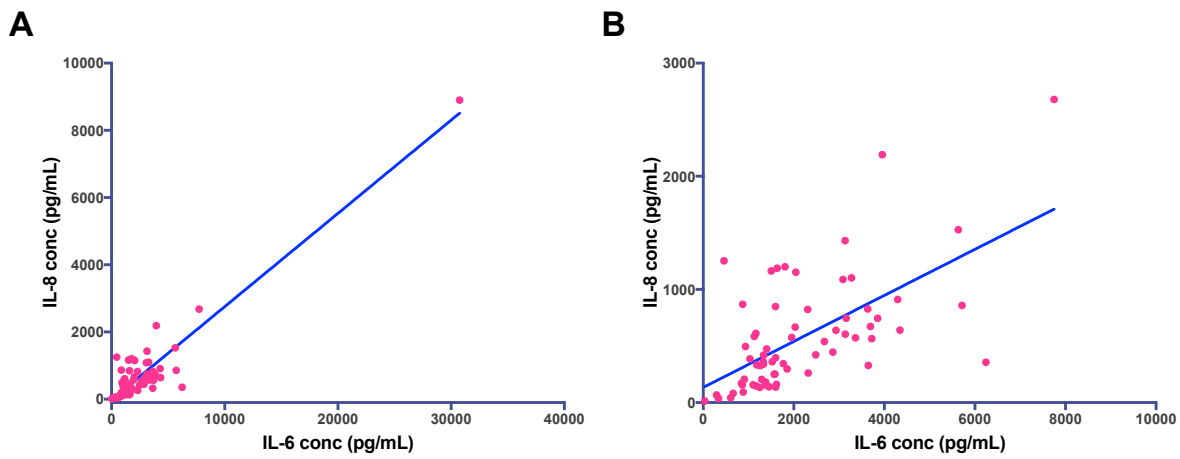

Figure 5.9; correlation between IL-6 and IL-8: **(A)** all samples (N = 68), linear regression line y = 0.2774x - 20.6, r^2 = 0.869, **(B)** largest outlier excluded (N = 67), linear regression line y = 0.2034x + 133.6, r^2 = 0.381.

IL-6 and IL-8 have previously been shown to be significantly higher in recurrent CSDH compared with primary CSDH (Frati et al., 2004), a finding also seen in this study; IL-6 primary CSDH median 1569 pg/mL versus recurrence median 4294 pg/mL (p = 0.0008); IL-8 primary CSDH median 396 pg/mL versus recurrence median 911 pg/mL (p = 0.0179) (Figure 5.10A&B). Again, this was repeated with largest outlier excluded (which was a recurrence sample for which no primary sample had been collected), maintaining significance for IL-6 (p = 0.0041), but just missing it for IL-8 (p = 0.0625) (Figure 5.10C&D).

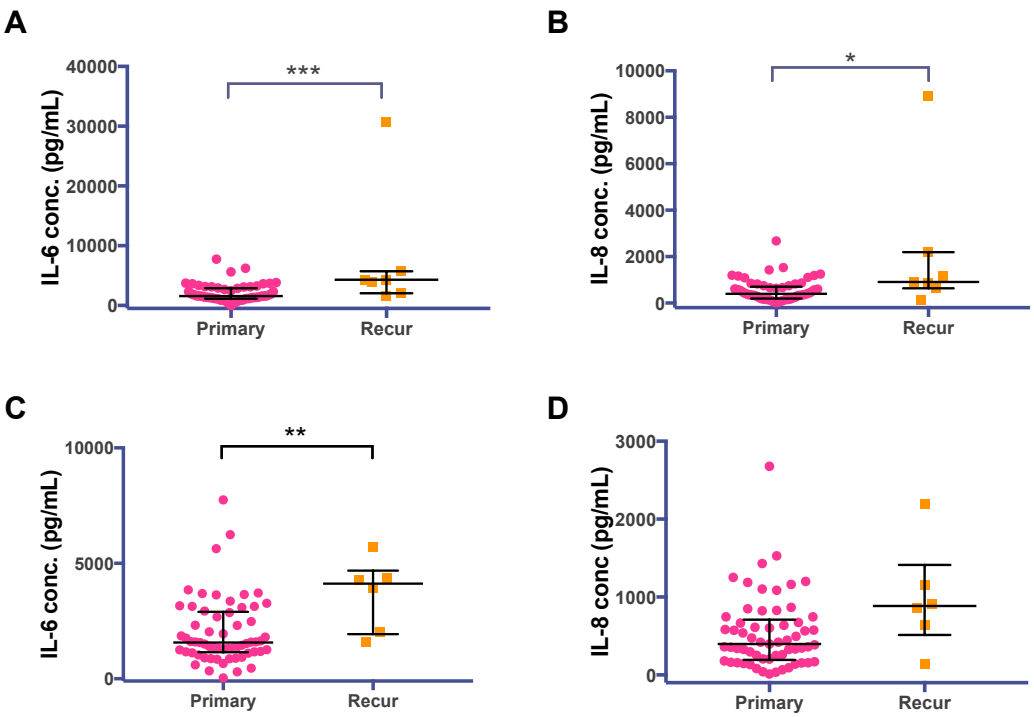

Figure 5.10; concentrations in all primary and recurrent CSDHs for**:** **(A)** IL-6, **(B)** IL-8, Primary n = 61, recurrence n = 7, **(C)** IL-6 excluding outlier, **(D)** IL-8 excluding outlier, Primary n = 61, recurrence n = 6. Line (median), bars (IQR), statistically significant differences denoted as; P ≤ 0.05 (*), P ≤ 0.005 (**), P ≤ 0.001 (***), (Recur = recurrence).

IL-10 showed no significant difference in CSDH concentration relating to recurrence (p = 0.3610) and there was no difference in the plasma levels of IL-6, IL-8 or IL-10 in recurrent or primary samples (data not shown). Analysis of the six, paired primary and recurrent CSDH samples showed a significant difference with IL-8 (p = 0.0312) and a non-significant trend to higher IL-6 (p = 0.0625) and IL-10 (p = 0.0625) in the recurrence samples (Figure 5.11).

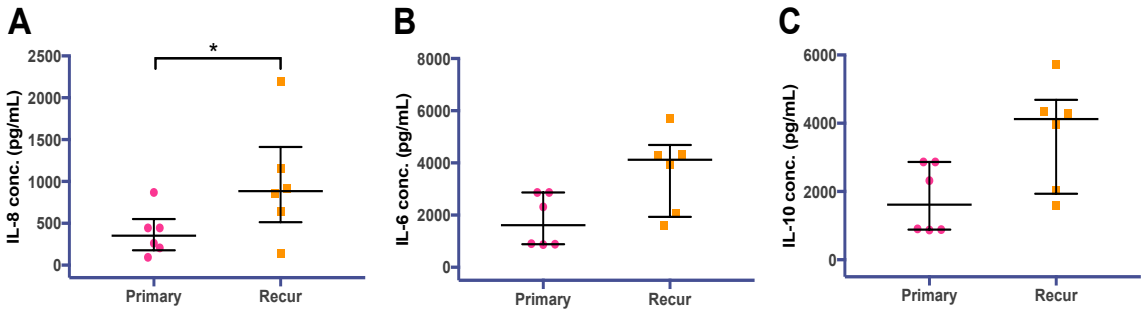

Figure 5.11; primary and recurrent CSDH paired samples: **(A)** IL-8, **(B)** IL-6, **(C)** IL-10. N = 6. Line (median), bars (IQR), statistically significant differences denoted as; P ≤ 0.05, (Recur = recurrence).

When assessing primary CSDH samples to see whether they were predictive of recurrence, no significant difference was seen for IL-6 or IL-8 (data not shown). However, IL-10 was significantly lower in the recurrent primaries ($p = 0.0015$) (Figure 5.12), which remained significant with removal of outliers ($p = 0.0020$). Lower concentrations of IL-10 may increase the risk of recurrence by reducing the normal anti-inflammatory response needed to counter-act pro-inflammatory cytokines, thus leading to more uncontrolled, persistent inflammation.

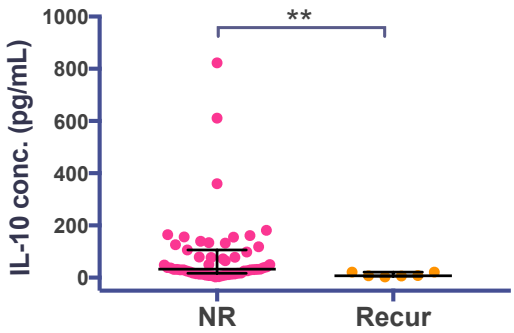

Figure 5.12; IL-10 in primary CSDH samples for non-recurrent and recurrent cases. Non-recurrence n = 55, recurrence n = 6. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.005$ (**), (NR = no recurrence, Recur = recurrence).

5.3.3 MMP-9

Matrix Metalloprotease-9 (MMP-9) is the only marker with significantly higher concentrations in plasma than CSDH ($p < 0.0001$). There were no significant differences between primary and recurrent samples for CSDH ($p = 0.2407$) or plasma ($p = 0.6458$) (Figure 5.13A). However, paired primary and recurrent CSDH samples showed a strong trend towards higher MMP-9 in the recurrent samples ($p = 0.0625$, Figure 5.13B). When all the primary CSDH samples were compared, those that went on to recur were not significantly different to those that did not ($p = 0.1837$, data not shown).

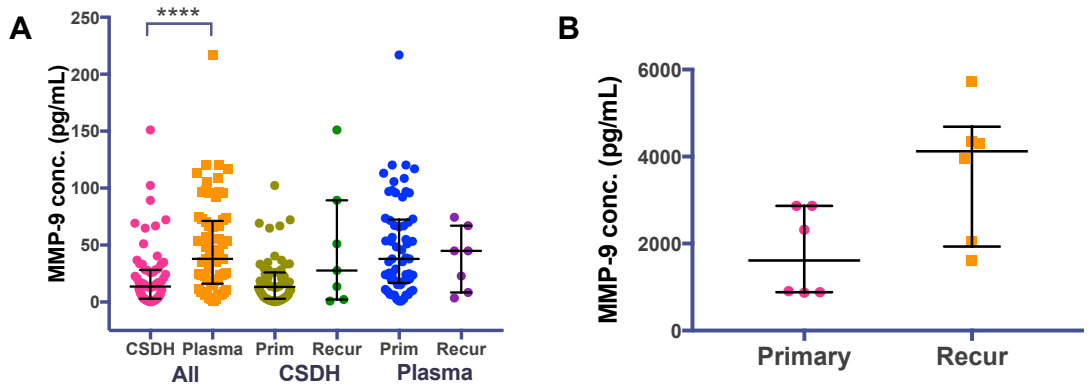

Figure 5.13; MMP-9 concentration in primary and recurrent CSDH and plasma: **(A)** all samples, N = 68, **(B)** paired primary and recurrent samples. N = 6. Line (median), bars (IQR), statistical significant differences denoted as $P \leq 0.0001$ (****), (Prim = primary, Recur = recurrence).

Interestingly MMP-9 was the only marker where the relationship between CSDH and plasma concentrations were not the same in all patients. There were three different patterns of MMP-9 observed, and when analysing all the primary CSDH samples; in 68% (46/68) MMP-9 was higher in plasma (HP), in 20% (14/68) it was higher in CSDH (HC) and in 12% (8/68) it was comparable with CSDH concentrations (within 5 pg/mL, Figure 5.14A). The median differences between plasma and CSDH were 33pg/mL for HP and -15pg/mL for HC. A significant difference was found in these values between primary and recurrent CSDHs, with higher CSDH concentrations compared to plasma in the recurrent cases ($p = 0.0241$, data not shown). This is also reflected in reversal of the MMP-9 plasma/CSDH patterns in the recurrent samples, with 4/7 (57%) HC, 2/7 (29%) HP and 1/7 (14%) comparable (Figure 5.14B). This increase in MMP-9 within the CSDH at recurrence (compared to plasma), suggests that MMP-9 is more active in recurrence than primary CSDH and may be because MMP-9 is important in the early stages of membrane formation, which has often long passed by the time of primary CSDH operation. However, recurrence could be partly driven by re-activation of MMP-9 post-operatively, leading to further new membrane formation and bleeding.

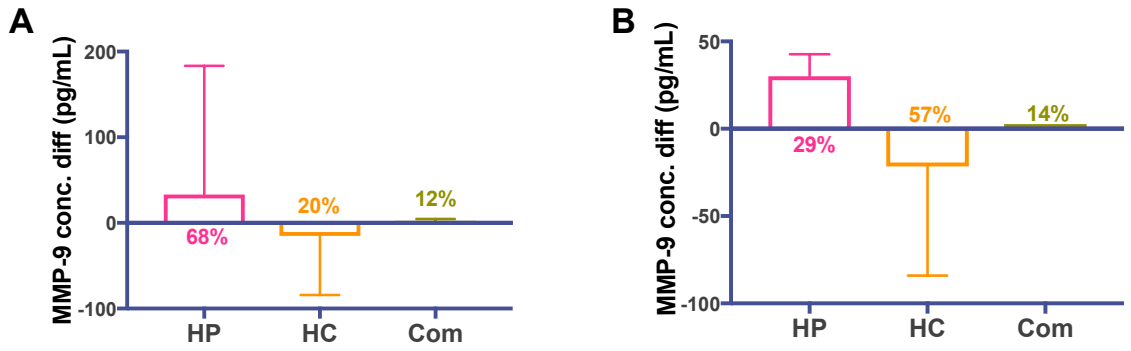

Figure 5.14; (A) MMP-9 subgroups in primary CSDH. Conc. diff is the concentration difference as shown by plasma concentration minus CSDH concentration. N=68, **(B)** MMP-9 sub-groups in recurrent CSDH. N = 7. Box (median), whiskers (range), (HP = higher plasma, HC = Higher CSDH, Com = comparable).

There was a moderate positive correlation between MMP-9 and MIP-1β (Spearman r = 0.499, $p < 0.0001$) and a moderate negative correlation between MMP-9 and IL-1α (Spearman r = -0.455, $p < 0.0001$) (Figure 5.15). A positive correlation with VEGF is reported earlier (Figure 5.7D), but all remaining analytes were weakly or not significantly correlated with MMP-9 (data not shown). Previous reports have suggested that MMP-9 can have pro or anti-inflammatory actions (Manicone & McGuire, 2008), in general the positive correlation with VEGF and MIP-1β suggest it is pro-inflammatory, although it is not clear why there is a negative correlation with IL-1α, which is generally accepted as a "pro-inflammatory" cytokine (Di Paolo & Shayakhmetov, 2016).

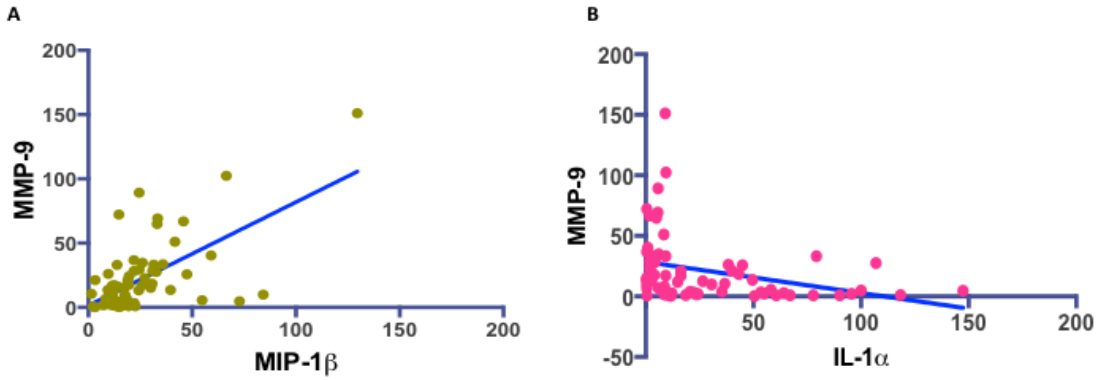

Figure 5.15; correlations of MMP-9 with: **(A)** MIP-1β, linear regression y = 0.8043x + 1.454, r^2= 0.3753, **(B)** IL-1α, linear regression y = -0.2567 + 28.48, r^2= 0.1018. N = 68.

5.3.4 Other analytes

The same analysis as shown in the above sections (3.3.1-3.3.3) was performed on all other analytes. Only the significant results are shown in this section with a summary of all findings.

MIP-1β and TNF-α

MIP-1β and TNF-α were significantly higher in recurrence samples compared with all primary samples (MIP-1β p = 0.02; TNF-α p = 0.0371) (Figure 5.16). However, significance was lost when comparing only the paired primary and recurrent samples and primary samples were not predictive of recurrence (data not shown).

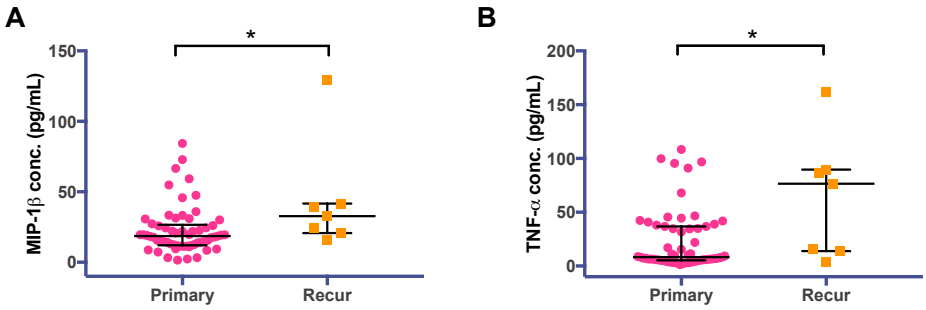

Figure 5.16; all primary and recurrent CSDH samples: **(A)** MIP-1β, **(B)** TNF-α. N = 68. Line (median), bars (IQR), statistically significant differences denoted as P ≤ 0.05 (*), (Recur = recurrence).

IL-1α and IL-1β

IL-1α and β are secreted by macrophages in the early stages of inflammation and can induce expression of pro-inflammatory genes for other cytokines and MMPs (Apte & Voronov, 2008). Severe inflammation, with necrotizing cells, is required for IL-1α to become detectable, therefore it is not surprising that circulating plasma levels, as seen in Table 5.2 are very low (Apte & Voronov, 2008). However, there was a significant difference in IL-1α concentration between paired primary and recurrent CSDH samples (p = 0.0312), but only a trend comparing all primary with recurrent samples (p = 0.1075) (Figure 5.17A&B). For IL-1β, there were significantly higher concentrations in all recurrence versus primary samples (p = 0.0045) but this was lost for the paired samples (p = 0.4375) (Figure 5.17C&D).

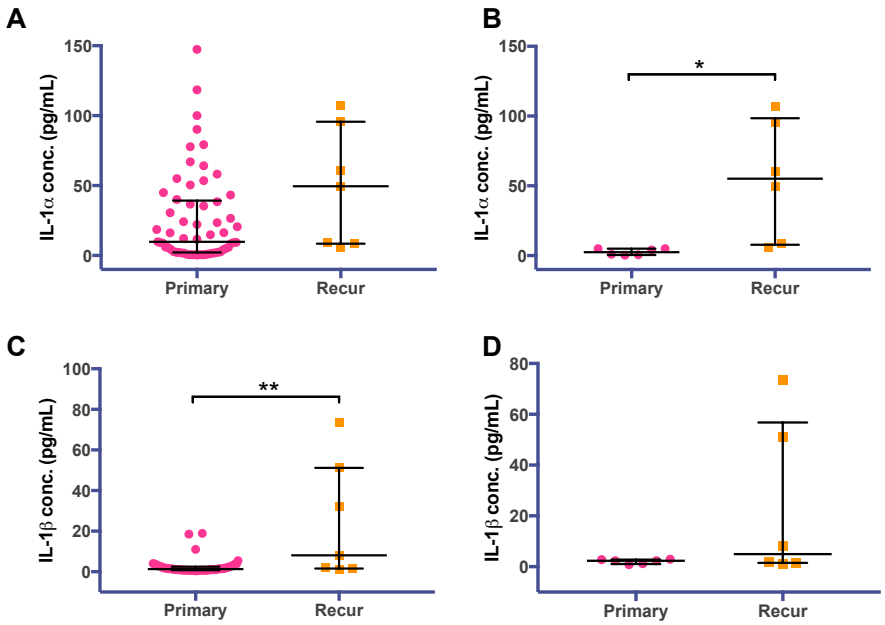

Figure 5.17; primary and recurrent CSDH samples: **(A)** IL-1α in all samples (n = 68), **(B)** IL-1α in paired samples (n = 6), **(C)** IL-1β in all samples (n = 68), **(D)** IL-1β in paired samples, (n = 6). Line (median), bars (IQR), statistically significant differences denote as P ≤ 0.05 (*), P ≤ 0.005, (Recur = recurrence).

IL-1 and TNF-α are often considered to simulate each other's production (Apte & Voronov, 2008). This is seen with the moderate positive correlation between IL-1α and TNF-α (Spearman r = 0.5341, p < 0.0001, Figure 5.18), although interestingly IL-1β was not significantly correlated to TNF-α nor to IL-1α (data not shown). IL-1α also has significant moderate positive correlation with IL-8 (r = 0.534, p < 0.0001), all other correlations were weak or non-significant.

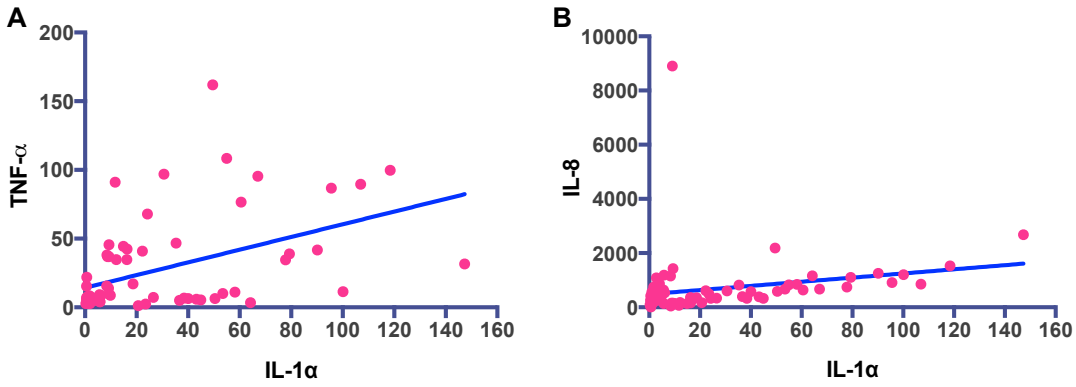

Figure 5.18; correlation of IL-1α with: **(A)** TNF-α (r^2 = 0.2159, y = 0.4623x + 14.22), **(B)** IL-8 (r^2 = 0.05, y = 7.65x + 483.

MCP-1, MIP-1α and IP-10

No significant differences between primary, recurrent or paired samples were found for these markers.

5.3.5 Bilateral CSDH

There were 10 cases of bilateral CSDH with no significant differences between the paired sides, suggesting contralateral sides are comparable for inflammatory profiles within individual patients (Figure 5.19). This is further validated by significant correlations between sides for MCP-1, VEGF, TNF-α, IL-1α and IL-1β (Table 5.3 and Figure 5.20). Correlation was close to significance for the remaining markers, but some are skewed by one or two outlying patients with large differences between sides. To assess the difference further, the side with the larger value was taken as the "baseline" and the percentage decrease to the smaller value calculated, with an overall mean difference of 30% between sides. In general, despite the collections being considered individual due to division by the midline falx cerebri and each CSDH membrane, this suggests there is reasonable congruity between sides. This is much greater than that seen between individual patients, as seen with large coefficients of variation (Table 5.3). The explanation for this is either the CSDH fluid can communicate across the membranes and falx cerebri, or that each patient mounts a similar level of inflammatory response on contralateral sides despite each CSDH being self-contained.

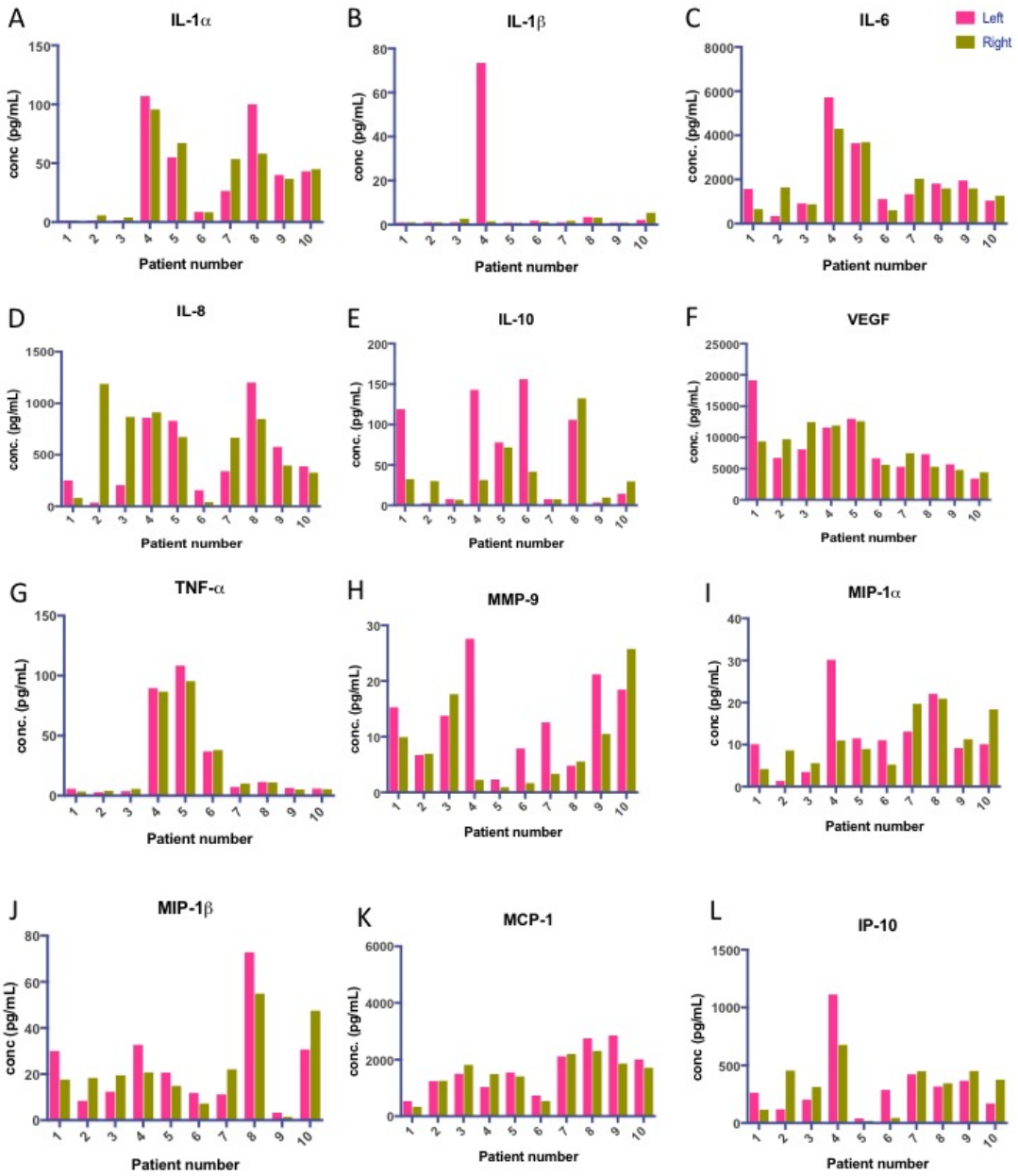

Figure 5.19; analyte concentrations for individual patients with bilateral CSDH for all analytes: **(A)** IL-1α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-8, **(E)** IL-10, **(F)** VEGF, **(G)** TNF- α, **(H)** MMP-9, **(I)** MIP-1α, **(J)** MIP-1β, **(K)** MCP-1, **(L)** IP-10. N = 10.

Table 5.3: correlation of analyte concentrations and percentage difference between sides of bilateral CSDH. Rows highlighted in blue show significant correlation.

Analyte	Correlation between sides of bilateral CSDH	Mean % decrease between sides	Coefficient of variation in all samples
IL-1α	R = 0.930 (p = 0.0003)	30%	122%
IL-1β	R = 0.748 (p = 0.016)	33%	248%
IL-6	R = 0.515 (p = 0.133)	30%	146%
IL-8	R = 0.139 (p = 0.707)	46%	162%
IL-10	R = 0.636 (p = 0.054)	47%	177%
VEGF	R = 0.733 (p = 0.020)	23%	64%
TNF-α	R = 0.891 (p = 0.001)	18%	124%
MMP-9	R = 0.478 (p = 0.166)	46%	127%
MIP-1α	R = 0.418 (p = 0.233)	42%	63%
MIP-1β	R = 0.624 (p = 0.060)	40%	84%
MCP-1	R = 0.879 (p = 0.002)	21%	45%
IP-10	R = 0.479 (p = 0.166)	44%	67%
Mean of all analytes	**N/A**	**35%**	**119%**

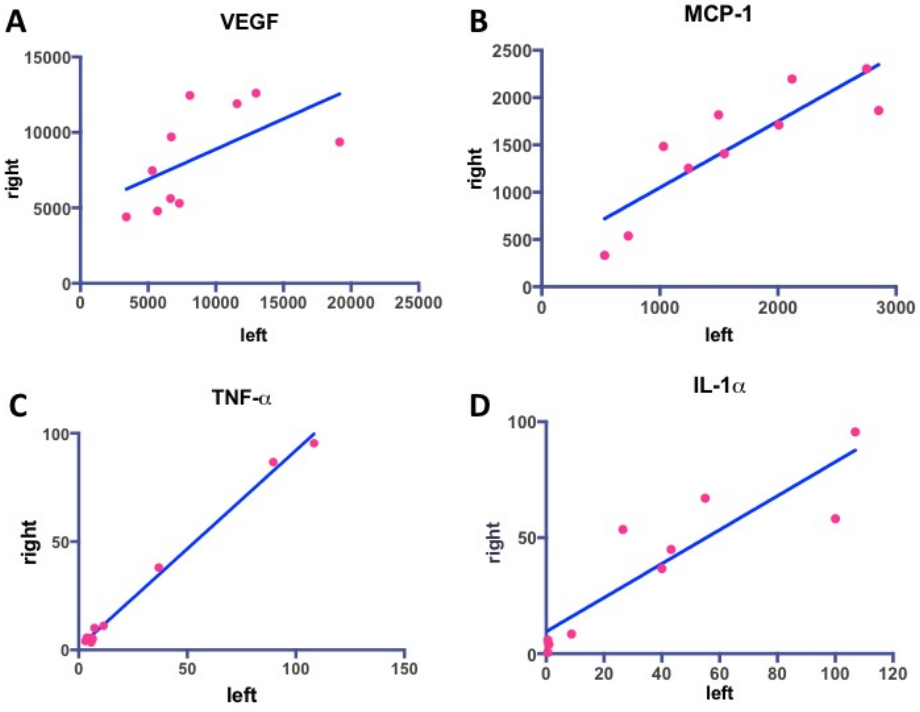

Figure 5.20; significant correlations for bilateral CSDH: **(A)** VEGF, **(B)** MCP-1, **(C)** TNF-α, **(D)** IL-1α. N = 10. For R and p values see Table 5.3.

All individual bilateral CSDHs (including those where only one side was sampled) were compared to all the unilateral CSDHs to see whether they behaved differently. The unilateral CSDHs had significantly higher concentrations of VEGF ($p = 0.0399$) and MIP-1α ($p = 0.0386$) (Figure 5.21). There was also a trend to higher concentration of IL-6 ($p = 0.0616$), but no difference for any other analyte (data not shown). One explanation for higher inflammatory markers in unilateral cases could be the dilution of the inflammatory markers in bilateral patients, were the body is required to mount an inflammatory response over a much larger surface area of brain (combined volumes of bilateral CSDHs are significantly more than unilateral, see imaging chapter seven). This might be particularly relevant if the pool of inflammatory cells is recruited locally from the skull bone marrow, as has been demonstrated in murine models, where the production per surface area of brain is potentially limited (Herisson et al., 2018).

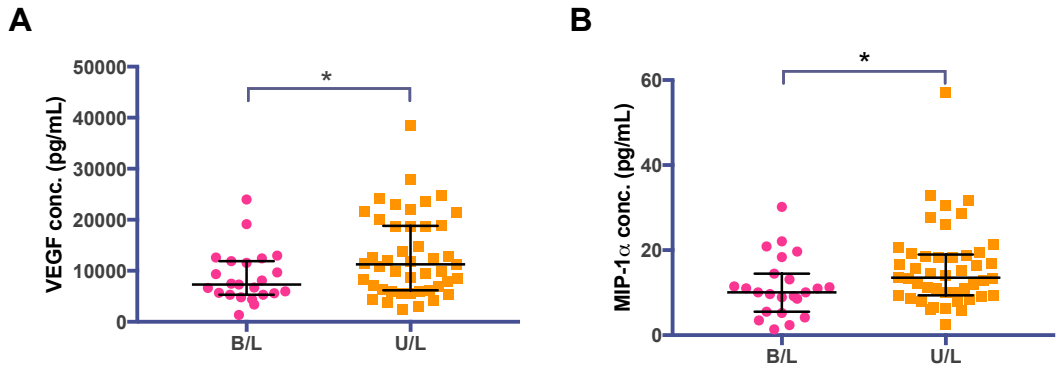

Figure 5.21; comparison between bilateral (B/L) and unilateral (U/L) CSDHs for: **(A)** VEGF, **(B)** MIP-1α, B/L n = 23, U/L n = 45. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.05$ (*).

In one patient two samples were taken from a unilateral CSDH; one from the anterior burr hole, the other from posterior burr hole. This was because the fluid in the collection looked very different between the anterior and posterior portions seen under each burr hole. This is usually due to different patterns of blood within the CSDH, which is discussed further in the imaging chapter (chapter seven). The posterior sample had higher concentrations of all analytes (shown by orange extension), apart from IL-1α and TNF-α, than the anterior sample (pink bar) (Figure 5.22). When all the markers were grouped and paired, there was a significant difference between the anterior and posterior concentrations (Wilcoxon test $p = 0.0342$). For all other analysis throughout this chapter, these two samples were counted as

two different CSDHs (as with each side of bilateral samples), as the samples were so different and it was not possibly to determine which was more representative. This raises the issue of regional CSDH inflammatory variation, which is unsurprising considering the different patterns of density seen within many CSDHs (see imaging chapter seven). The burr hole positioning could therefore have a significant influence on the inflammatory markers observed in the fluid collected. For future studies, it may be beneficial to take samples from both burr holes (if two are made) to help understand if these regional differences occur in all CSDHs.

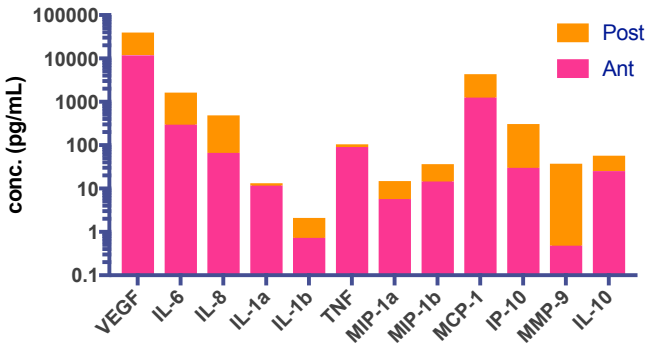

Figure 5.22; difference in analyte concentration between anterior and posterior samples within one CSDH.

5.3.6 Intra-operative data summary

General findings from the intra-operative CSDH and plasma fluid data can be surmised as;

i. All analytes were significantly elevated in CSDH compared to plasma, apart from MMP-9. This supports the established theory that the inflammatory response in CSDH is overwhelmingly a local phenomenon.

ii. VEGF was the marker with the greatest concentration by far within CSDH, supporting the hypothesis that this is the primary mediator of inflammation.

iii. MMP-9 had significantly higher plasma levels in the majority of primary CSDHs, however this was reversed for recurrent CSDH, and may indicate a role for MMP-9 in the early development of CSDH which is re-activated during recurrence.

iv. The majority of pro-inflammatory markers (IL-6, IL-8, IL-1α, IL-1β, MIP-1β, TNF-α and MMP-9) are elevated in CSDH recurrence samples suggesting that escalating, uncontrolled inflammation may be responsible for driving recurrence.

v. The only marker where concentrations at the primary operation could help predict recurrence was IL-10, where it was significantly lower in CSDHs that went on to recur. This suggests a poorly balanced anti-inflammatory response in CSDHs that recur.

vi. Several of the pro-inflammatory cytokines are moderately well correlated, particularly VEGF, IL-6 and IL-8 suggesting they are working in synergistic cascades (see Table 5.4)

vii. Bilateral CSDHs are more similar to one another than CSDHs between different patients, suggesting they either communicate or that each patient's cellular inflammatory response is similar regardless of the collection being self-contained on the opposite side of brain.

Table 5.4; summary of correlations between individual inflammatory markers

	IL-1β	**VEGF**	**IL-6**	**IL-8**	**IL-10**	**TNF-α**	**MCP-1**	**IP-10**	**MIP-1α**	**MIP-1β**	**MMP-9**
IL-1α	/	/	+	++	/	++	/	/	+	/	+
IL-1β		/	/	/	/	/	/	+	+	+	+
VEGF			++	+	/	/	/	/	/	++	+
IL-6				++	/	+	/	/	+	+	/
IL-8					/	/	+	/	+	+	/
IL-10						+	-	/	/	/	/
TNF-α							/	/	/	/	/
MCP-1								/	/	+	+
IP-10									+	/	/
MIP-1α										+	/
MIP-1β											+

R <0.3 no correlation (even if p significant) = /

R = 0.30 - 0.49 and p<0.05; significant weak correlation = + (positive) or - (negative)

R = 0.50 - 0.70 and p<0.05; significant moderate correlation = ++ (positive)

5.4 Post-operative CSDH drain samples

Post-operative drain samples were collected from 28 CSDHs in 25 patients (three bilateral), with comparable demographic data to the patients who had intra-operative sampling (Figure 5.23).

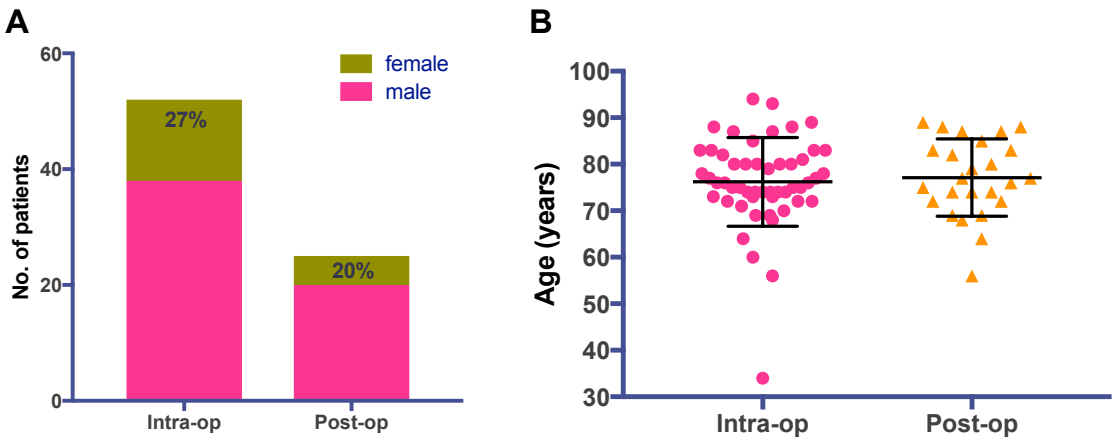

Figure 5.23; demographic data on patients with drain samples collected compared to all patients: **(A)** gender distribution, **(B)** age distribution. Intra-op n = 68, post=op n = 25, line (mean), bars (S.D.), (op = operative).

All 28 CSDHs had the drain sampled and emptied within 8-24hours of the operation, 10 of these drains were emptied and sampled again within the next 16-hour time window (25-40 hours), four of whom were emptied and sampled in the final 24-hour time window (41-65 hours). More drain samples were collected but were excluded if they were not sampled at all time-points, as this would allow accumulation of markers over multiple time points and skew the data. The drainage fluid contained, in differing ratios, a mixture of residual CSDH fluid, intra-operative irrigation fluid (0.9% normal saline), and fresh haemorrhage from the surgical wound. However, considering that the plasma concentrations were low for all analytes other than MMP-9, any increases in inflammatory markers is largely considered to be due to new, local production within the subdural space rather than from operative haemorrhage. This is further corroborated by the low-rate of acute bleeding seen on post-operative CT scans (see imaging chapter seven). The data in this chapter is also potentially limited by the variations in volume of fluid output for each drain. To get truly reliable readings the concentration of analytes needs to be related to the drainage rate of the fluid.

5.4.1 VEGF

The trend in the percentage-change in VEGF concentration from baseline (intra-operative) can be seen in Figure 5.24. The median percentage change was -33.5% (mean -23.6%) in samples collected at 8-24 hrs, although there was a wide range (-86% to +80%) with 71% (20/28) of CSDHs showing a drop from baseline. This exemplifies what was hypothesised to occur in the immediate post-operative period, as the majority of the VEGF should have been washed out during surgical evacuation of the CSDH, leading to a decrease in concentration. However, as the CSDH membranes remain in-situ, there will be on-going production of VEGF post-operatively and infiltrating inflammatory cells produced in response to the surgical trauma, explaining the positive trend seen in some patients. From 25-40 hrs the median percentage change was 22% (mean 15.5%), showing a significant shift from the 8-24 hr values (unpaired t-test, $p = 0.0176$), to an increased production of VEGF. This time 70% (7/10) of samples showed an increase compared to baseline, suggesting this is the most reactive pro-inflammatory time period. Finally, between 41-65 hrs the median percentage change was -26% (mean -20.3%), with 75% (3/4) showing a negative trend once again. As post-operative drains are removed within 48-72 hrs it is impossible to assess the trend after this time point. Therefore, the levels of VEGF could be cyclical or more likely, levels are depleted during surgical evacuation but on-going production leads to an acuumulation of VEGF at around 25-40 hours post-operatively which then declines, presumably continually, until resolution of the CSDH occurs (unless there is a recurrence).

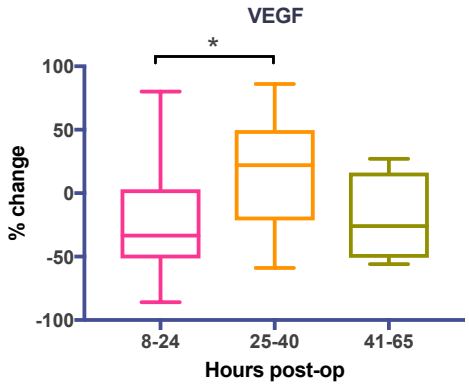

Figure 5.24; percentage change in VEGF in post-operative drain samples. Line (median), box (IQR), whiskers (range), statistically significant differences denoted as $P \leq 0.05$ (*).

5.4.2 IL-6, IL-8 and IL-10

No significant changes were seen at any of the post-operative time-points with regard to the percentage change in IL-6 or IL-8, although again the range of changes was wide. A few patients showed massive increases in IL-6/IL-8 production, again mostly at the 25-40 hr time point (Figure 5.25).

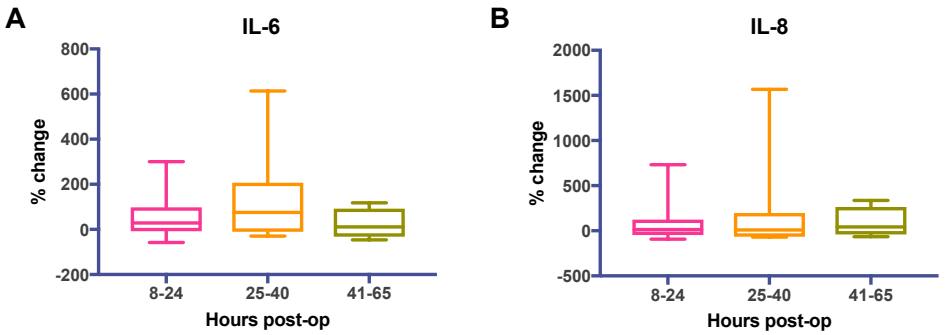

Figure 5.25; percentage change in post-operative drain samples: **(A)** IL-6, **(B)** IL-8. Line (median), box (IQR), whiskers (range).

No significant differences were seen at different time points post-operatively for the full IL-10 data set, however there were six outliers at the 8-24 hr time point. This included a patient with an intra-operative IL-10 concentration of only 3.58 pg/mL which increased by a massive 3714% to 136.57 pg/mL post-operatively. Interestingly, this was one side of a bilateral CSDH and the contralateral side also increased substantially, by 1282% from 9.81 pg/mL to 135.69 pg/mL. Again, this suggests that either the fluid on both sides is in communication, or that the patient's inflammatory response is mirrored on each side of the head. This is a surprising response as IL-10 is considered to primarily be anti-inflammatory, and such a huge surge of anti-inflammatory action would not be expected following an inflammatory stimulus such as surgery. Exclusion of the six outliers, makes it easier to observe the peak in concentrations at 25-40 hrs, which is significant without outliers (unpaired T test, $p = 0.0049$) (Figure 5.26).

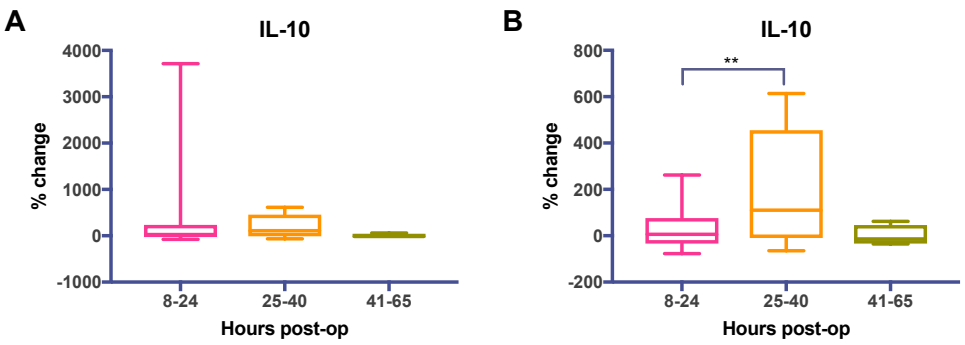

Figure 5.26; percentage changes in IL-10 in post-operative drain samples: **(A)** all data, **(B)** excluding outliers. Line (median), box (IQR), whiskers (range), statistically significant differences denoted as P ≤ 0.005 (**).

In-keeping with the intra-operative samples, a significant positive correlation was found between the percentage changes in drain samples of IL-6 and IL-8 at 8-24 hrs (Pearson's r = 0.554, p = 0.0022) and 25-40 hrs (Pearson's r = 0.8856, p = 0.0007). There were not enough time points at 41-65 hrs to assess correlation and there was no correlation with either marker and IL-10 or VEGF at any time point (Figure 5.27).

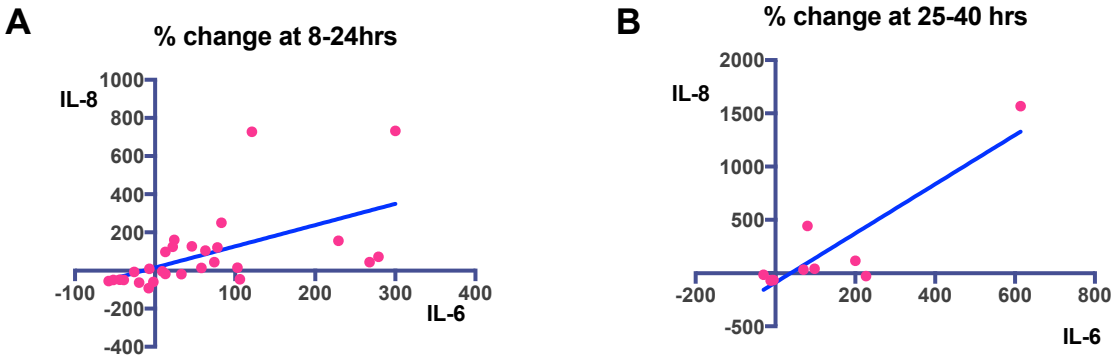

Figure 5.27; correlation of IL-6 and IL-8 in post-operative drain samples at: **(A)** 8-24 hrs, linear regression line y = 1.115x + 15.21, **(B)** 25-40 hrs, linear regression line y = 2.309x - 88.98.

5.4.3 MMP-9

The drain samples showed escalating, and in some cases massive concentrations of MMP-9 post-operatively, with 96% showing a positive change at 8-24 hours, but with wide ranges and no significant differences (Figure 5.28). This, to some extent, explains why MMP-9 was higher in CSDH than plasma in the recurrent samples, as clearly it is re-activated following surgery for CSDH.

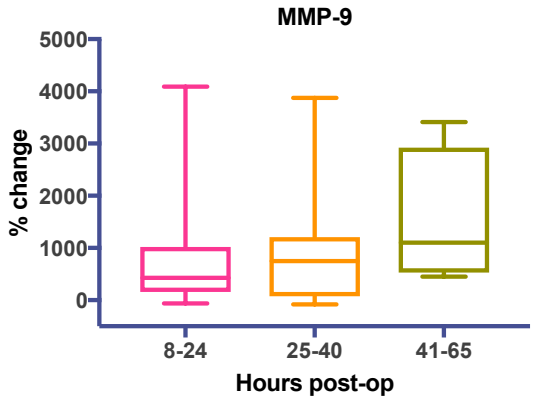

Figure 5.28; percentage change in MMP-9 in post-operative drain samples. Line (median), box (IQR), whiskers (range).

5.4.4 Other analytes

None of the remaining markers showed significant changes post-operatively, but the patient numbers are also small. Perhaps more informative are the patterns in mean percentage-change post-operatively (Figure 5.29). VEGF and IP-10 were the only markers to show a majority decrease in concentration at 8-24 hrs, whilst all other markers showed a majority increase at the earliest time point. In some cases, this was extremely large, such as the MIP-1β, where the mean increase was 4187%. This contradicts the hypothesis of surgery washing out and diluting all the inflammatory markers, and instead surgery appears to stimulate production of most markers. Several of the key markers such as VEGF, IL-6 and IL-8 increase up to 25-40 hrs and then start to show a decline, although concentrations are still higher than the intra-operative values at 41-65 hrs for all markers except VEGF. Most of the remaining markers continue to increase up to 41-65 hrs, which may mean that they take longer to decline post-operatively or that it is the change in balance of pro- and anti-inflammatory markers, rather than the absolute values, which switch the environment from progressive inflammatory CSDH expansion to repair and CSDH resolution.

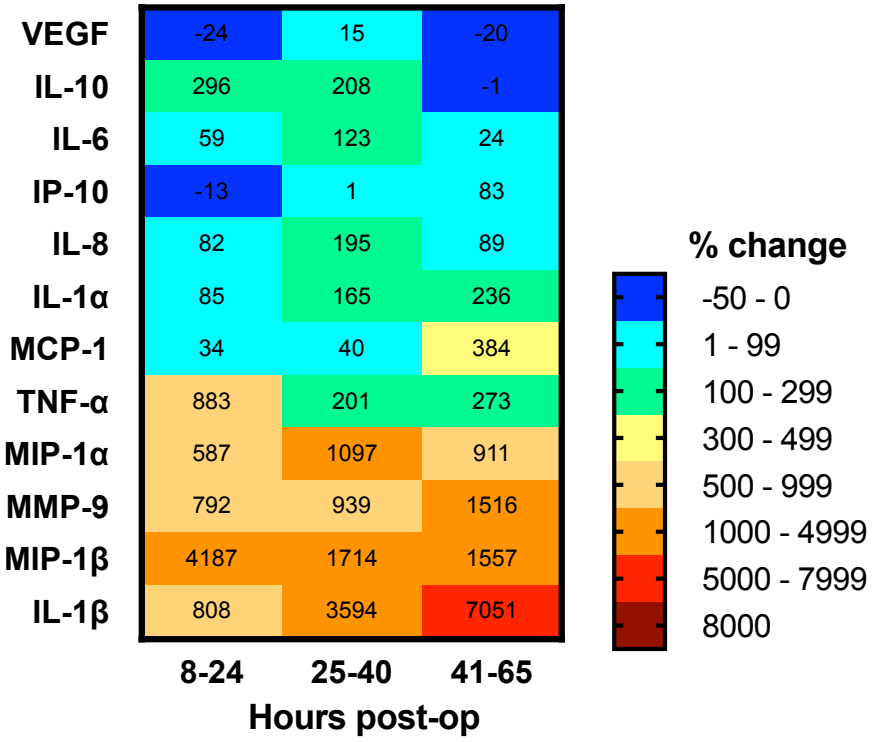

Figure 5.29; mean analyte percentage changes in post-operative drain samples.

5.4.5 Post-operative drain samples and recurrence

One might expect higher post-operative levels of inflammatory markers in patients that go on to develop a recurrence. However, a significant difference between recurrent (n=4) and non-recurrent (n=22) primary post-operative samples at 8-24 hrs was seen for only one marker, MCP-1 (Unpaired t-test, p = 0.0092) (Figure 5.30). Only one patient who went on to have a recurrence was sampled after 24 hours, therefore no further comparisons could be made at the later time-points.

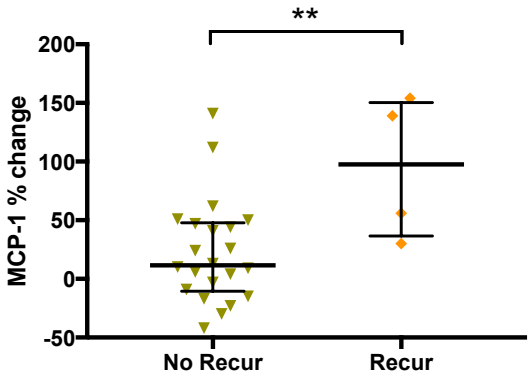

Figure 5.30; percentage change in MCP-1 in post-operative drain samples at 8-24 hrs in primary CSDHs that lead to recurrence (n=4) or no recurrence (n=22). Line (median), box (IQR), whiskers (range), statistically significant differences denoted as P ≤ 0.005 (**), (Recur = recurrence).

5.5 Conclusions

A panel of cytokines and chemokines was selected for assessment in CSDH pathophysiology. The selection was based on previous evidence of molecules that are likely to contribute to CSDH or have been shown to be involved in neuro-inflammatory processes in other types of head injury. The aim was to determine which factors are most significant in CSDH pathophysiology and recurrence, and thus could be potential targets for treatments such as dexamethasone. Only IL-10 was included as an anti-inflammatory marker, due to previous evidence of its role in predicting lower recurrence (Wada et al., 2006). MMP-9 has been shown to act in both pro- and anti-inflammatory roles but also has a key role in angiogenesis **(Manicone & McGuire, 2008)**, a process essential to CSDH membrane formation.

Limitations of work

As only approximately 10% of CSDHs recur it is challenging to obtain sufficient patient numbers to make meaningful comparisons between recurrent and non-recurrent CSDH. CSDH collections can also be loculated and poorly mixed, meaning that the region of sampling might influence to some degree the inflammatory response observed, as seen in the case with anterior and posterior samples. Multi-regional sampling might help overcome this in future studies.

Summary of findings

Analysis of the intra-operative CSDH fluid led to findings agreeing with previous literature that VEGF, IL-6, IL-8, IL-10, IP-10, MCP-1 and IL-1β were significantly raised compared to plasma. Novel data showed that IL-1α, MIP-1α and MIP-1β were also raised, implying they contribute to the inflammatory cascade in CSDH. TNF-α levels were also found to be significantly higher in CSDH, whereas previous literature has suggested they are not raised (Pripp & Stanisic, 2014; Stanisic, Aasen, et al., 2012).

MMP-9 was the only marker with overall significantly higher concentrations in plasma than CSDH, although this was not the case in all patients. This contradicts previous findings in the literature where consistently significantly higher levels of MMP-9 were found in CSDH compared to serum (Hua et al., 2016). Positive MMP-9 staining has also been displayed in CSDH outer membranes, suggesting it has a key role in CSDH pathophysiology (Nakagawa, Kodera, & Kubota, 2000). The percentage of patients with higher MMP-9 in CSDH

compared to plasma was greater in recurrent CSDHs (57%) compared to primary CSDH (20%). There were also huge increases in MMP-9 post-operatively for most patients (as high as 4000% increase), which continued to increase up to 65 hrs post-operatively. This has led to a new hypothesis that MMP-9 is important in the early stages of CSDH membrane development and angiogenesis and may have decreased (below plasma levels) by the time of primary surgery in most patients. However, surgery appears to "re-activate" MMP-9 which may be an important cause of further membrane/vessel development and subsequent recurrence.

Higher concentrations of several other markers were also linked to recurrence; VEGF, IL-6, IL-8, IL-1α, IL-1β, MIP-1β, TNF-α, giving credence to the theory that on-going, uncontrolled inflammation drives CSDH recurrence. Indeed, post-operative drain samples suggest that rather than "washing" out these markers, most are increased by the stimulus of surgery. Unfortunately, the concentrations of most analytes at the time of primary CSDH surgery, and post-operatively, do not appear to be *predictive* of recurrence. Only a decreased level of IL-10 appeared to relate to a higher risk of recurrence, and therefore might be valuable for predicting high-risk patients.

This is the first time that congruity in inflammatory response between sides of bilateral CSDHs has been shown, and is surprising since each side is often assumed to be an entirely independent collection. Either the inflammatory response from the body is co-ordinated in such a way that the response is the same on both sides, or possibly the fluid communicates across membranes and through CSF pathways, translating the inflammatory response from one side to the other. There was one clear outlier with highly different levels of IL-6, IL-8 and IL-10 between sides, and there are often clinical cases where only one side of a bilateral CSDH needs treating, whilst the other is small and dormant, therefore clearly situations exist where the inflammatory response is not equivalent bilaterally. This could potentially relate to CSDHs which are more encapsulated or membranous.

In summary, the hypotheses that VEGF would be the most significant marker, and that this, and other markers, would be further elevated in recurrent CSDH was confirmed. However, the hypothesis that surgery would result in significant dilution of markers was not confirmed, with all markers showing some elevation at one or more time-points post-operatively. VEGF

was the marker with the greatest overall decrease, although there was still a peak at 25-40 hrs. Several of the other markers remained significantly elevated throughout the post-operative period. This may indicate that the early post-operative period is an important target time for anti-inflammatory therapies such as dexamethasone, as there is actually an on-going and in some cases elevated inflammatory response following surgery. Dampening down this response may help to prevent an on-going tide of new inflammatory marker activation which leads to recurrence. This will be investigated further in the next chapter.

5.6 References

Apte, R. N., & Voronov, E. (2008). Is interleukin-1 a good or bad 'guy' in tumor immunobiology and immunotherapy? *Immunol Rev, 222*, 222-241. doi:10.1111/j.1600-065X.2008.00615.x

Dahl, J., Ormstad, H., Aass, H. C., Malt, U. F., Bendz, L. T., Sandvik, L., . . . Andreassen, O. A. (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology, 45*, 77-86. doi:10.1016/j.psyneuen.2014.03.019

Di Iorio, A. (2003). Serum IL-1 β levels in health and disease: a population-based study. 'The InCHIANTI study'. *Cytokine, 22*(6), 198-205. doi:10.1016/s1043-4666(03)00152-2

Di Paolo, N. C., & Shayakhmetov, D. M. (2016). Interleukin 1alpha and the inflammatory process. *Nat Immunol, 17*(8), 906-913. doi:10.1038/ni.3503

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctot, K. L. (2010). A meta-analysis of cytokines in major depression. *Biol Psychiatry, 67*(5), 446-457. doi:10.1016/j.biopsych.2009.09.033

Frati, A., Salvati, M., Mainiero, F., Ippoliti, F., Rocchi, G., Raco, A., . . . Delfini, R. (2004). Inflammation markers and risk factors for recurrence in 35 patients with a posttraumatic chronic subdural hematoma: a prospective study. *J Neurosurg, 100*(1), 24-32. doi:10.3171/jns.2004.100.1.0024

Herisson, F., Frodermann, V., Courties, G., Rohde, D., Sun, Y., Vandoorne, K., . . . Nahrendorf, M. (2018). Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. *Nat Neurosci, 21*(9), 1209-1217. doi:10.1038/s41593-018-0213-2

Hohenstein, A., Erber, R., Schilling, L., & Weigel, R. (2005). Increased mRNA expression of VEGF within the hematoma and imbalance of angiopoietin-1 and -2 mRNA within the neomembranes of chronic subdural hematoma. *J Neurotrauma, 22*(5), 518-528. doi:10.1089/neu.2005.22.518

Hong, H. J., Kim, Y. J., Yi, H. J., Ko, Y., Oh, S. J., & Kim, J. M. (2009). Role of angiogenic growth factors and inflammatory cytokine on recurrence of chronic subdural hematoma. *Surg Neurol, 71*(2), 161-165; discussion 165-166. doi:10.1016/j.surneu.2008.01.023

Hua, C., Zhao, G., Feng, Y., Yuan, H., Song, H., & Bie, L. (2016). Role of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, and Vascular Endothelial Growth Factor in the Development of Chronic Subdural Hematoma. *J Neurotrauma, 33*(1), 65-70. doi:10.1089/neu.2014.3724

Jonsson, A., Hjalmarsson, C., Falk, P., & Ivarsson, M. L. (2016). Levels of matrix metalloproteinases differ in plasma and serum - aspects regarding analysis of biological markers in cancer. *Br J Cancer, 115*(6), 703-706. doi:10.1038/bjc.2016.127

Kishimoto, T., Akira, S., & Taga, T. (1992). Interleukin-6 and its receptor: a paradigm for cytokines. *Science, 258*(5082), 593-597.

Kleiner, G., Marcuzzi, A., Zanin, V., Monasta, L., & Zauli, G. (2013). Cytokine levels in the serum of healthy subjects. *Mediators Inflamm, 2013*, 434010. doi:10.1155/2013/434010

Kusumanto, Y. H., Meijer, C., Dam, W., Mulder, N. H., & Hospers, G. A. (2007). Circulating vascular endothelial growth factor (VEGF) levels in advanced stage cancer patients compared to normal controls and diabetes mellitus patients with critical ischemia. *Drug Target Insights, 2*, 105-109.

Kut, C., Mac Gabhann, F., & Popel, A. S. (2007). Where is VEGF in the body? A meta-analysis of VEGF distribution in cancer. *Br J Cancer, 97*(7), 978-985. doi:10.1038/sj.bjc.6603923

Li, T., Jing, J. J., Yang, J., Sun, L. P., Gong, Y. H., Xin, S. J., & Yuan, Y. (2018). Serum levels of matrix metalloproteinase 9 and toll-like receptor 4 in acute aortic dissection: a case-control study. *BMC Cardiovasc Disord, 18*(1), 219. doi:10.1186/s12872-018-0958-2

Lip, P. L., Belgore, F., Blann, A. D., Hope-Ross, M. W., Gibson, J. M., & Lip, G. Y. (2000). Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. *Invest Ophthalmol Vis Sci, 41*(8), 2115-2119.

Malecaze, F., Clamens, S., Simorre-Pinatel, V., Mathis, A., Chollet, P., Favard, C., . . . Plouet, J. (1994). Detection of vascular endothelial growth factor messenger RNA and vascular endothelial growth factor-like activity in proliferative diabetic retinopathy. *Arch Ophthalmol, 112*(11), 1476-1482.

Manicone, A., & McGuire, J. (2008). Matrix metalloproteinases as modulators of inflammation. *Semin Cell Dev Biol, 19*(1), 34-41. doi:10.1016/j.semcdb.2007.07.003

Nakagawa, T., Kodera, T., & Kubota, T. (2000). Expression of matrix metalloproteinases in the chronic subdural haematoma membrane. *Acta Neurochir (Wien), 142*(1), 61-66.

Nanko, N., Tanikawa, M., Mase, M., Fujita, M., Tateyama, H., Miyati, T., & Yamada, K. (2009). Involvement of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in the mechanism of development of chronic subdural hematoma. *Neurol Med Chir (Tokyo), 49*(9), 379-385.

Pripp, A. H., & Stanisic, M. (2014). The correlation between pro- and anti-inflammatory cytokines in chronic subdural hematoma patients assessed with factor analysis. *PLoS One, 9*(2), e90149. doi:10.1371/journal.pone.0090149

Salvi, M., Pedrazzoni, M., Girasole, G., Giuliani, N., Minelli, R., Wall, J. R., & Roti, E. (2000). Serum concentrations of proinflammatory cytokines in Graves' disease: effect of treatment, thyroid function, ophthalmopathy and cigarette smoking. *Eur J Endocrinol, 143*(2), 197-202.

Shoab, S. S., Scurr, J. H., & Coleridge-Smith, P. D. (1998). Increased plasma vascular endothelial growth factor among patients with chronic venous disease. *J Vasc Surg, 28*(3), 535-540.

Shono, T., Inamura, T., Morioka, T., Matsumoto, K., Suzuki, S. O., Ikezaki, K., . . . Fukui, M. (2001). Vascular endothelial growth factor in chronic subdural haematomas. *J Clin Neurosci, 8*(5), 411-415. doi:10.1054/jocn.2000.0951

Stanisic, M., Aasen, A. O., Pripp, A. H., Lindegaard, K. F., Ramm-Pettersen, J., Lyngstadaas, S. P., . . . Saehle, T. (2012). Local and systemic pro-inflammatory and anti-inflammatory cytokine patterns in patients with chronic subdural hematoma: a prospective study. *Inflamm Res, 61*(8), 845-852. doi:10.1007/s00011-012-0476-0

Stanisic, M., Lyngstadaas, S. P., Pripp, A. H., Aasen, A. O., Lindegaard, K. F., Ivanovic, J., . . . Saehle, T. (2012). Chemokines as markers of local inflammation and angiogenesis in patients with chronic subdural hematoma: a prospective study. *Acta Neurochir (Wien), 154*(1), 113-120; discussion 120. doi:10.1007/s00701-011-1203-2

van Breemen, M. J., de Fost, M., Voerman, J. S., Laman, J. D., Boot, R. G., Maas, M., . . . Rezaee, F. (2007). Increased plasma macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels in type 1 Gaucher disease. *Biochim Biophys Acta, 1772*(7), 788-796. doi:10.1016/j.bbadis.2007.04.002

Wada, T., Kuroda, K., Yoshida, Y., Ogasawara, K., Ogawa, A., & Endo, S. (2006). Local elevation of the anti-inflammatory interleukin-10 in the pathogenesis of chronic subdural hematoma. *Neurosurg Rev, 29*(3), 242-245. doi:10.1007/s10143-006-0019-7

Weigel, R., Schilling, L., & Schmiedek, P. (2001). Specific pattern of growth factor distribution in chronic subdural hematoma (CSH): evidence for an angiogenic disease. *Acta Neurochir (Wien), 143*(8), 811-818; discussion 819.

Chapter 6; Dexamethasone and clinical correlations of inflammation

6.1 Introduction

Firstly, this chapter will explore the hypothesis that dexamethasone aids resolution of CSDH through reducing inflammation within the subdural space. This will be investigated by comparing concentrations of the inflammatory markers discussed in chapter five in the intra-operative and post-operative CSDH fluid samples from both placebo and dexamethasone-treated patients. It is hypothesised that dexamethasone-treated patients will have lower concentrations of all inflammatory markers compared with placebo patients; particularly VEGF, the marker present in highest concentrations in CSDH. It is also anticipated that the concentration of inflammatory markers will be lower in the post-operative than the intra-operative samples, due to the cumulative effect of dexamethasone dosing over time. Likewise, patients who have received higher doses of dexamethasone pre-operatively are expected to have lower concentrations of inflammatory markers than those with lower cumulative pre-operative dosing.

The second main hypothesis of this chapter is that the level of inflammatory response within a CSDH correlates to both the time since traumatic injury and patient outcome. If the inflammatory response in CSDH continues to escalate over time, as is suspected, then those patients with the longest time delay from trauma to surgery should have the highest levels of inflammation. Similarly, those patients with higher levels of inflammation present are more likely to have a poorer outcome, as measure by GCS on discharge and mRS at three and six months. Further clinical data which may be relevant to the inflammatory response, such as patient age and prior anti-platelet or anti-coagulant use are also investigated.

6.2 Methods

All samples were collected as per the method reported in chapter five and the same 68 samples from 52 patients are reviewed in this chapter. All patients recruited to the neurochemistry sub-study had been previously randomised to the study IMP (dexamethasone or placebo), and this allocation was blinded throughout the collection and analysis of all neurochemistry samples. Unblinding for final results analysis only occurred once all patients had completed their final study follow-up at six months, and this information was restricted to the neurochemistry team.

At the time of inclusion in the neurochemistry study, details on how much trial medication was taken pre-operatively and post-operatively, during drainage sample collection, was recorded. Relevant demographic and clinical data was also collected on all patients. There was no significant difference between the treatment groups for time from randomisation to operation, with most patients operated on the same or following day.

Of the 52 patients included in the neurochemistry study, 22 received dexamethasone and 30 received placebo. Only 11 of the dexamethasone patients had received any IMP prior to surgery, with the remaining 11 patients starting it post-operatively (Table 6.1). Those patients who had not received any pre-operative IMP were grouped with the placebo patients into a "no dexamethasone" category for the intra-operative fluid analysis.

Table 6.1; pre-operative doses of dexamethasone and recurrence

No. of patients	Dexamethasone dose pre-op	Recurrence
11	0 mg	1 (Day 41 + 50)
2	8 mg	0
2	16 mg	0
1	32 mg	1 (Day 14)
1	40 mg	0
1	48 mg	0
1	54 mg	0
1	60 mg	0
1	72 mg	0
1	124 mg (full course)	0

There were two recurrences in the 22 patients who were randomised to dexamethasone (9%), only one of which was sampled at the time of recurrence (the other only had sampling of the primary CSDH). There were four recurrences in the 30 patients on placebo (13%), all of which were sampled. One of the recurrence patients who was on dexamethasone, received no drug pre-operatively and stopped the course early on day seven (receiving a total of 92 mg). This patient was treated for recurrent CSDH 41 days after primary surgery, with a second recurrence a further nine days later. The second patient on dexamethasone with recurrence received two full days of pre-operative dexamethasone (total 32 mg) and was treated for recurrence 14 days after primary surgery, having completed the full dexamethasone course (a total of 124 mg). Of the four recurrence patients treated with placebo; one occurred eight days after primary surgery, one at 11 days, one at 13 days and one at 14 days. Therefore, the median time to recurrence was 14 days (mean 18.6 days) in all patients, but was later in the dexamethasone group due to the recurrence in one patient on day 41.

A one-off intravenous dose of 6.6 mg intra-operative dexamethasone (IOD) was allowed in trial patients, as this was routine clinical practice before the trial commenced, for anti-emesis as part of the anaesthetic. There were eight patients who were given IOD; four from the dexamethasone treatment arm (two with bilateral CSDH, making a total of six samples) and four from the placebo treatment arm (one with bilateral CSDH, making a total of five samples). The potential impact of IOD on the inflammatory profiles of intra-operative CDSH fluid are reviewed.

6.3 Dexamethasone and the inflammatory profile results

6.3.1 Intra-operative samples

All primary CSDH samples were grouped into "no dexamethasone" (ND, n=49), where the patient was either on placebo or received no dexamethasone pre-operatively, and dexamethasone (Dex, n=12), where they received pre-operative dexamethasone. It was anticipated that dexamethasone worked as a treatment for CSDH by reducing the concentration of key inflammatory markers, particularly those previously identified as higher in recurrent CSDH (i.e. VEGF, IL-6, IL-8, MIP-1β, TNF-α, IL-1α, IL-1β). However, the opposite trend was seen for all these markers, with significantly higher levels in the dexamethasone treated group for IL-6 ($p = 0.0107$), IL-8 ($p = 0.0041$), MIP-1β ($p = 0.0147$), TNF-α ($p = 0.0217$) and IL-1α ($p = 0.0274$) and a non-significant trend to higher levels for VEGF ($p = 0.2954$) (Figure 6.1). Smaller, non-significant trends were seen for IL-10, MIP-1α and IP-10 and no differences were seen for MCP-1, IL-1β and MMP-9 (data not shown).

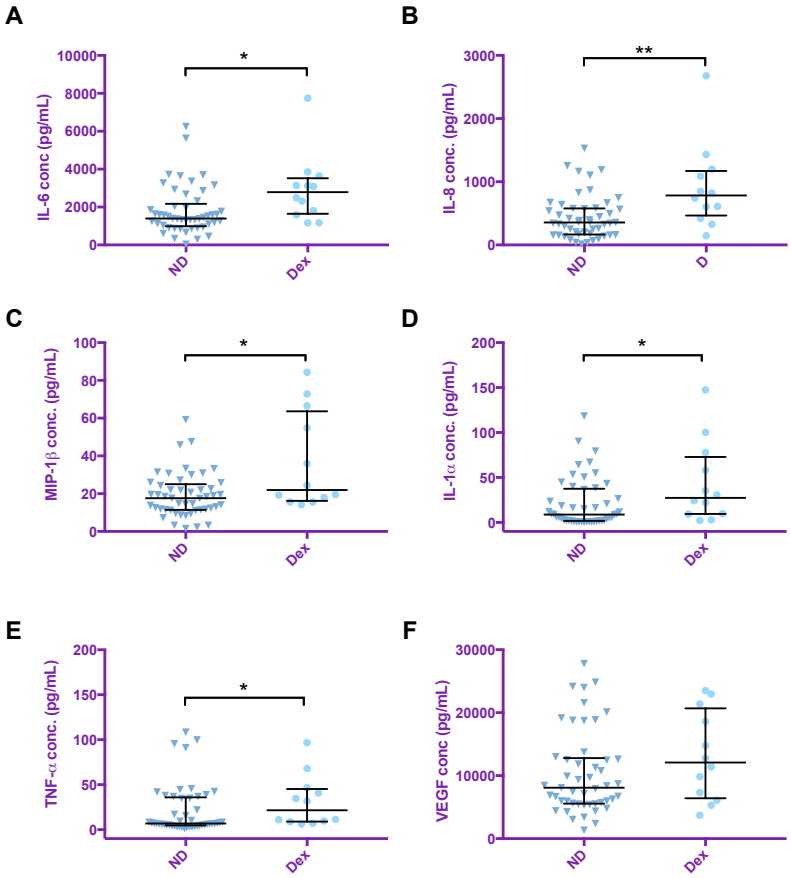

Figure 6.1; comparison inflammatory profiles in no-dexamethasone (ND) and dexamethasone (D) samples: **(A)** IL-6, **(B)** IL-8, **(C)** MIP-1β, **(D)** IL-1α, **(E)** TNF-α, **(F)** VEGF. ND = 49, Dex = 12. Line (median), bars (IQR), statistically significant differences denoted as $P \leq 0.05$ (*), $P \leq 0.005$ (**).

Further analysis of the dexamethasone group, by total cumulative dose received pre-operatively, was performed (Figure 6.2). One patient, who received 124 mg (the complete course) pre-operatively, was excluded, as the surgery was performed 44 days after completion of the course. This is a sufficient time-lag for any anti-inflammatory action of the dexamethasone to have resolved and the inflammatory profile to change significantly.

VEGF, MMP-9 and to a lesser extent IL-6, all showed a continued rise in concentration with increasing doses of dexamethasone (Figure 6.2A-C). All other analytes generally showed an increase in concentration up to 48 mg dexamethasone, but a decrease when the maximum pre-operative dose of 54-72 mg had been given (equivalent to 4 or 5 days of treatment). This was most apparent for IP-10, TNF-α, IL-1α and IL-1β (Figure 6.2H-K). This may suggest that the effect of dexamethasone in reducing inflammation in CSDH only becomes apparent at higher (>48 mg) or longer (>3 days) dosing. It may be that the doses given were insufficient to see a change for the key markers such as VEGF and IL-6, as these were also the markers at the highest concentrations within the fluid. Alternatively it is possible that the markers only appear higher in concentration because there is less fluid, and that the dexamethasone is working to reduce fluid production resulting in less dilution of markers. Further work is needed sampling CSDH fluid in patients who have received longer course of dexamethasone to further clarify this.

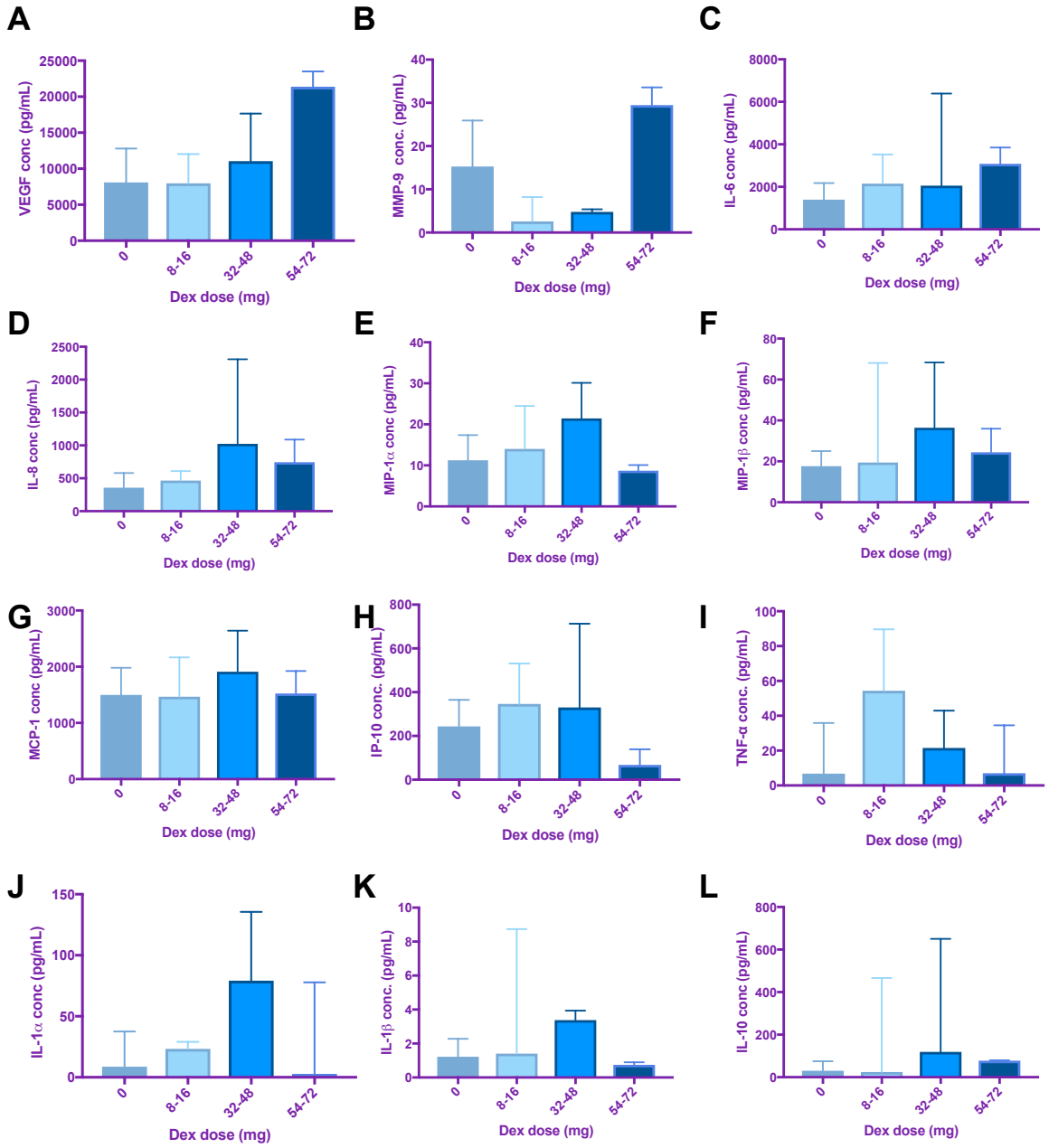

Figure 6.2; analyte concentrations in relation to cumulative pre-operative dexamethasone dose: **(A)** VEGF, **(B)** MMP-9, **(C)** IL-6, **(D)** IL-8, **(E)** MIP-1α, **(F)** MIP-1β, **(G)** MCP-1, **(H)** IP-10, **(I)** TNF-α, **(J)** IL-1α, **(K)** IL-1β, **(L)** IL-10. Dexamethasone doses; 0 mg (n = 49), 8-16 mg (n = 4), 32-48 mg (n = 4), 54-72 mg (n = 3). Bar (median), line (IQR), (Dex = dexamethasone).

Intra-operative dexamethasone

Including only the "no-dexamethasone" patients, those given 6.6 mg of IOD were compared with the those given none (ND). IL-1α was the only analyte to show significantly higher concentrations in the IOD compared to ND group, but with such a wide range this is likely due to chance (Figure 6.3A). The overwhelming trend with all other markers is that IOD has

no effect on the analyte concentration (Figure 6.3B-D), which is as expected with a small, one-off dose of dexamethasone given immediately prior to surgery.

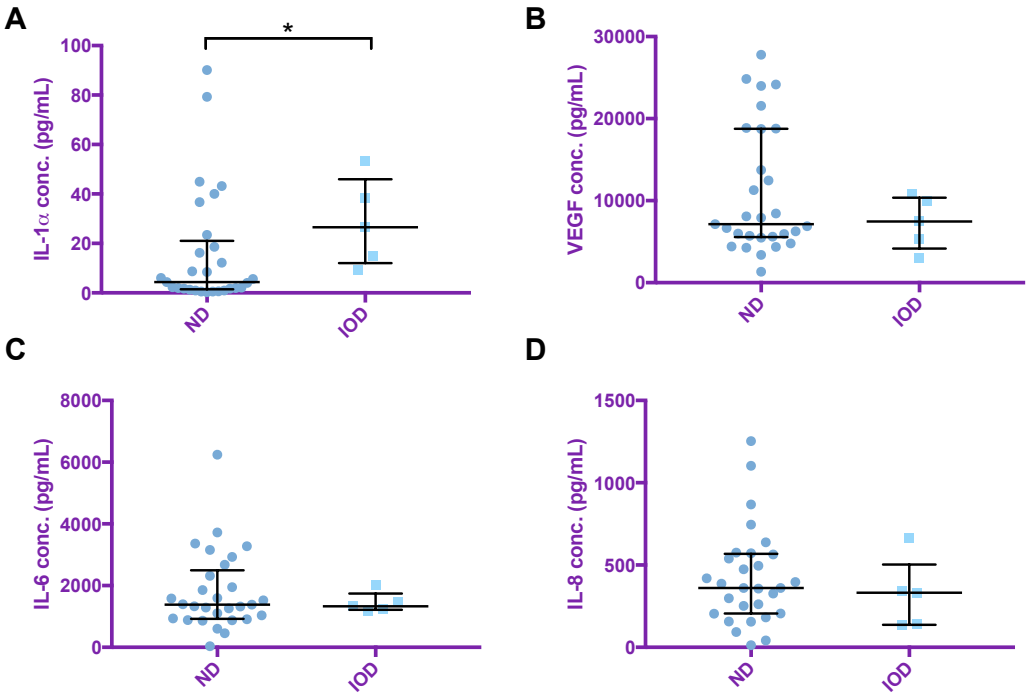

Figure 6.3; placebo patients given intra-operative dexamethasone (IOD) and no dexamethasone (ND): **(A)** IL-1α, **(B)** VEGF, **(C)** IL-6, **(D)** IL-8**.** ND (n = 29), IOD (n= 5). Line (median), bar (IQR), statistically significant difference denoted as $P \leq 0.05$ (*).

6.3.2 Post-operative drain samples

Dexamethasone may be more active post-operatively once the large collection of inflammatory cells and fluid has been drained. This is supported by the data on post-operative drain samples for the majority of analytes. In the placebo samples, there is usually a peak in the inflammatory markers at 25-40 hours, this is significantly reduced in the dexamethasone group for VEGF ($p = 0.0095$), and shows a non-significant trend in reduction for IL-6, IL-8, IL-10, IL-1α, IL-1β, MIP-1α and MIP-1β (Figure 6.4). There are also reduced percentage increases in these inflammatory markers across all the time points, which gives an overall picture of a "dampening down" of the inflammatory response post-operatively. It may be this effect which is essential to promoting CSDH resolution, rather than the intra-operative changes discussed above. No significant differences or patterns were observed for MMP-9, MCP-1, TNF-α, IP-10 in relation to dexamethasone post-operatively (data not shown).

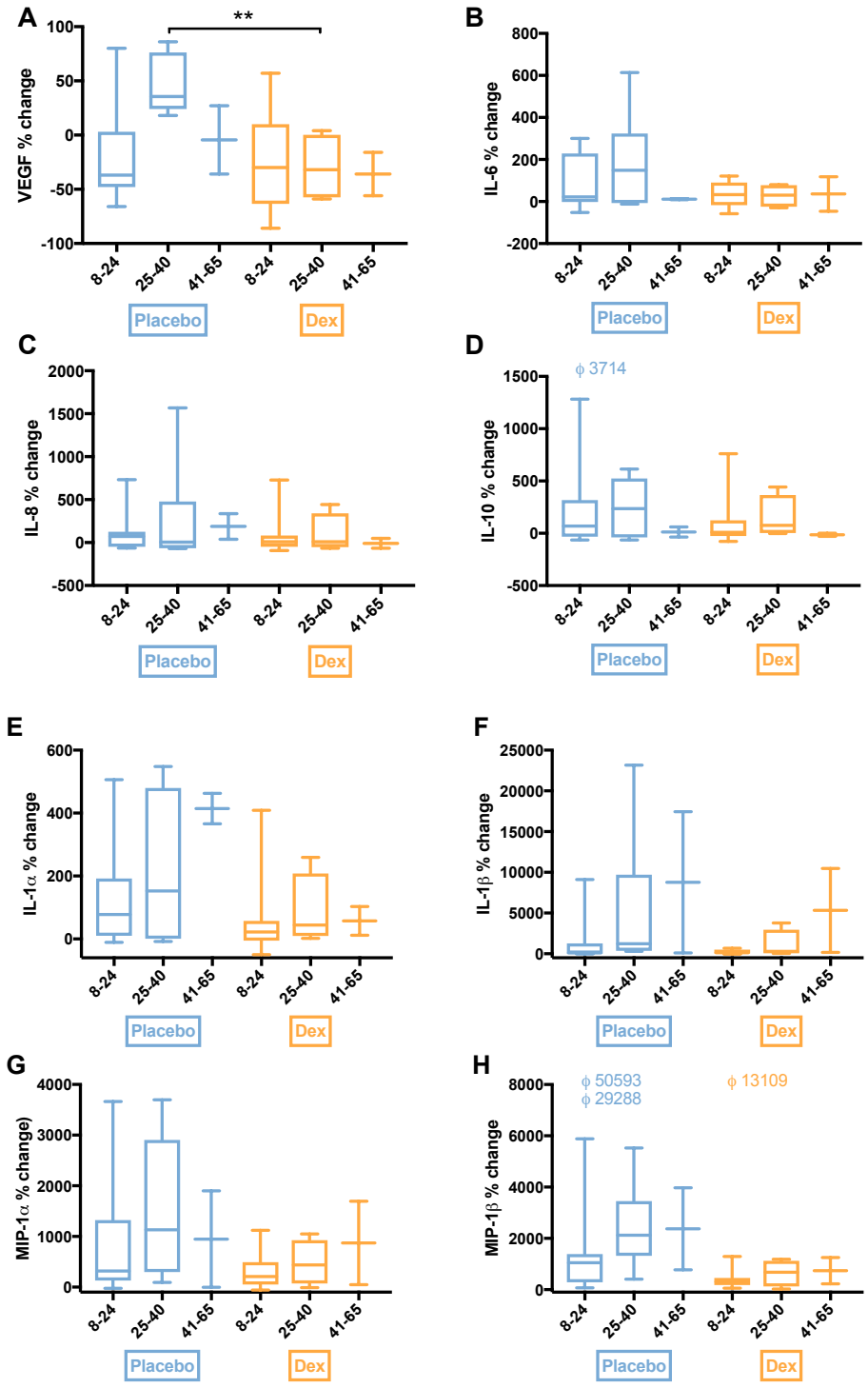

Figure 6.4; percentage-change of analyte concentrations in post-operative drain samples by treatment group: **(A)** VEGF, **(B)** IL-6, **(C)** IL-8, **(D)** IL-10, **(E)** IL-1α, **(F)** IL-1β, **(G)** MIP-1α, **(H)** MIP-1β. Line (mean), box (IQR), whiskers (range), statistically significant differences denoted as $P \leq 0.005$ (**), ϕ = outlier, Dex = dexamethasone.

6.3.3 Paired primary and recurrence samples

The paired primary and recurrence samples were compared between the treatment groups. There were two paired samples in the dexamethasone arm; from the same patient who had two recurrences, both of which were paired to the primary CSDH sample. This patient had also not received any dexamethasone prior to the primary surgery and the recurrences were late (day 41 and 50). There were four paired samples from three patients (one bilateral CSDH) in the placebo arm. Due to these small patient numbers no statistical significance is assessed, but the patterns are shown below.

VEGF, IL-6, IL-10 and IL-1β showed relatively stable concentrations between primary and recurrence samples in the dexamethasone patient compared to increases in most of the placebo samples at recurrence (Figure 6.5).

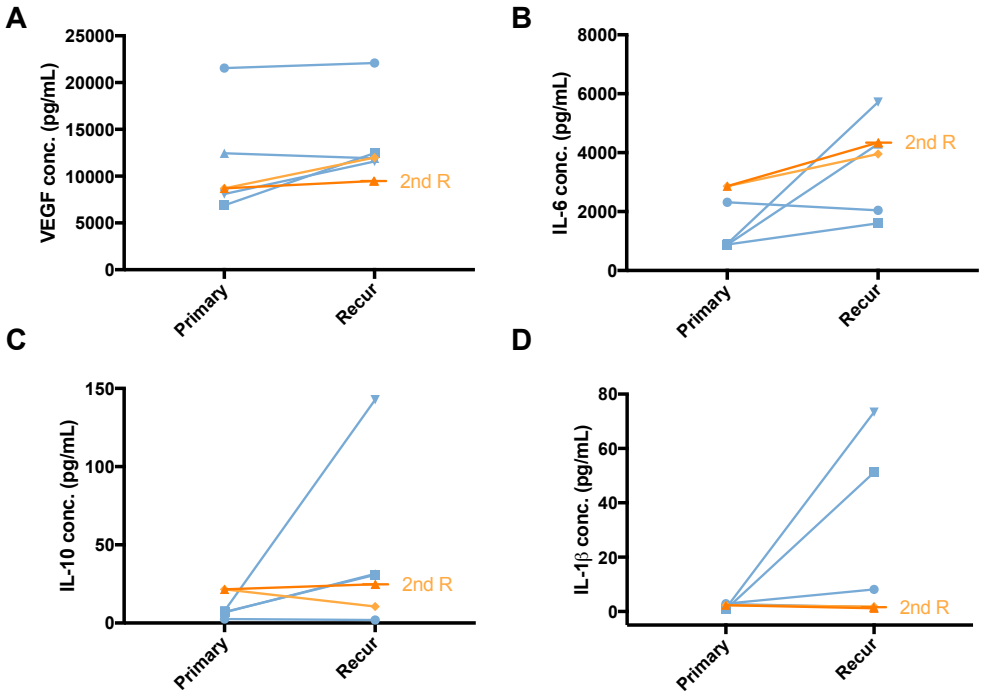

Figure 6.5; paired primary and recurrence samples: **(A)** VEGF, **(B)** IL-6, **(C)** IL-10, **(D)** IL-1β. Placebo (blue, N = 4), dexamethasone (orange, N = 2), (2nd R = second recurrence, Recur = recurrence).

IL-1α and TNF-α showed increases in concentration between primary and recurrence samples in the dexamethasone patient and increase or stability in the placebo samples (Figure 6.6A-B). MCP-1, IP-10, MIP-1α, MIP-1β and IL-8 all showed increases in first recurrence

but decreased or stable levels in the second recurrence in the dexamethasone patient, whilst placebo patients increased in most apart from with MCP-1 (Figure 6.6B-G). MMP-9 was the only analyte to show a marked decrease in both dexamethasone recurrences, with varied responses in the placebo patients (Figure 6.6H).

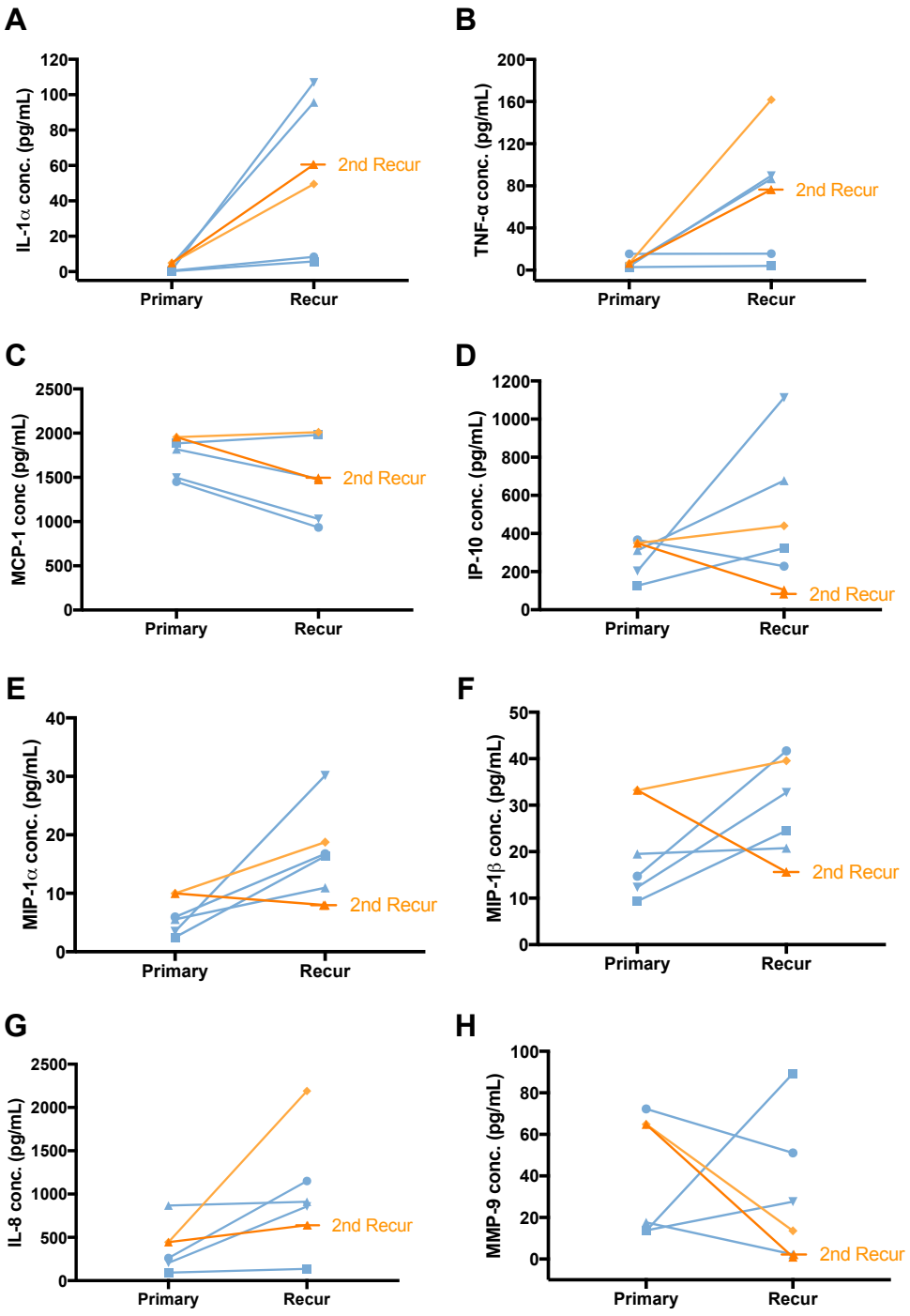

Figure 6.6; paired primary and recurrence samples: **(A)** IL-1α, **(B)** TNF-α, **(C)** MCP-1, **(D)** IP-10, **(E)** MIP-1α, **(F)** MIP-1β, **(G)** IL-8, **(H)** MMP-9. Placebo (blue, N = 4), dexamethasone (orange, N = 2), (Recur = recurrence).

The change of each analyte from primary to recurrent CSDH is very different in each patient, regardless of treatment group. Therefore, it is difficult to identify any clear trends. This is exemplified by comparing the changes in IL-6 and IL-8, which are considered to work synergistically and concentrations normally correlate well to one another (see chapter five), but show very different patterns from primary to recurrent CSDH (Figure 6.7). In one patient (P1) there was a large increase in IL-8 at recurrence, but a simultaneous decrease in IL-6, whilst others (P3-R) showed the opposite trend.

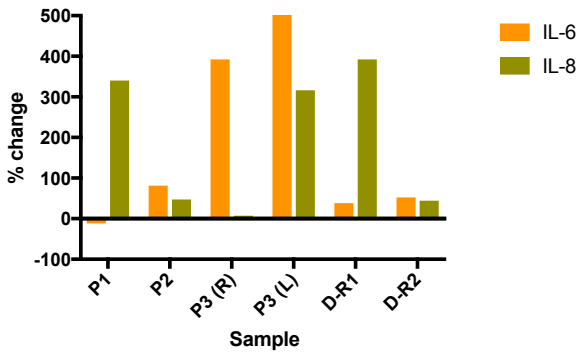

Figure 6.7; percentage change between paired primary and recurrent CSDHs in placebo (P) and dexamethasone (D) treated samples, (L= left, R= right, R1 = 1st recurrence, R2 = 2nd recurrence).

6.4 Clinical correlations

6.4.1 Trauma and time course

Clinical data was available on all 52 CSDH patients, as reported in chapter five the mean age was 76 years and 27% were females. The inflammatory profiles for all markers were compared between primary CSDH samples from patients aged under 76 and those 76 and above, and no significant differences were found (data not shown). This suggests that age alone does not alter the inflammatory response.

The patient and/or next-of-kin were asked whether there had been a recent history of head trauma within the past 6 months and this was reported as present in 32/52 (62%) patients. Although more male patients reported no history of trauma, there was no significant difference between genders (Fishers exact, p = 0.1978) (Figure 6.8).

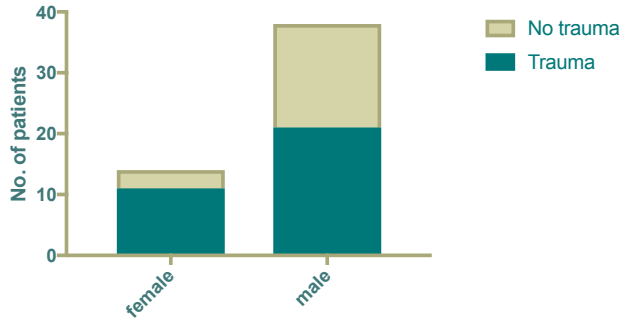

Figure 6.8; Gender and reported history of trauma, female n = 14, male n = 38.

The time interval between trauma and CSDH diagnosis was known for 28/32 trauma patients, with the shortest interval of two weeks, and maximum 12 weeks (Figure 6.9A). The mean time interval from trauma to CSDH was five weeks (median four weeks) and three time-periods were made to assist further analyses, dividing at the median of four weeks and with a later time point at >8 weeks, as a few patients had very long time delays (see Figure 6.9B).

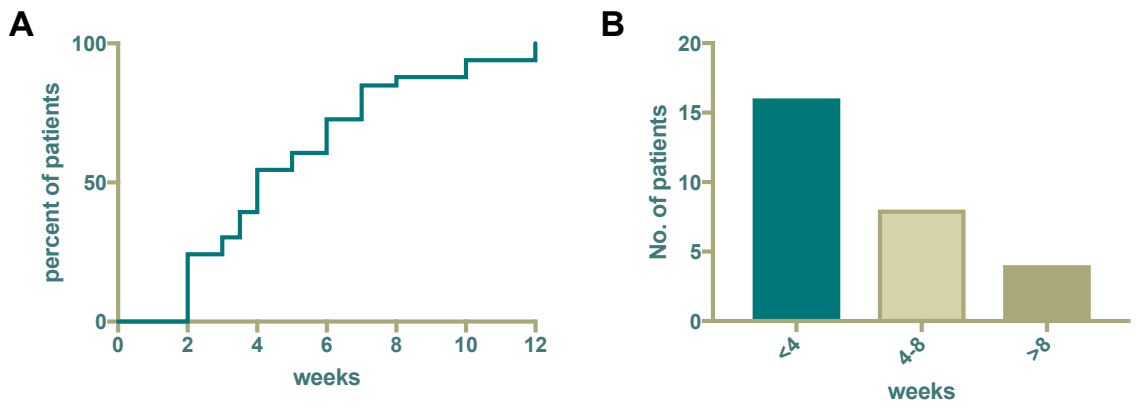

Figure 6.9; (A) survival curve of time from trauma to CSDH diagnosis (N = 28), **(B)** Time-groups from trauma to CSDH diagnosis, <4 weeks (n = 16), 4-8 weeks (n = 8) and after 8-weeks (n =4).

The 61 primary CSDH samples (counting each side of bilateral and anterior/posterior as individual CSDHs) were reviewed for each analyte, comparing those with a reported history of trauma (n = 36) and those without (n = 25). The only markers which showed a significant difference between these two groups were IL-1α (p = 0.0245) and TNF-α (p = 0.0263) (Figure 6.10). This suggests that the method of CSDH initiation (trauma or no trauma) makes little difference to the ensuing inflammatory reaction, as most of the key markers showed no differences. There is also possible bias from the fact that some patients cannot remember trauma despite it occurring.

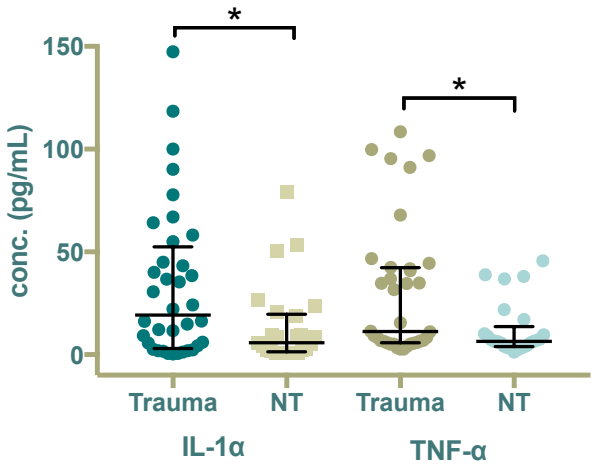

Figure 6.10; IL-1α and TNF-α in trauma and non-trauma patients. Trauma n = 36, Non-Trauma (NT) n = 25, statistically significant differences denoted as P ≤ 0.05 (*).

The time interval from trauma to CSDH diagnosis was known in 32/36 samples and grouped as per Figure 6.9B. A significant difference between the three time intervals was found for VEGF concentration (Kruskal-Wallis test, p = 0.0057), with a dip at 4-8weeks suggesting perhaps a more dormant period of inflammation with an early and late peak either side (Figure 6.11A). A increase across the three time points, with a much higher peak at the latest time point, was seen for IL-6, IL-8, IL-1α and MIP-1α (Figure 6.11B-E). This supports the hypothesis of escalating inflammation over time following trauma. Only MMP-9 followed a similar trend to VEGF, with a peak in CSDHs diagnosed <4 weeks after trauma. This suggests that these two inflammatory markers may be important in the early stages of CSDH development, with VEGF re-activated later as well. No trends or significant differences were seen for the remaining markers (data not shown).

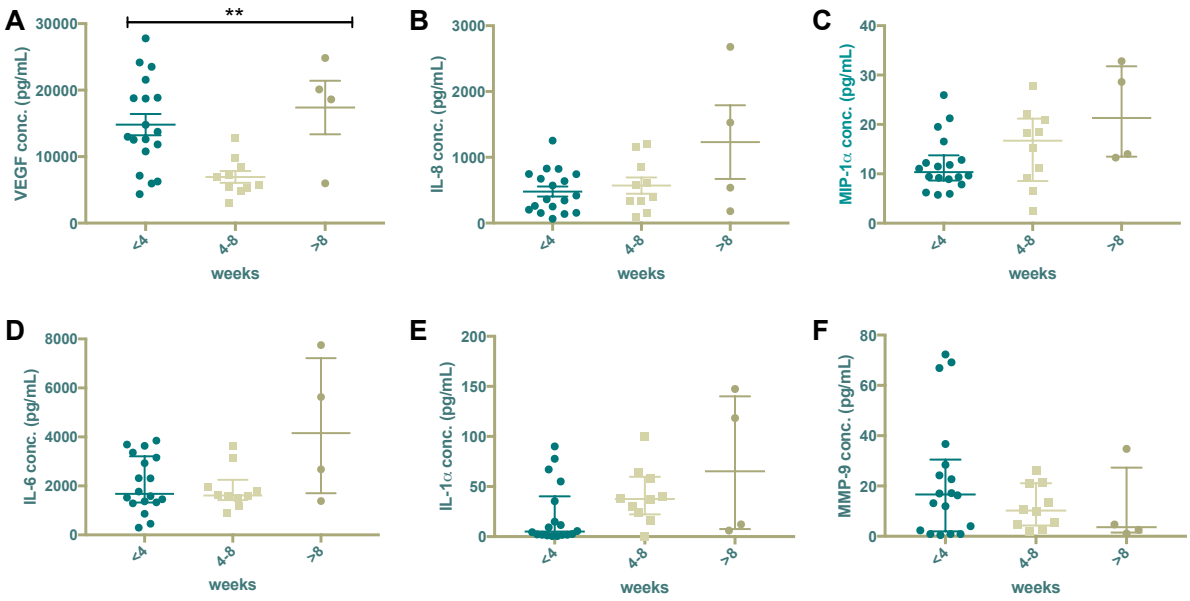

Figure 6.11; Analyte concentrations at time intervals post-trauma: **(A)** VEGF, **(B)** IL-8, **(C)** MIP-1α, **(D)** IL-6, **(E)** IL-1α, **(F)** MMP-9, <4 weeks (n = 18), 4-8 weeks (n = 10), >8-weeks (n =4). Line (median), bar (IQR), statistically significant differences denoted as P ≤ 0.005 (**).

The peak in VEGF in CSDHs diagnosed <4 weeks from trauma may also relate to the pathophysiological sub-type (as discussed in Chapter two). It has already been demonstrated that CSDHs transformed from acute subdural haematomas (acute transformed; CSDH-AT) present earlier following trauma than those that appear to form de-novo (CSDH-DN); with a mean of 16 days in the former versus 57 days in the latter. Thus, VEGF may be key in the period of early conversion and expansion from ASDH to CSDH-AT. When reviewing the 11

patients with imaging confirmation of CSDH-AT (n = 5) compared to CSDH-DN (n = 6), higher VEGF was seen in all but one outlying CSDH-AT patient (Figure 6.12). Excluding this outlier, a significant difference is seen between VEGF levels which are higher in CSDH-AT than CSDH-DN (p = 0.0121). No differences were seen between the sub-types for any other marker, including MMP-9 (data not shown).

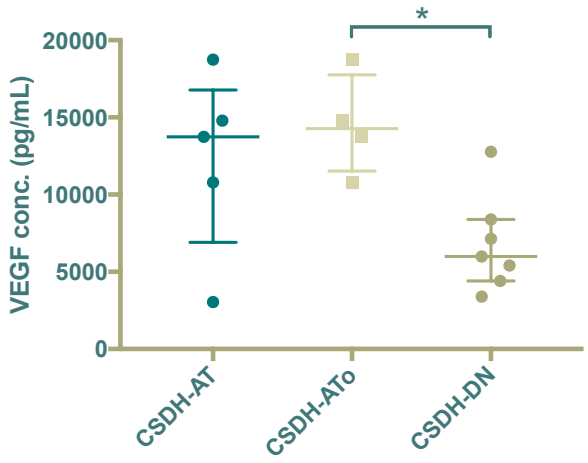

Figure 6.12; difference in VEGF between CSDH sub-types. Median (line), IQR (bars), (AT = acute transformed, CSDH = chronic subdural haematoma, DN = De Novo, o = outlier excluded).

6.4.2 Anti-platelet and anti-coagulant treatment

Patient data on anti-coagulants and anti-platelets is shown in Table 6.2. There were not enough patients on aspirin or clopidogrel to compare these medications, therefore they are grouped together under anti-platelets despite their different mechanisms of action. Whether the patient was on anti-platelets or anti-coagulants prior to CSDH surgery appeared to have no effect on the inflammatory profile with no significant differences between the two groups for any marker. An example of the equal distribution is shown with VEGF in Figure 6.13. This is perhaps surprising, as both aspirin and clopidogrel have been shown to have anti-inflammatory properties (Schrottmaier, Kral, Badrnya, & Assinger, 2015; Sternberg et al., 2016; Thomas & Storey, 2015; Vane & Botting, 1998). However, there were several confounding factors including variation in the duration of time from stopping treatment to surgery and whether any reversal therapies (e.g. platelets, vitamin K) were given. Unfortunately, detailed data on this was not available to allow comparison. It is certainly an area for future research to look at the profiles more carefully in patients who are naïve to any anti-platelet treatment compared with very recent exposure.

Table 6.2: anti-coagulant and anti-platelet data

Type of treatment	No. out of 61 primary samples (%)
None	26 (43%)
Anti-platelets	13 (21%)
Aspirin	8
Clopidogrel	4
Combination treatment	1
Anti-Coagulants	22 (36%)
Warfarin	17
Novel Oral Anti-coagulants	5

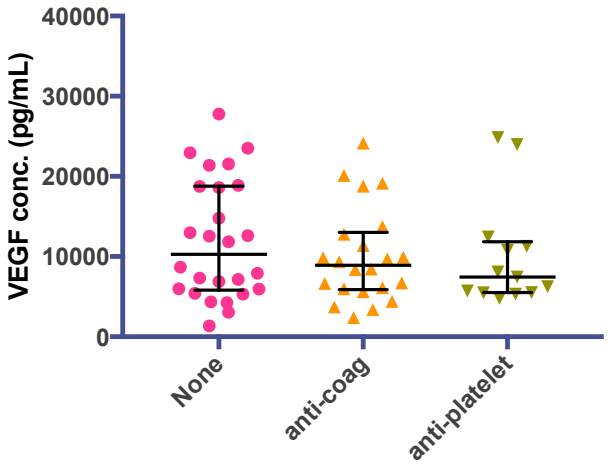

Figure 6.13; VEGF and anti-platelet and anti-coagulant (anti-coag) treatments, n as per Table 6.2, line (median), bars (IQR).

6.4.3 Admission and outcome data

The Glasgow Coma Scale (GCS) is a universal measure of the degree of coma. It is made up of three component parts assessing response by eyes, verbal and motor function which are added together to a total score ranging from 3 - 15 (Table 6.3).

Table 6.3; breakdown of GCS scoring (Teasdale)

Eye response	Verbal score	Motor score
4; spontaneously	5; orientated	6; obeys commands
3; to verbal command	4; confused	5; localises to pain
2; to pain	3; inappropriate words	4; flexion to pain
1; no eye opening	2; incomprehensible sounds	3; abnormal flexion to pain
	1; no verbal response	2; extension to pain
		1; no motor response

The GCS was assessed in all patients on admission and discharge from the neurosurgical unit and always either improved or stayed the same (if originally 14 or 15) (Figure 6.14A). The median GCS was 15 on admission and discharge (Figure 6.14B), exemplifying that all patients recruited to the neurochemistry sub-study were relatively well neurologically, probably because there is often not enough time to recruit the sickest patients due to delays with consent and the need for immediate surgery.

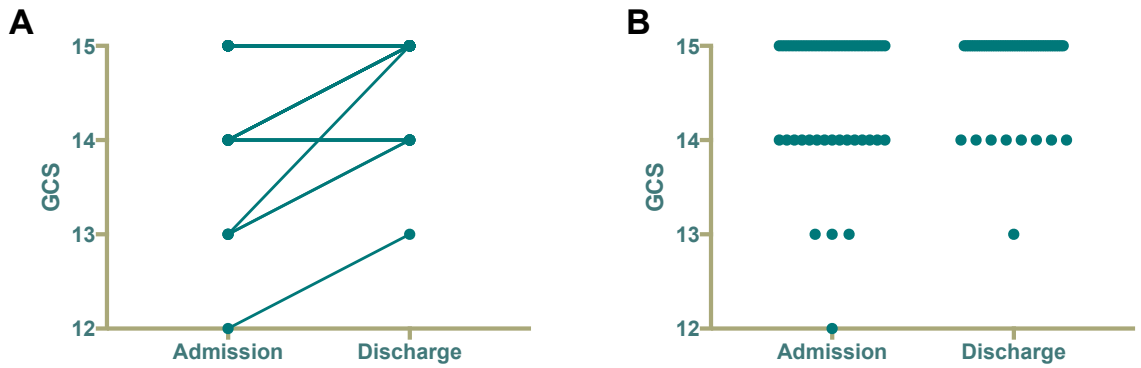

Figure 6.14; Admission and discharge GCS: **(A)** paired admission and discharge GCS patterns, **(B)** individual patient numbers of GCS on admission and discharge, n = 52.

One might expect more rapidly expanding CSDH to cause a lower GCS and be associated with a greater escalation of inflammatory markers. The limited number of patients with a low GCS make it difficult to determine this, but no significant differences were found in relation to GCS and any inflammatory marker. In fact, the pattern seen with some of the key inflammatory markers (e.g. VEGF, IL-6) showed the opposite, with similar, or lower, concentrations in the five samples from patients with the lowest GCS scores (12-13), see Figure 6.15.

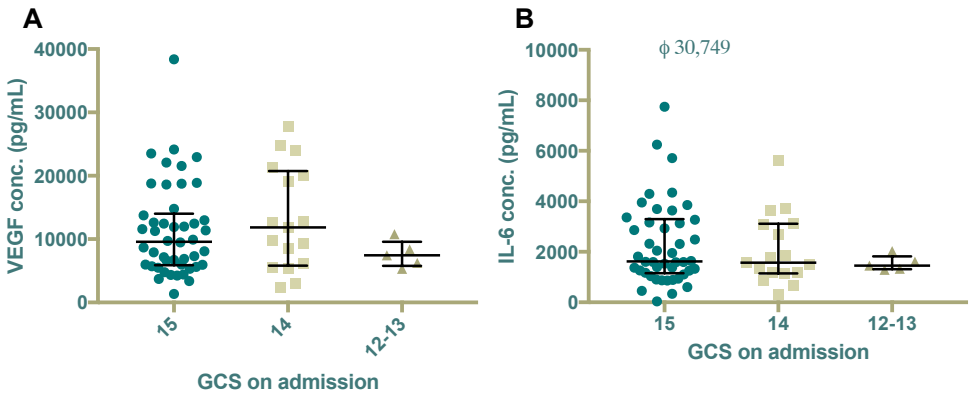

Figure 6.15; GCS on admission related to: **(A)** VEGF, **(B)** IL-6. GCS 15 n = 46, GCS 14 n = 17, GCS 12-13 n = 5. Line (median), bars (IQR), ϕ = outlier.

Long-term outcome was assessed at three and six months with the modified Rankin Scale (mRS); dichotomised to good outcome (mRS 0-3) and poor outcome (mRS 4-6) (see chapter eight for more details). Two patients had follow-up missing at three months and one bilateral CSDH was lost-to follow-up at six months, leaving 66 CSDH samples with mRS outcomes at each time point.

VEGF was significantly lower in patients with a poorer outcome at both three months (p = 0.0224) and six months (p = 0.0031) (Figure 6.16A&B). A similar pattern was seen for IL-6 at six months (p = 0.0181, Figure 6.16C) but not for any other analyte (data not shown). The data was also reviewed by individual ordinal mRS score, but this did not add any further insight to the dichotomisation of VEGF concentrations above and below mRS scores of three (Figure 6.16D). As there are very few patients with an mRS 4-6 it is difficult to make any definite conclusions about this. One possible explanation is that poor outcome from CSDH is higher in patients with co-morbidities and poorer health in general, such patients may not be capable of mounting a high immune response at the time of CSDH, thus resulting in the lower levels of key inflammatory cytokines such as VEGF and IL-6.

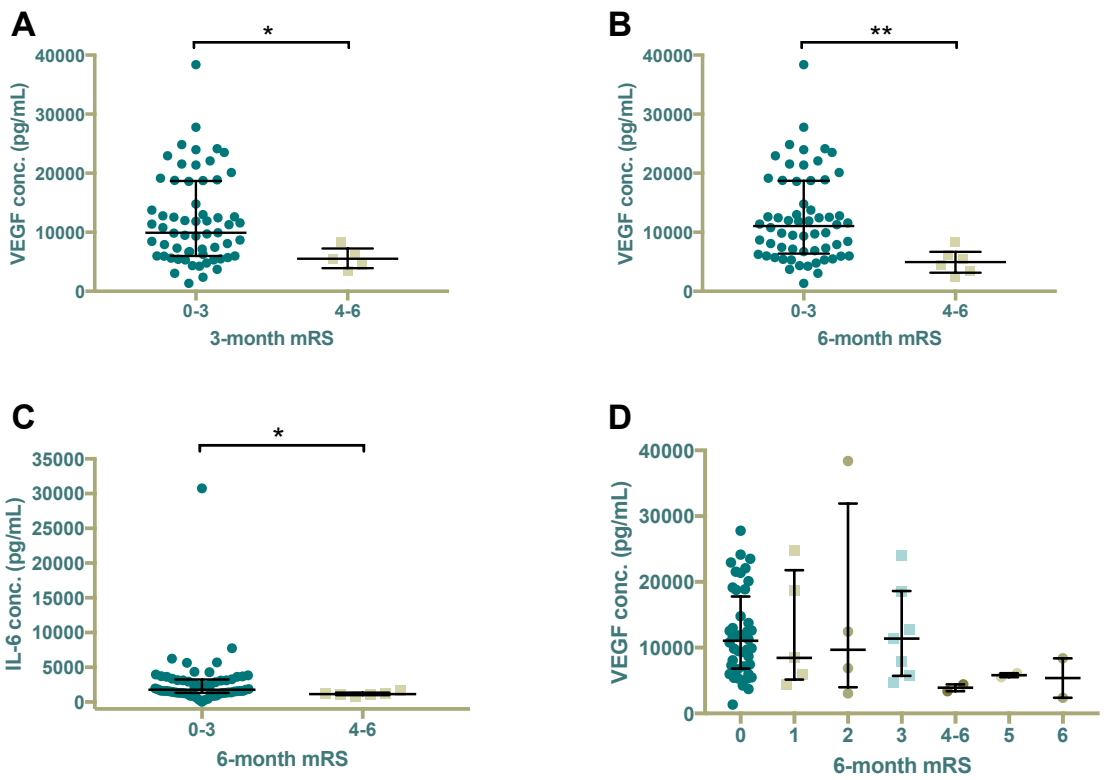

Figure 6.16; **(A)** VEGF and mRS at 3 months, mRS 0-3 n = 61, mRS 4-6 n = 5, **(B)** VEGF and mRS at 6 months, mRS 0-3 n = 60, mRS 4-6 n = 6, **(C)** IL-6 and mRS at 6 months, n as per B, **(D)** VEGF and all mRS categories at 6 months. Line (median) and bar (IQR), statistically significant differences denoted as $P \leq 0.05$ (*), $P \leq 0.005$ (**), (mRS = modified Rankin Scale).

6.5 Conclusions

The data on treatment groups shows a surprising elevation of several of the key inflammatory markers (although not significant for VEGF) in patients who have received dexamethasone compared to those that haven't. This appears to continue despite higher cumulative pre-operative dosing of dexamethasone for most markers. Some markers do appear to fall in patients who have received more than 48mg (or three days) of pre-operative dexamethasone, suggesting this may be the minimum dose required to initiate an effect on inflammation in CSDH. Overall, the findings contradict the original hypothesis that dexamethasone would result in reduced levels of inflammation in CSDH fluid. However, this is not to say that dexamethasone doesn't have an anti-inflammatory effect, and timing of doses and sampling may be important.

As the collection of subdural fluid is sufficiently large to require surgery, it provides a substantial pool of inflammatory cells and mediators. Chapter four evidences that dexamethasone does not accumulate within the subdural space and therefore its effects may occur from down-stream genomic effects on the cells in the subdural membranes. The combination of these two factors may mean that the pre-existing CSDH fluid collections take some time to change their inflammatory profile. The post-operative drain data does show reduced concentrations of most inflammatory markers across all time points in patients treated with dexamethasone, suggesting once the milieu of inflammation is removed with surgery, the drug has a more rapid effect. If the post-operative peak in inflammation is the driver to recurrence, this would explain how dexamethasone works to reduce post-operative recurrence. However, this does not explain why the levels of some markers, including IL-6 and IL-8, are significantly *higher* in the intra-operative samples. It is possible that the anti-inflammatory action of dexamethasone initially stimulates a rise in some markers, but that these are switching to a "reparative" role.

Dexamethasone has also been reported to work successfully as a conservative treatment option for some patients with CSDH, therefore surgical drainage is not always necessary (Sun, Boet, & Poon, 2005). As some of the inflammatory markers showed a decline in the intra-operative CSDH fluid for patients who had received a minimum of 48mg (three days) of dexamethasone, this may indicate that inflammation can be controlled with sufficient cumulative dexamethasone treatment. This is corroborated to some degree by Berghauser's

study which found that the recurrence risk with CSDH was lower, the longer the pre-operative course of dexamethasone given, with five days being the median duration in non-recurrence patients (Berghauser Pont, Dammers, Schouten, Lingsma, & Dirven, 2012). Therefore, perhaps if a longer course of dexamethasone were given prior to surgery then different changes would have been observed. It may also be the case that surgery is more successful if performed once inflammation is already reducing, thus making the case for a more prolonged trial of dexamethasone treatment for CSDH prior to surgery, in patients that are neurologically stable.

Finally, review of clinical data does appear to confirm the hypothesis that the inflammatory response escalates over time, with higher levels of most markers in patients who present the longest time-period after trauma. VEGF and MMP-9 appear particularly relevant in the early pathophysiological stages, which is interesting as both are also implicated in CSDH recurrence (see Chapter five). This may mean that recurrence occurs through a re-initiation of CSDH inflammatory cycle, similar to that which occurs at the very origin of the CSDH initially. VEGF was also significantly higher in acute transformed (CSDH-AT) compared to de-novo (CSDH-DN), the latter forming more insidiously over time.

Contrary to expectations, patients with a worse outcome, as measured by the mRS, appear to have lower inflammatory profiles. This may be a reflection of the general immunosuppressed status of more unwell patients, who have poorer recovery in the long-term. This is also confounded by the small numbers of patients with a poor outcome, and therefore needs further investigation in larger patient groups.

6.6 References

Berghauser Pont, L. M., Dammers, R., Schouten, J. W., Lingsma, H. F., & Dirven, C. M. (2012). Clinical factors associated with outcome in chronic subdural hematoma: a retrospective cohort study of patients on preoperative corticosteroid therapy. *Neurosurgery, 70*(4), 873-880; discussion 880. doi:10.1227/NEU.0b013e31823672ad

Schrottmaier, W. C., Kral, J. B., Badrnya, S., & Assinger, A. (2015). Aspirin and P2Y12 Inhibitors in platelet-mediated activation of neutrophils and monocytes. *Thromb Haemost, 114*(3), 478-489. doi:10.1160/th14-11-0943

Sternberg, Z., Chichelli, T., Sternberg, D., Sawyer, R., Ching, M., Janicke, D., . . . Munschauer, F. (2016). Relationship between Inflammation and Aspirin and Clopidogrel Antiplatelet Responses in Acute Ischemic Stroke. *J Stroke Cerebrovasc Dis, 25*(2), 327-334. doi:10.1016/j.jstrokecerebrovasdis.2015.10.001

Sun, T. F., Boet, R., & Poon, W. S. (2005). Non-surgical primary treatment of chronic subdural haematoma: Preliminary results of using dexamethasone. *Br J Neurosurg, 19*(4), 327-333. doi:10.1080/02688690500305332

Teasdale, G. Glasgow Coma Scale/Score (GCS). Retrieved from https://www.mdcalc.com/glasgow-coma-scale-score-gcs

Thomas, M. R., & Storey, R. F. (2015). Effect of P2Y12 inhibitors on inflammation and immunity. *Thromb Haemost, 114*(3), 490-497. doi:10.1160/th14-12-1068

Vane, J. R., & Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflamm Res, 47 Suppl 2*, S78-87.

Chapter 7; Imaging analysis in CSDH

7.1 Introduction

Previous studies have shown some evidence that imaging characteristics of CSDH, such as the laterality, volume, mid-line shift and pattern of density, may be useful in predicting the risk of recurrence, however the data is conflicting. A sub-group of 164 patients recruited to the Dex-CSDH study, had baseline diagnostic computed tomography (CT) imaging available for analysis. Just over half of these patients (86/164) also had post-operative CT imaging available for comparison. Clinical data was available on all 164 patients, and all patients in the neurochemistry sub-study (discussed in chapters five and six) were also included for comparison of imaging to inflammatory profile data.

The first aim of this chapter was to establish a reliable and accurate methodology for analysing CT imaging with regard to volume and density. Such that, this method could be applied to analysing all the CT imaging from patients in this study.

The first hypothesis was that larger CSDHs would be more likely to recur and have a poorer outcome. This assumed that larger CSDHs are more likely to be incompletely evacuated and therefore residual inflammatory molecules could increase the risk of recurrence and poor outcome. Similarly, it was hypothesised that post-operative CT imaging would be useful in predicting recurrence, with higher recurrence rates in patients with larger residual volumes of pneumocephalus. This is based on the theory that pneumocephalus prevents adequate, timely brain re-expansion and thus allows a persistent space for re-accumulation of the CSDH to occur. Finally, it was hypothesised that CSDHs containing a density pattern suggesting recent new haemorrhage within the CSDH (i.e. high density) would also be more likely to recur. This is because haemorrhage from membranes may suggest a more “active” stage of inflammation and membrane development, and thus would also likely be correlated to higher inflammatory markers within the fluid.

7.2 Imaging Methods

The first aim was to quantify the volume of all CSDHs in this study. Two different methods for calculating the volume of CSDHs and ASDHs are referred to in the literature and were assessed before designing the volume analysis method for all scans. The first method for assessing volume is the ABC/2 technique, and has been published as reliable for CSDH volume calculation when using the maximum width x maximum length (both on any slice) x depth (no. of slices x slice thickness) (Sucu, Gokmen, & Gelal, 2005). The second method, referred to here as slice-by-slice (SBS), involves calculating the volume by tracing the haemtoma on each slice (area) and multiplying by the slice thickness (Huang, Lin, Lu, & Chen, 2014; Yan, Yang, & Huang, 2018). This technique is used more commonly in the literature, as intuitively it would be more accurate, but it is also more time consuming. Thus, the ABC/2 technique would be more efficient for analysing large imaging data sets, such as in this study, as long as accuracy can be maintained. The methods were compared for accuracy and agreement on a sub-set of images before determining the imaging analysis protocol for the full CSDH data set.

7.2.1 Semi-automated volume calculation

To make the SBS volume calculation quicker and more efficient, a semi-automated computer programme was developed to aid the volume calculation. Images were downloaded into ITK-snap (Yushkevich et al., 2006) and a newly developed programme called “watershed” was used to segment each scan into regions by density. Areas of different density could then be grouped together by point and click, thus only highlighting the area correlating to CSDH (not normal brain). This was done on each slice of the scan so that eventually the volume of area selected (i.e. CSDH) from each slice was combined to calculate the overall volume of the CSDH (Figure 7.1).

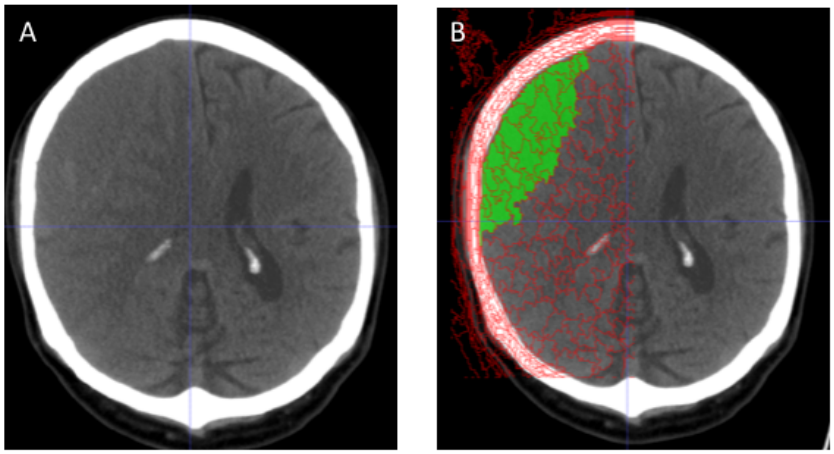

Figure 7.1; (A) Original CSDH scan, **(B)** Automated segmentation by software (red lines) with region of CSDH highlighted in green.

The software can help delineate CSDHs that are isodense, where the user may have difficulty finding the distinction between brain and CSDH. The regions that are automatically segmented can also be manually edited by the user if needed. The programme is also able to assess the density within the highlighted area, and therefore can split the CSDH into areas of "high" (red) and "low" (green) density, giving an objective assessment of density (Figure 7.2). It can also create a final map of the volume once all slices have been highlighted (Figure 7.2C).

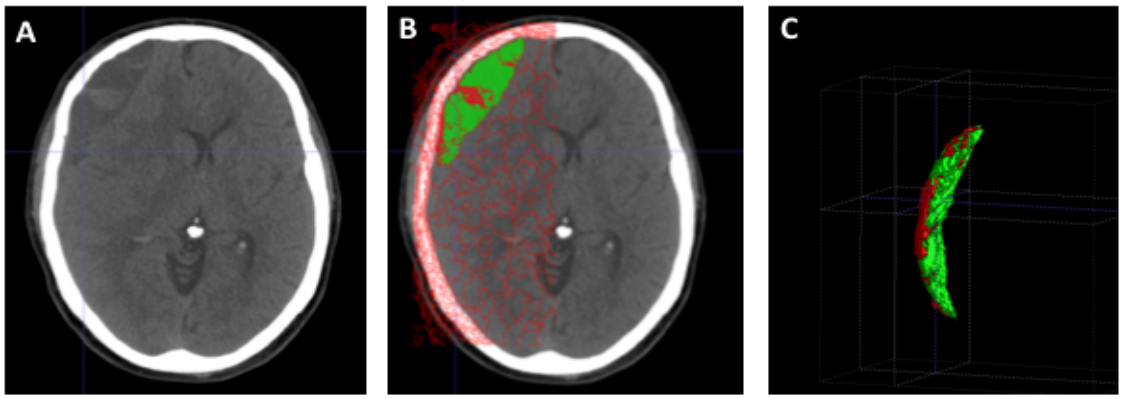

Figure 7.2; (A) original CSDH scan, **(B)** automated segmentation with CSDH highlighted and areas split into high (red) and low (green) density, **(C)** final net of CSDH volume.

7.2.2 Agreement between methods for volume calculation

Agreement between the two different measurement methods (ABC/2 and SBS) for ASDH volume calculation (n = 15) were assessed by comparing the average volumes with the differences between them, as per Figure 7.3 (Bland & Altman, 1986).

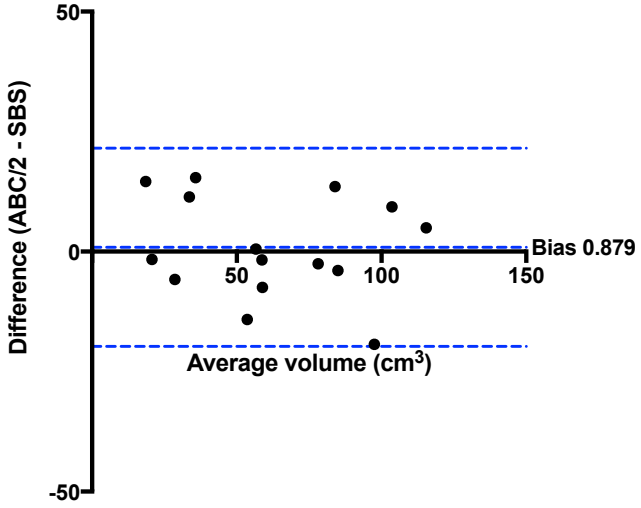

Figure 7.3; Bland-Altman plot of volume calculation with ABC/2 and SBS, upper and lower 95% confidence intervals shown with blue lines, n = 15.

The bias, or mean difference, is only 0.879cm^3 (S.D 10.53), with the ABC/2 tending to slightly overestimate. However, the 95% limits of agreement from -19.77 to 21.53 cm^3 is wide, which can be particularly problematic for small ASDHs. This is exemplified by one patient in whom the ABC/2 method overestimated the volume by 130% (25.8cm^3) compared to SBS (11.3cm^3). With this in mind, the distribution of ASDH volume was plotted, with an obvious division between the smallest five ASDHs (all under 40cm^3) and the larger 10 CSDHs (Figure 7.4A). The percentage difference between the methods was significantly lower in the larger volume ASDHs (>40cm^3) compared to smaller ASDHs (unpaired T test, p = 0.0188) (Figure 7.4B). Therefore, it would seem sensible not to apply the ABC/2 technique to ASDHs smaller than 40cm^3.

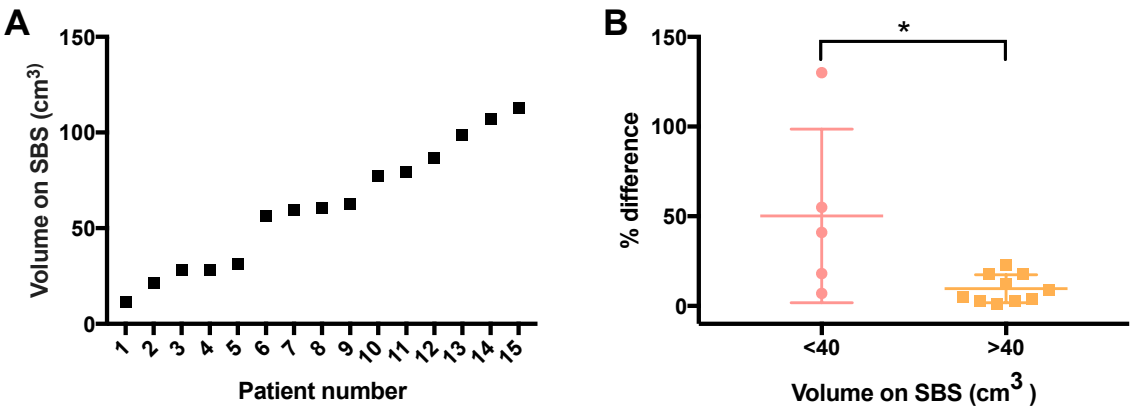

Figure 7.4; (A) distribution of ASDHs by volume, n = 15, **(B)** comparison of percentage-difference in ASDHs below and above 40cm^3 in volume on SBS, statistically significant differences denoted as $p \leq 0.005$ = **.

Agreement between the two methods for calculating the CSDH volumes (n = 50) was assessed with the same method and showed a larger bias of 12.97 cm^3 (S.D. 32.25), again with the ABC/2 method over-estimating (Figure 7.5). The CSDH volumes measured were often greater than the ASDHs and unlike the ASDHs, large and small volume CSDHs were equally poorly estimated with the ABC/2 method.

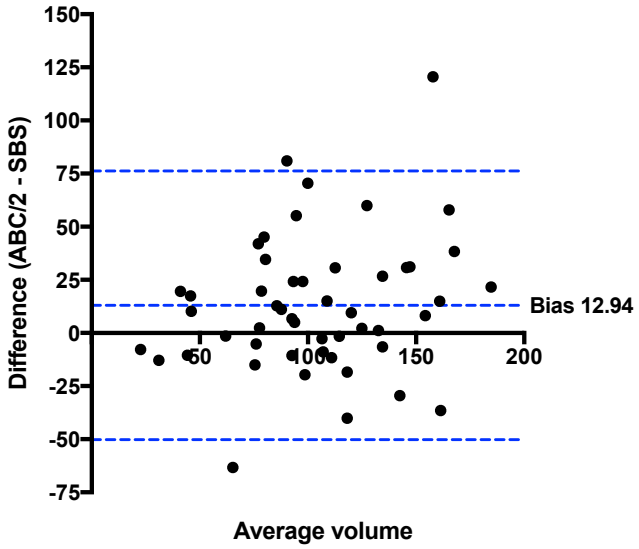

Figure 7.5; Bland-Altman plot of volume calculation with ABC/2 and SBS. Upper and lower 95% confidence intervals shown with blue lines, n = 50.

Intra- and Inter-rater reliability

The intra-rater reliability for the SBS and ABC/2 methods was calculated by assessing five CSDH images (differing in density) and three ASDH images twice by a single rater (E

Edlmann). The intra-class coefficients can be seen in Figure 7.6 and clearly show that the SBS method was more reliable than ABC/2 for both types of subdural, but particularly for CSDH volume calculation.

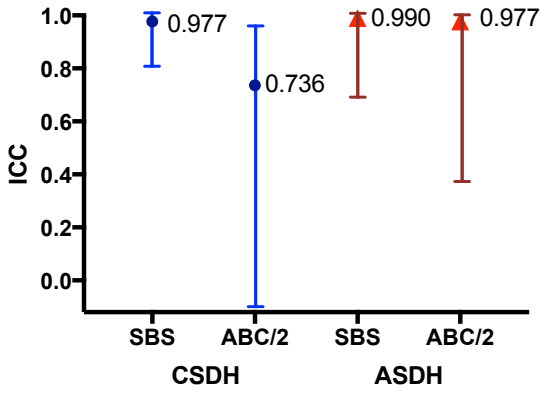

Figure 7.6; intra-rater reliability for CSDH and ASDH volume assessment by SBS and ABC/2 methods, as measured by intra-class correlation coefficient (ICC), bars = 95% confidence intervals.

The inter-rater reliability of the both methods was also assessed with four different raters; one junior neurosurgeon, two senior neurosurgeons and one neuro-radiologist. Four different CSDH images (varying in density) and two ASDH images were assessed by all raters. The ICC for inter-rater variability was similar between the two methods; ABC/2 = 0.911 and SBS = 0.929. However, the SBS method was marginally superior, and this can be seen in the pattern of measurements displayed in Figure 7.7, with the ABC/2 method showing wider variation in readings, which often over-estimate the volume.

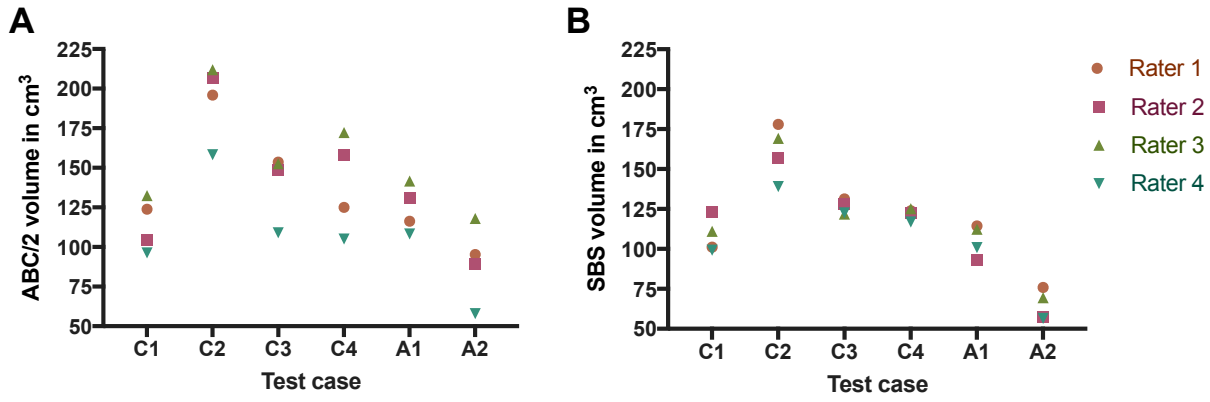

Figure 7.7; inter-rater reliability for CSDH (C1-4) and ASDH (A1-2) test cases assessed by four raters with; **(A)** ABC/2 method, **(B)** slice-by-slice (SBS) method.

Overall, although quicker, it was evident that only small changes in one measurement with the ABC/2 method could result in significant changes in volume. A single rater (E Edlmann) would be used to assess all images in this study, therefore the SBS technique was applied for its superior accuracy and repeatability.

7.2.3 Methods for density assessment

There is varying literature on different "types" of CSDH as classified on CT. The most common classification system referred to was first reported by Nakaguchi et al. and has seven categories within two main sub-types; homogenous (hyperdense, isodense and hypodense) and mixed (trabecular, laminar, gradation and separated) (Figure 7.8A-G) (Nakaguchi, Tanishima, & Yoshimasu, 2001).

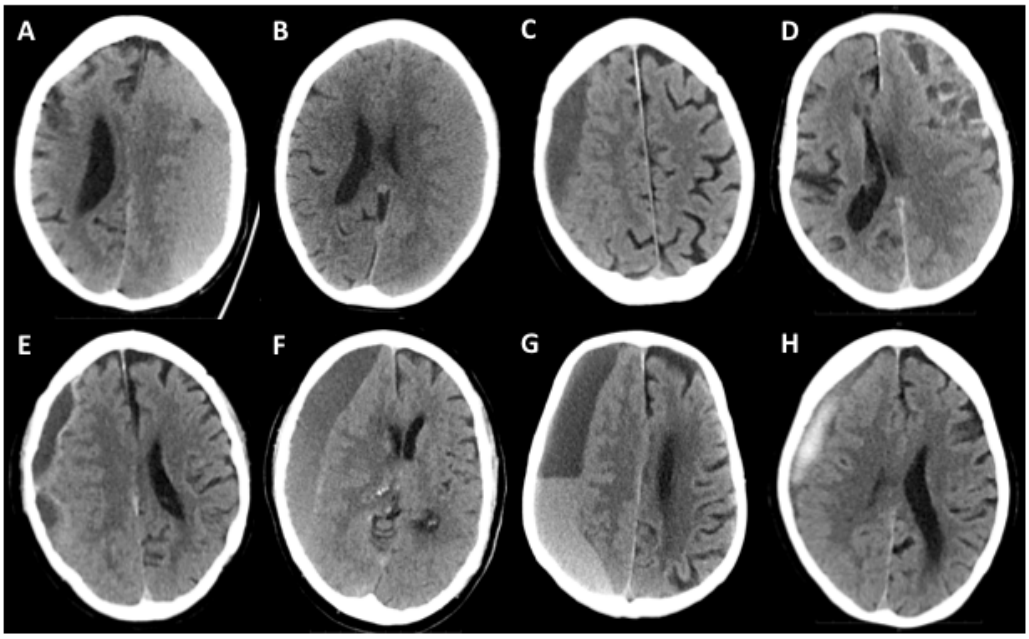

Figure 7.8; Nakaguchi classification: **(A)** homogenous hyperdense, **(B)** homogenous isodense, **(C)** homogenous hypodense, **(D)** mixed trabecular, **(E)** mixed laminar, **(F)** mixed gradation, **(G)** mixed separated, **(H)** mixed sub-type not described by Nakaguchi et al.

Nakaguchi postulated that the sub-types represented different stages in CSDH evolution, each with varying bleeding tendencies. The homogenous types were considered the earliest stage, some of which are "laminar" with an inner layer of hyperdensity. The CSDH then progresses to the separated stage and finally the trabecular stage. The lowest recurrence, of 0%, was seen

in the latest stage (trabecular), which is considered a "resolution stage". Higher recurrence was seen in the other stages; homogenous (15%) and laminar (19%), with the highest recurrence in separated CSDHs (36%), considered the most "active" period of bleeding. This was corroborated by Ohba et al. who found significantly higher recurrence in separated compared to trabecular CSDHs and Huang et al. who found higher recurrence with "layered" (or separated) CSDHs (Huang et al., 2014; Ohba, Kinoshita, Nakagawa, & Murakami, 2013). Yamamoto et al. used a slightly different classification, but also found lower recurrence rates in hematomas with "multiplicity" of density, most similar to the trabecular type (Yamamoto et al., 2003). However, conversely CSDH with septations (which could be considered trabecular) have been linked to a higher risk of recurrence (Jack, O'Kelly, McDougall, & Findlay, 2015), as have CSDHs categorised as "mixed density" (which also includes those that appear trabecular) (Yan et al., 2018). Some studies have also suggested that hyper- and isodense CSDHs, from the homogenous sub-group, have a higher risk of recurrence alongside the separated group (Stanisic & Pripp, 2017).

Overall, density classifications are subjective and it is difficult to know how uniformly the definitions are applied, even when the same classification is referred to. A large degree of judgment is required as many CSDHs do not fit neatly into the pre-defined classification. For example, there are variations within the "mixed density" group that do not fit into any of the Nakaguchi categories (see Figure 7.8H), where there is some acute blood but with no pattern. There are also many CSDHs that could largely be considered homogenous but may have some areas of mixed or higher density around the skull base (the most dependent area), whether this is significant enough to change to a mixed category depends on the assessor.

It was therefore anticipated that an objective measure of CSDH density would be a stronger predictor of the recurrence risk than the current subjective classifications. This was done using the same software as the volume calculation (Yushkevich et al., 2006), where the watershed programme automatically calculates the mean density of the area highlighted. It can also perform a density-split, where the exact volume of high and low density is calculated. This split was then converted into a percentage of high density (percentage-hyperdense) with the formula;

% hyperdense = (volume of high density/total volume of CSDH) x 100

The main drawback to this technique is that the percentage of hyperdensity gives no indication about the distribution of the hyperdensity, as would be provided in the subjective descriptions (e.g. trabecular versus separated). Therefore, the percentage of hyperdensity could be the same for Figures 7.9A&B, despite different patterns of blood.

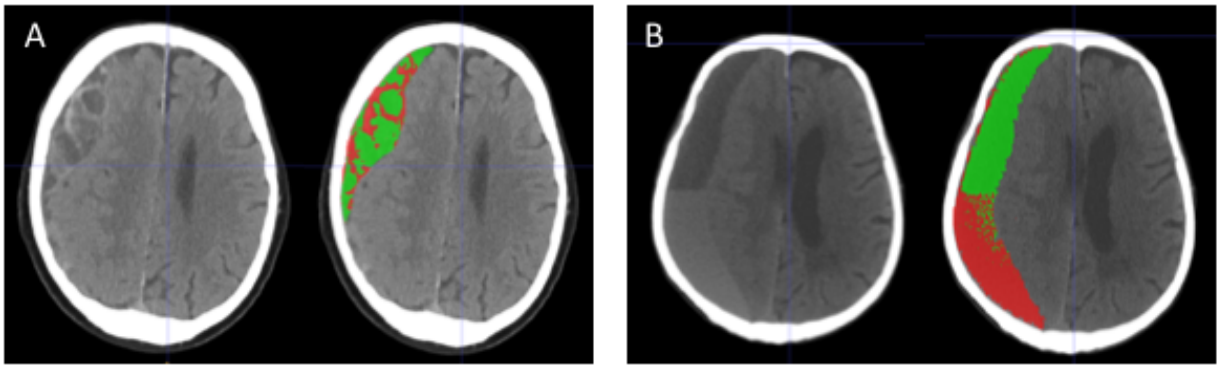

Figure 7.9; CSDH scans with semi-automated segmentation and density split, high density areas shown in red and low density in green: **(A)** Trabecular CSDH, **(B)** Separated CSDH.

7.2.4 Agreement between methods for density assessment

To test inter-rater and intra-rater reliability, 50 CSDH patients were chosen at random for repeat assessment of the density category (as per Figure 7.8). Four patients had bilateral CSDHs, which made a total of 54 CSDHs analysed. Each CSDH was assessed as homogenous (H) or mixed (M) and then further sub-grouped as either hypodense (HYPO), isodense (ISO) or hyperdense (HYPER) in the homogenous group and laminar (LAM), trabecular (TRA), separated (SEP) or gradation (GRA) in the mixed group.

The intra-rater reliability for density category was assessed twice by a single rater (E.Edlmann) at least seven days apart and with the same 54 CSDHs assessed in a different order. The classifications agreed in 44/54 CSDHs (Table 7.1; blue cells), giving Cohens kappa coefficient of 0.768 (standard error 0.064) showing reasonable agreement between the two ratings (where 1 is complete agreement). This was limited by assessing only one rater and this rater had experience in using the classification in CSDH, therefore one might expect less agreement with raters not experienced with it. Of the 10 ratings that did not agree these were most commonly in CSDHs classified as hyper or isodense on at least one of the ratings,

suggesting these are the most difficult sub-types to delineate. A case example of a CSDH initially classified as isodense and later classified as hyperdense can be seen in Figure 7.10.

Table 7.1; intra-rater categorisations of density sub-types with pink boxes highlighting cases with disagreement between the two assessments.

1st/2nd	HYPO	ISO	HYPER	TRA	LAM	GRAD	SEP
HYPO	**13**						
ISO	1	**4**	1				
HYPER		3	**4**			2	
TRA		1	1	**15**	1		
LAM					**3**		
GRAD						**3**	
SEP							**2**

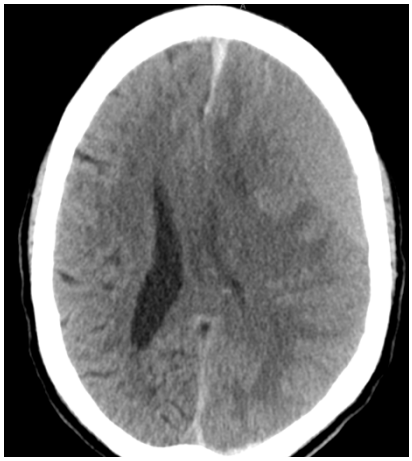

Figure 7.10; CSDH case example classified as both hyperdense and isodense by the same rater.

The inter-rater reliability was assessed using two raters, both neurosurgeons experienced with assessment of CSDH, on the same 54 CSDHs. The agreement between the two different raters was poor, with a Cohens kappa coefficient of 0.397 (standard error 0.75). This shows the significant limitation of applying the Nakaguchi classification, with different users interpreting it very differently. As there was reasonable intra-rater reliability with the single rater, all the images in this study were still grouped by the Nakaguchi classification in order to compare this with the objective density calculations.

7.3 Results on primary CSDH volume

Diagnostic CT imaging was reviewed on 189 CSDHs in 164 patients recruited to the Dex-CSDH trial. There were 25 patients with bilateral CSDHs and of the remaining 139 unilateral CSDHs, 65 were left-sided and 74 right-sided. Small contralateral CSDHs which did not require any treatment were not included in the analysis.

7.3.1 CSDH volume in all patients

The volumes for all 189 CSDHs can be seen in Figure 7.11, with a median volume of 103cm^3 (I.Q.R 69 - 138cm^3). The volumes were not normally distributed; therefore, all statistical analyses used non-parametric tests.

Surgery was performed as first-line treatment for 172/189 (91%) CSDHs in 149 patients (23 bilateral CSDHs). First line conservative medical management was used for 17 CSDHs in 15 patients (two bilateral CSDHs), of which four CSDHs in three patients (one bilateral) failed, requiring subsequent surgery at days 15, 28 and 36 after the original diagnosis.

As one would expect, there was a significantly lower volume in the patients initially treated conservatively (median 74cm^3) compared with those initially treated surgically (median 106cm^3), (Mann Whitney test, p = 0.0196), with one clear outlier in the conservative group.

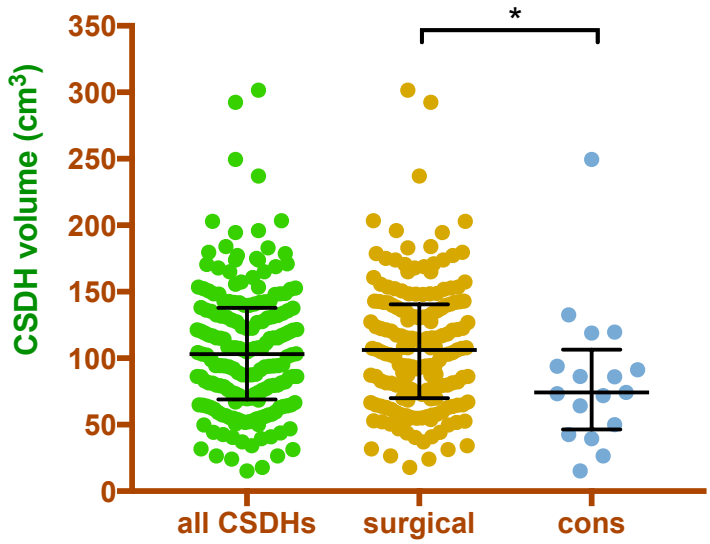

Figure 7.11; CSDH volumes in all patients, those treated surgically (surgical) and those treated conservatively (cons). Statistically significant differences denoted as p < 0.05 = *.

In clinical practice, midline shift is often measured on CT scans (as this is easy to do) and used as a correlate for how large the CSDH is and how urgently it should be operated on. However, when assessing midline shift and volume in all operative unilateral CSDHs (n = 126), there was only a very weak, although significant, correlation with volume (Spearman r = 0.2145, p = 0.0159) (Figure 7.12A). This is likely to be because whilst midline shift is affected by CSDH volume, it is also highly dependent on the amount of cerebral atrophy, and thus redundant space, within the cranial vault. This is exemplified by grouping the patients by age into ≤65 years (n = 16) and >65 years (n = 110), where there was a much stronger significant correlation in the younger age group (Spearman r = 0.6618, p = 0.0065) and no correlation in the older group (Spearman r = 0.1213, p = 0.2070) (Figure 7.12B&C). This loss of correlation is likely to be due to the greater variation in atrophy in older patients.

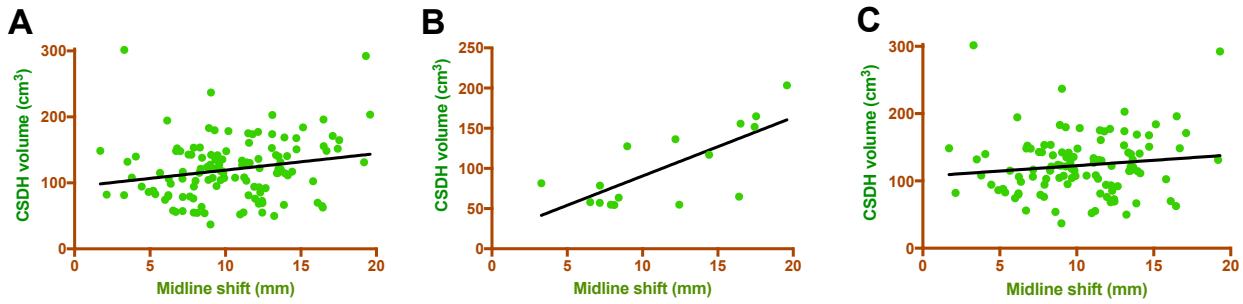

Figure 7.12; correlation between CSDH volume and midline shift: **(A)** all unilateral operative patients, n = 126, **(B)** all patients ≤65 years, n = 16, **(C)** all patients >65 years, n = 110.

The 50 individual bilateral CSDHs were significantly smaller in volume than the 139 unilateral CSDHs (Mann Whitney, p < 0.0001, Figure 7.13A). However, if the volume from both sides was combined in the bilateral cases, then they were significantly larger than unilateral cases (Mann Whitney, p< 0.0041, Figure 7.13B), showing a larger, combined, intra-cranial burden in patients suffering with bilateral CSDH.

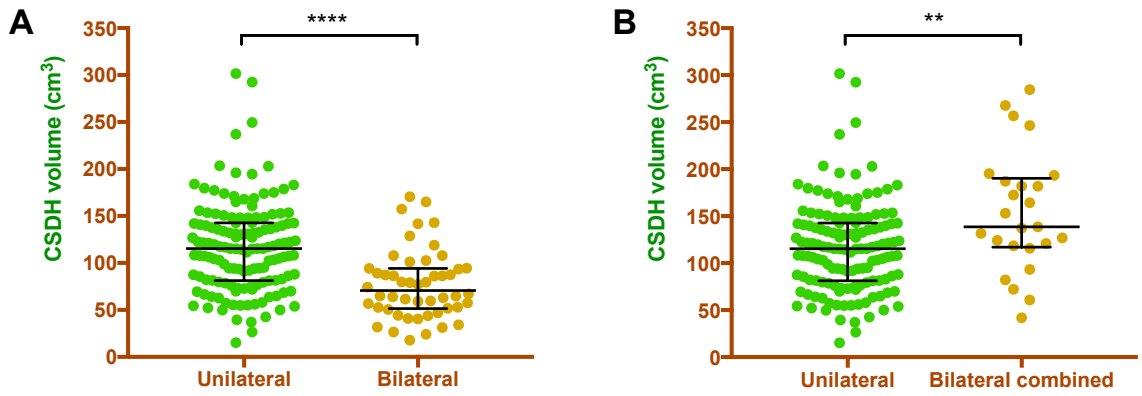

Figure 7.13; (A) unilateral and bilateral (each side separately) CSDHs, **(B)** unilateral and bilateral (both sides combined) CSDHs. Line (Median), bar (IQR), statistical significant differences denoted as $p<0.0001$ = ****, $p<0.01$ = **.

7.3.2 Recurrence and outcome

It has been previously suggested that larger diameter CSDHs are more likely to recur (Bartek et al., 2017). More specifically, CSDHs over 120-130mls in volume pre-operatively have been reported as a moderate predictor of recurrence (Stanisic & Pripp, 2017; Yan et al., 2018). Greater pre-operative midline shift has also been shown as a predictor of recurrence (Kuhn et al., 2018). Therefore, it was hypothesised that larger pre-operative CSDHs (excluding conservatively managed CSDHs) would have a higher risk of recurrence, with recurrence measured as patients requiring further surgery for CSDH on the same side as previously operated within six months. The handling of bilateral CSDHs is important. As already displayed the volumes are significantly higher or lower than unilateral CSDHs depending on if each side is considered combined or seperately. Many studies exclude or do not mention bilateral CSDHs, but there are also several studies which report that bilateral CSDHs themselves have a higher risk of recurrence (Bartek et al., 2017; Han et al., 2017; Schwarz et al., 2015; Torihashi et al., 2008). In this study only 1/25 (4%) patients with bilateral CSDH experienced recurrence, compared to 13/126 (10%) unilateral operative CSDHs, suggesting the opposite is true. As the bilateral CSDHs were too few to analyse separately, three analyses were performed; unilateral CSDHs only (n = 113 non-recurrent, n = 13 recurrent), unilateral and bilateral with each side separately (n = 157 non-recurrent, n = 15 recurrent), unilateral and bilateral with each side combined (n = 135 non-recurrent, n = 14 recurrent). In all analyses, there were no obvious differences in volume between CSDHs that went on to recur and those that didn't, making a definite case that volume is not a risk factor for recurrence (Figure 7.14).

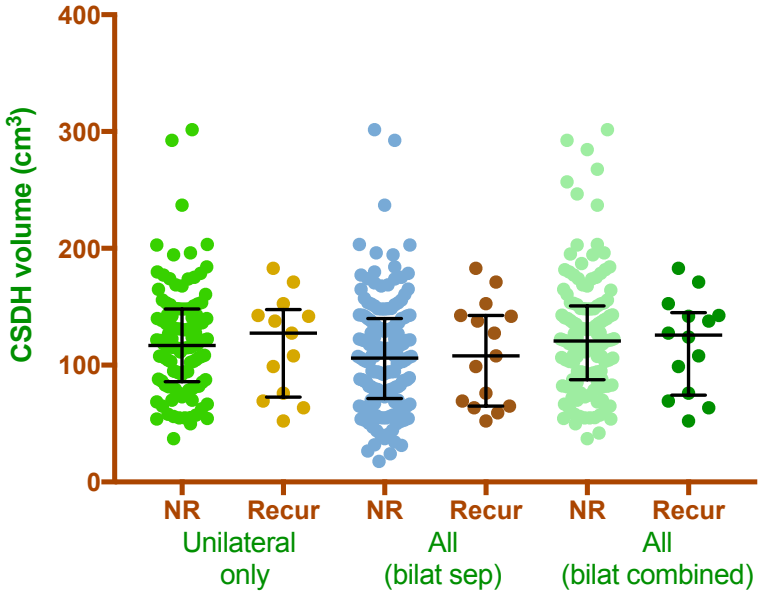

Figure 7.14; volume in non-recurrent (NR) and recurrent (Recur) CSDH. Line (Median), bar (IQR), (bilat = bilateral, sep = separately).

Further to this midline shift was also assessed in relation to recurrence but no significant differences were found (Figure 7.15).

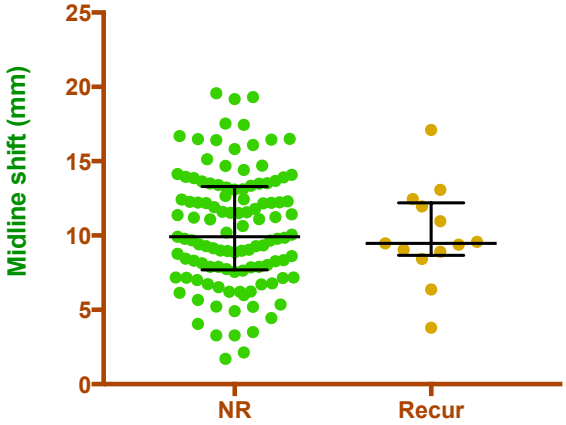

Figure 7.15; midline shift in non-recurrent (NR) and recurrent (Recur) unilateral CSDH. Line (Median), bar (IQR).

Some studies agree with the finding that pre-operative CSDH volume, width or associated midline shift are not associated with recurrence (Huang et al., 2014; Kim et al., 2015; Ohba et al., 2013). It has also been suggested that it is not the pre-operative volume per se that is important, but the residual volume post-operatively, and the association only arises because

larger CSDHs can be more difficult to drain, leading to higher post-operative residuals (Jack et al., 2015). This theory is explored further in analysis of the post-operative imaging below (section 5.3.3).

Haemtoma thickness above 3cm has been correlated with unfavourable outcome, as measured by an mRS of 2-6 (Kwon, Al-Awar, Richards, Izu, & Lengvenis, 2018). No studies have assessed outcome in relation to CSDH volume or midline shift. In this study, volume and MLS were compared between patients with an unfavourable (mRS 4-6) and a favourable (mRS 0-3) outcome. This dichotamisation is used throughout the Dex-CSDH study and is discussed further in chapter seven. Interestingly, despite the lack of significance in relation to recurrence, CSDH volume was significantly greater in patients with an unfavourable outcome compared to a favourable outcome at three months (Mann-Whitney $p = 0.0006$) and 6 months (Mann-Whitney $p = 0.0106$) (Figure 7.16A&B). When assessing the mRS on admission this also showed a strongly significant relationship between the volume of the CSDH and an unfavourable admission mRS score (Mann-Whitney, $p < 0.0001$, Figure 7.16C). This may explain to some degree the relationship between outcome and volume, as larger volume CSDHs can result in worsened neurological deficit, and thus a lower mRS on admission but also a poorer recovery in the long term. The significant correlation between admission and 3-month mRS can be seen in Figure 7.17 (Pearson $r = 0.3876$, $p < 0.0001$, chi squared test $p = 0.0150$). One previous study has already identified that the mRS score on admission is a significant predictor of death at six months (Santarius & Hutchinson, 2009)(**Santarius**). However, age was also significantly greater in the patients with an unfavourable outcome at three months (Mann-Whitney $p < 0.0001$). Therefore, although there was only a weak correlation between age and CSDH volume (see section 5.3.4), it may still be part of the driver of the poorer outcome in larger CSDHs.

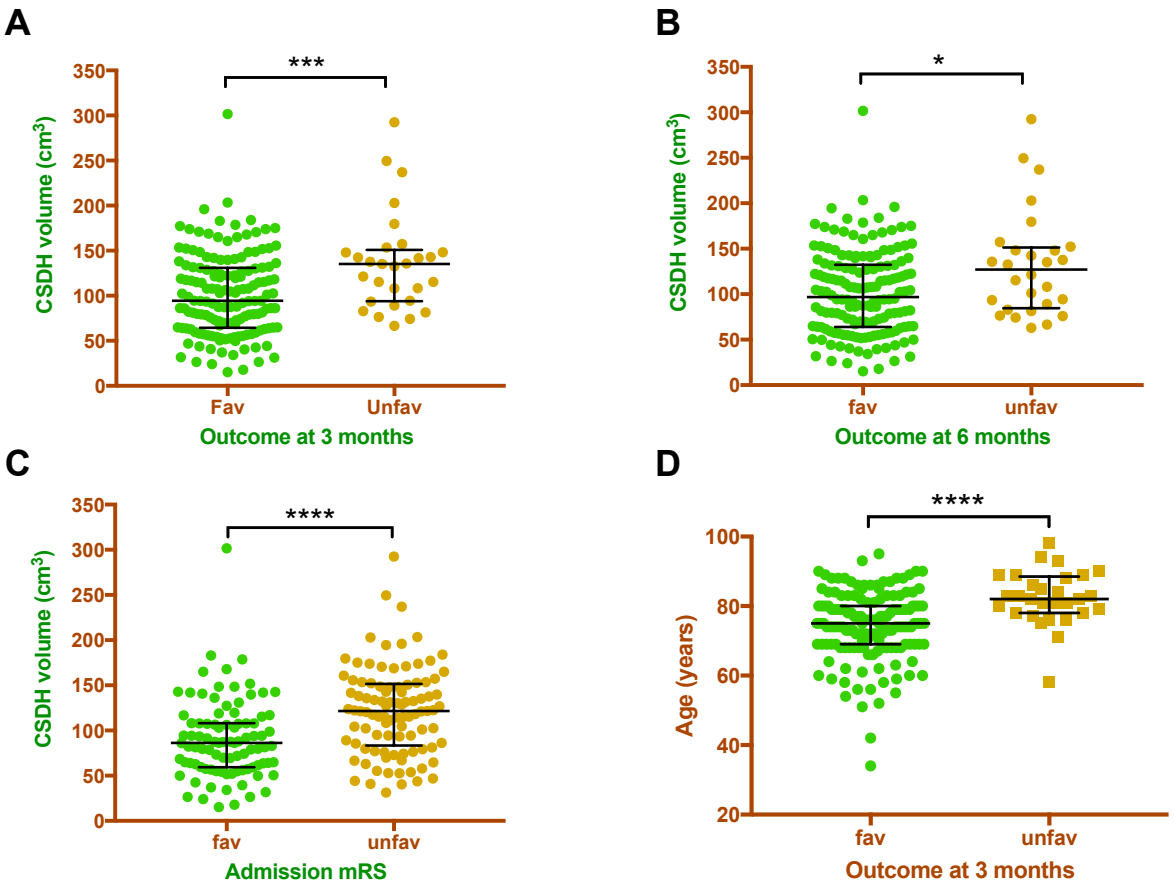

Figure 7.16; dichotomised modified rankin scale (mRS) into favourable (Fav) or unfavourable in relation to: **(A)** CSDH volume and outcome at three months (fav n =153, unfav n = 29, 7 no data), **(B)** CSDH volume and outcome at six months (fav n = 158, unfav n = 28, 3 no data), **(C)** CSDH volume and admission mRS (fav n = 92, unfav n = 97), **(D)** age and outcome and three months (fav n = 153, unfav n =29) . Line (Median), bars (IQR), statistically significant differences denoted as P≤0.0001 (****), P≤0.001 (***), P≤0.05 (*).

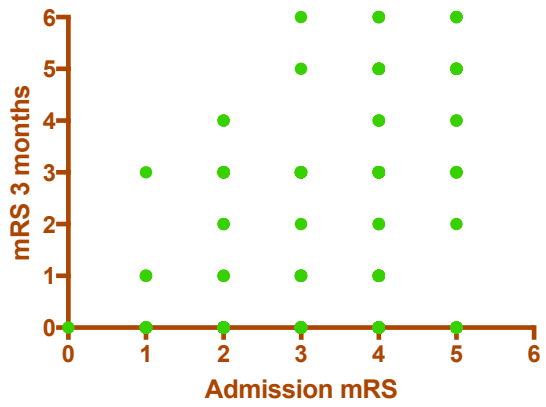

Figure 7.17; correlation between ax mRS and 3-month mRS.

7.3.3 Surgical techniques

All CSDHs treated primarily with surgery (n = 172) were assessed for surgical technique; one burr hole (1BH), two burr holes (2BH) or a mini-craniotomy (MC), as well as placement of a subdural or subgaleal drain (SDD and SGD respectively). Due to a publication in 2009 showing that SDD placement significantly reduces CSDH recurrence and in turn mortality, it has become common practice to place a SDD in the UK (Santarius & Hutchinson, 2009). The only circumstances where this is not done is if it is felt unsafe intra-operatively, usually due to brain re-expansion leaving insufficient subdural space to place a drain. A SDD was placed in 162/172 (94%) of surgical treated CSDHs, with one SGD placed and nine CSDHs with no drain placed.

When comparing recurrence rates, there were 2/10 (20%) recurrences in CSDHs without a SDD, and 13/162 (8%) in those with a SDD. The tiny number of patients without a drain makes any meaningful statistical comparison difficult but the 8% recurrence rate in the drain group is comparable to the literature on patients treated with drains (Almenawer et al., 2014; Brennan et al., 2017; Santarius & Hutchinson, 2009). CSDH volume was significantly lower in cases where no SDD was placed (Mann-Whitney, $p < 0.0001$), which is perhaps unsurprising as the brain is more likely to re-expand rapidly, preventing SDD placement in smaller CSDHs (Figure 7.18). However, given the evidence for drain placement reducing recurrence and the 20% recurrence rate in the no SDD group, placement of a SGD, overlying the burr hole but not risking entry to the brain, may be the next best option to promote drainage of any residual fluid and preventing recurrence. This may be determined by a randomised trial currently running which is comparing subdural and sub-periosteal (same as SGD) drains (Soleman, Lutz, Schaedelin, Mariani, & Fandino, 2016).

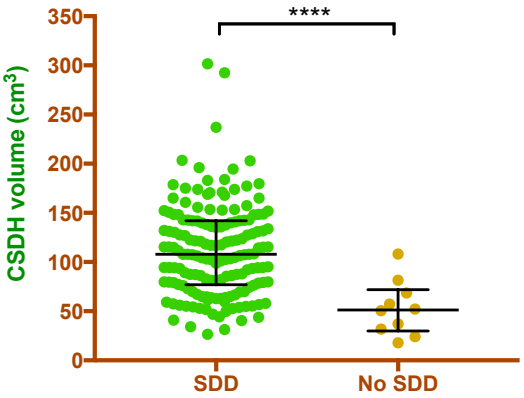

Figure 7.18; CSDH volume in relation to sub-dural drain (SDD) placement. Line (median), Bars (IQR), statistical significance shown as p<0.0001 = ****.

The most common surgical procedure was 2BHs, in 131/172 (76%) of operative CSDHs, followed by MC in 33/172 (19%) and 1BH in 8/172 (5%). In three cases the original operation was 2BH which was then converted into a MC, due to inability to evacuate acute blood or membranes through the BHs, but the remaining MCs were performed due to consultant preference. There was a trend to slightly lower CSDH volumes in the 1BH group compared to the other surgical groups, and may explain why 1BH was considered sufficient if the surface area of the CSDH was small (Figure 7.19). The highest volume outlier in the 1BH group (volume 237cm^3) was aged 98, therefore it may have been co-morbidities that led to a preference of a quicker operative procedure with 1BH in this patient. Otherwise the ages of patients were comparable between groups (data not shown). The two outliers in the MC group (292cm^3 and 301cm^3) were both patients with prior cerebral lesions, one with an arachnoid cyst and the other with an old stroke leading to significant cerebral atrophy. This provides anecdotal information on how pre-existing atrophy or enlarged subdural space can influence the volume of a CSDH. This was also assessed to some extent in chapter two, where a greater degree of cerebral atrophy (measured with bicaudate ratio) did correlate with larger CSDH volume.

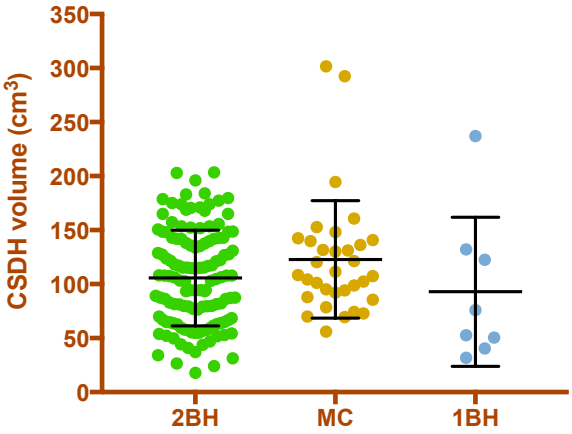

Figure 7.19; surgical techniques and CSDH volume. Line (median), bars (IQR), (BH = burr hole, MC = mini-craniotomy).

7.4 Primary CSDH density

7.4.1 Density assessments in all patients

The mean density within each CSDH was calculated for all 189 CSDHs (mean 40.81 HU, n = 189), and was similar between those who had surgery as first-line treatment (40.67 HU, n=172) and those initially treated conservatively (42.20 HU, n = 17) (Figure 7.20). The spread of data on density followed a normal distribution, therefore parametric statistical tests were used for all analyses.

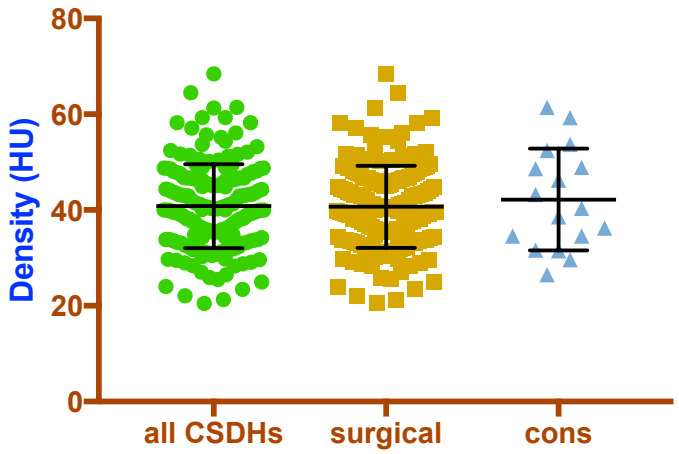

Figure 7.20 mean density within each CSDH for all cases, surgical treated and conservatively (cons) treated. Line (mean) and bars (S.D.).

Each CSDH was also categorised subjectively into one of the Nakaguchi classifications (as per Figure 7.8). The mean density calculations appeared to show the differences between the homogenous group classifications well, with a significant difference between hypodense, isodense and hyperdense CSDHs (One-way ANOVA, $p < 0.0001$, $F = 70.96$), although there was still considerable cross-over between groups (Figure 7.21). All the mixed density categories had similar mean densities, with an overall average sitting between isodense and hypodense.

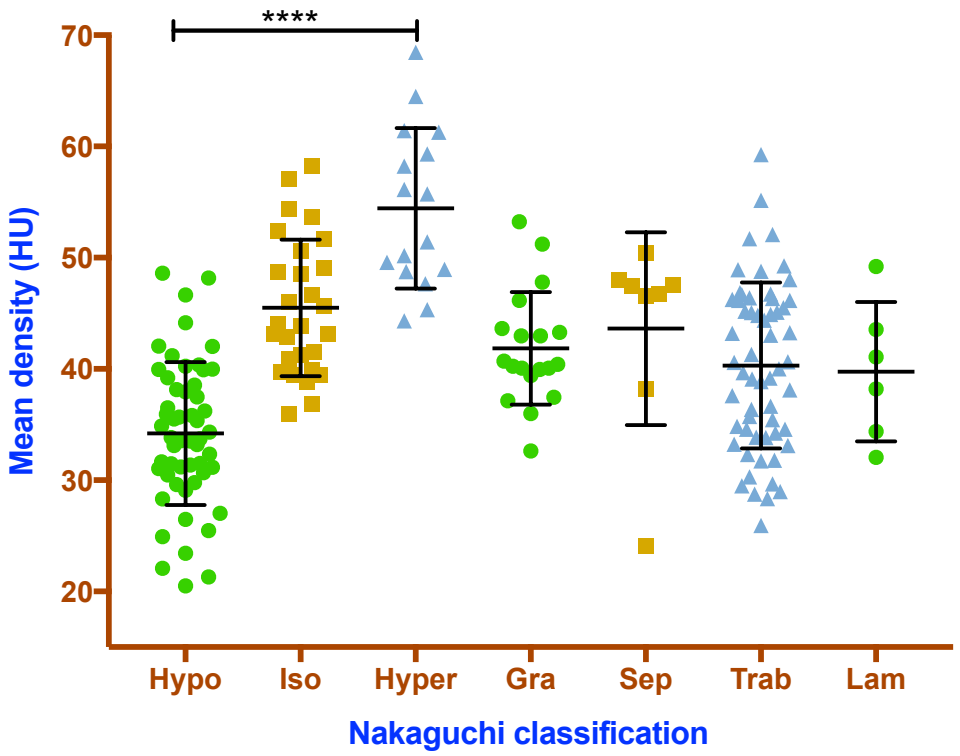

Figure 7.21; automated mean density calculation compared to Nakaguchi classification. (Hypodense (hypo) n = 54, Isodense (Iso) n = 28, Hyperdense (Hyper) n = 16, Gradation (Gra) n = 19, Separated (Sep) n = 8, Trabecular (Trab) n = 58, Laminar (Lam) n = 6). Line (mean), bars (S.D), statistically significant differences denotes as $p < 0.0001$ = ****

The automated split to calculate the percentage-hyperdense (see methods) was performed on 141/189 CSDHs, as this could not be calculated on imaging where there was any movement, air or artefact. The percentage-hyperdense was compared between the Nakaguchi classifications (Figure 7.22) and showed a similar pattern to the mean densities, with a significant difference between the homogenous categories (One-way ANOVA, $p < 0.0001$, $F = 64.12$). It also highlights how similar all the separated CSDHs are, with just over 50% hyperdense in all five cases, whilst the other mixed density CSDHs (gradation, trabecular and laminar) tend to have a lower percentage of hyperdensity. The percentage of hyperdensity in the isodense CSDHs had the widest range from around 15% hyperdense to almost 100% hyperdense (which one might have been expected to be classified as hyperdense, as was highlighted in the intra-rater variability testing).

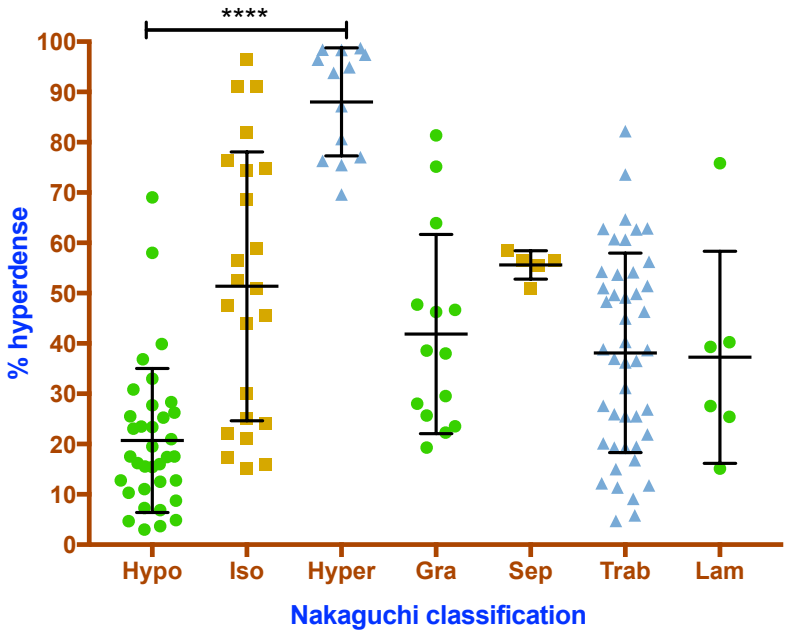

Figure 7.22; percentage hyperdense compared to Nakaguchi classification (Nakaguchi classification abbreviations and N as per Figure 7.21). Line (mean), bars (S.D.), statistically significant differences denoted as $p < 0.0001$ = ****

No correlation was found between the mean density or the percentage-hyperdense and the CSDH volume or midline shift (examples in Figure 7.23). The fact that the amount of high density, which is suggested to come from recent bleeding or membranes, does not relate to the volume or midline shift, suggests this happens throughout the life cycle of the CSDH and is not necessarily confined exclusively to the early stages (when CSDH is presumably smaller).

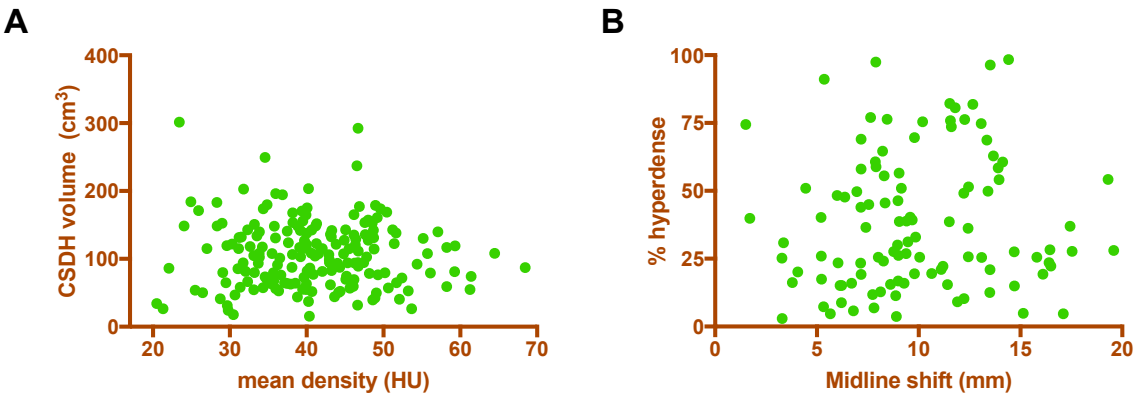

Figure 7.23; correlation of: **(A)** CSDH volume and mean density**, (B)** percentage (%) hyperdense and midline shift.

7.4.2 Density in relation to CSDH volume

When the Nakaguchi classification was used, there was a significant difference in volume between the category with the lowest median volume (hypodense) and the highest median volume (separated) (Mann-Whitney, p = 0.0018) (Figure 7.24). Interestingly, this shows the opposite of what Nakaguchi hypothesised, which was that the later stages of CSDH evolution, when one would also expect the volume to be the highest, have a dormant, hypodense appearance (Nakaguchi et al., 2001). However, with very few patients in the separated group, it is hard to rely on this significant difference.

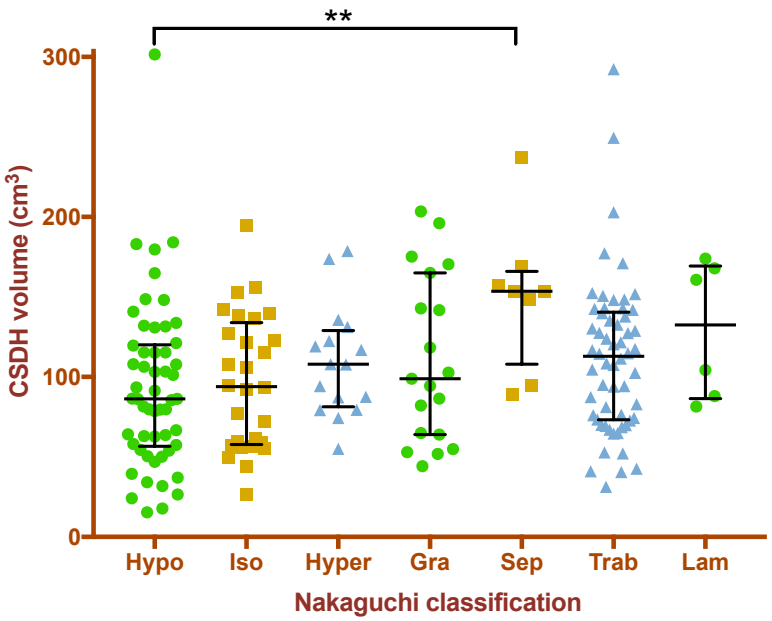

Figure 7.24; CSDH volume by Nakaguchi classification**.** Line (median), bars (IQR), statistically significant differences denoted as P≤0.005 (**), abbreviations as per Figure 7.21.

There was no difference in the mean density between unilateral and bilateral CSDHs (Figure 7.25).

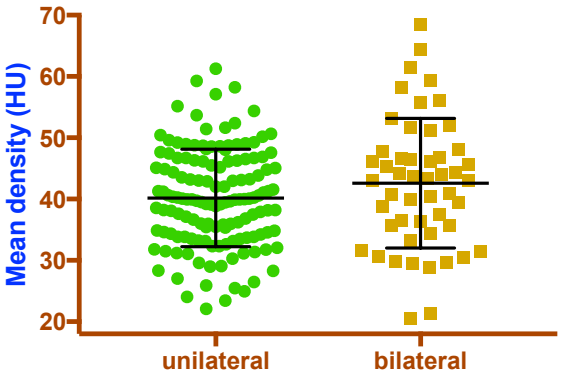

Figure 7.25; mean density in unilateral and bilateral CSDH. Line (mean), bars (S.D.).

7.4.3 Recurrence and outcome

There was no difference in the mean density or percentage-hyperdense between primary CSDHs treated surgically that went on to recur (n=15) and those that did not (n = 157) (Figure 7.26A&B). However, when using the Nakaguchi categories, there were more heterogenous (mixed) density CSDHs in those that went on to recur (10/15, 66%) compared to those that didn't recur (78/157, 50%), although the small numbers mean this does not reach statistical significance (Fishers's exact test p = 0.2843) (Figure 7.26C). When this was broken down by individual categories, there was a clear increase in trabecular sub-types in CSDHs that recurred (9/15, 60%) compared to those that did not (44/157, 28%), and no hyperdense, laminar or separated CSDHs were found in the recurrence group (Figure 7.26D).

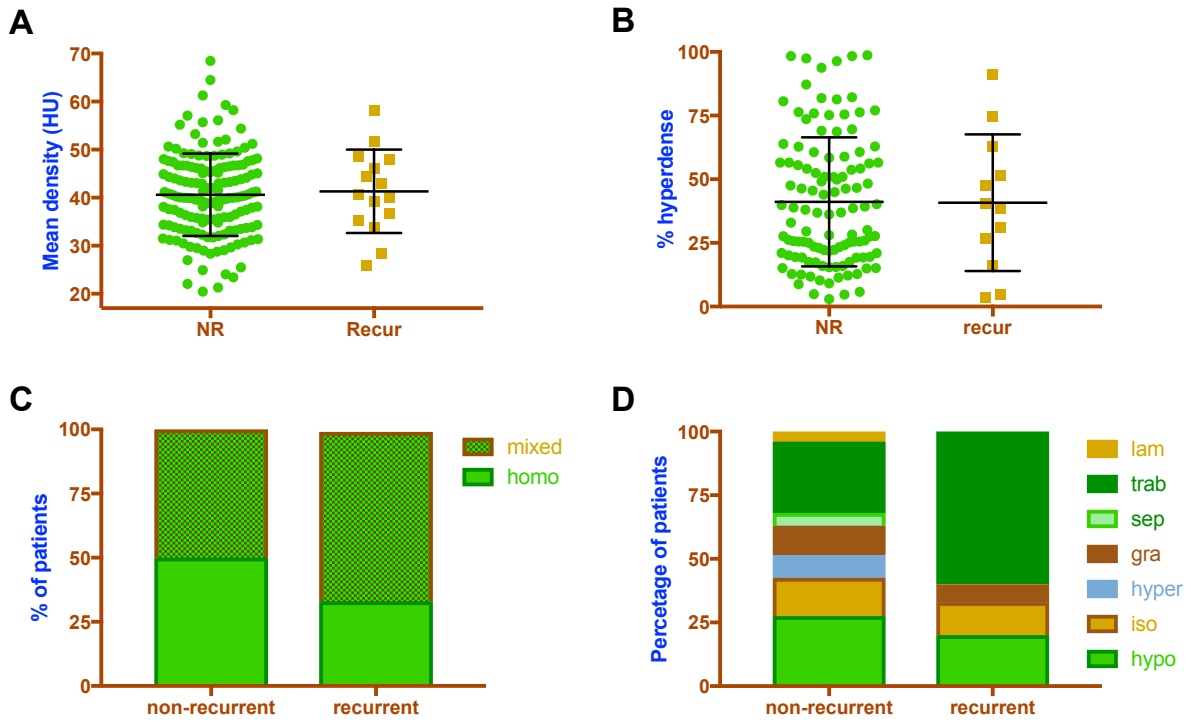

Figure 7.26; comparison between recurrent and non-recurrent CSDHs in: **(A)** mean density, **(B)** percentage-hyperdense, **(C)** percentage classified as homogenous or mixed density, **(D)** percentage by each density classification (abbreviations as per Figure 7.21). Line (mean), bars (S.D.), (Recur = recurrence, NR = non-recurrence).

The small number of CSDHs that went on to recur means that statistical analysis is limited. However, there appears to be a clear trend for trabecular CSDHs to be more likely to recur than any of the other density categories. This is the opposite finding to several previous studies which suggested the trabecular stage has the lowest recurrence risk, and it is highest

in separated CSDHs (Huang et al., 2014; Nakaguchi et al., 2001; Ohba et al., 2013). However, there are previous reports of higher recurrence in mixed density CSDH (Jack et al., 2015; Yan et al., 2018). Some of these differences in reporting may come from the variation in definition of trabecular or mixed density CSDHs. Clearly this is important, as the objective measurements show the actual breakdown of high and low density within the CSDH in not significant, but rather the *pattern* of density on imaging, which requires subjective interpretation. This may be because it is not hyperdensity from blood, but rather from membranes which is important, as these are a key difference seen in trabecular rather than other mixed density CDSHs. On the basis of these results, I would suggest that re-defining CSDHs into "membranous" and "non-membranous" may be a much more helpful classification in relation to predicting recurrence than the complex classification by Nakaguchi.

Due to the complexities of applying seven different sub-groups to each analysis, further discussion in this chapter divides the density classification into mixed (heterogeneous) or homogenous only. With consideration that the majority of the mixed group comprises of trabecular (58/91), or in many cases "membranous" CSDHs.

The breakdown between homogenous and mixed density CSDH was almost exactly equal in all surgical technique groups, with only a marginal increase of mixed density (60%) in the MC group (Figure 7.27). This may reflect the few patients where burr holes were converted to a mini-craniotomy to improve access to acute blood and membranes as 16/20 of the mixed CSDHs in the MC group were trabecular.

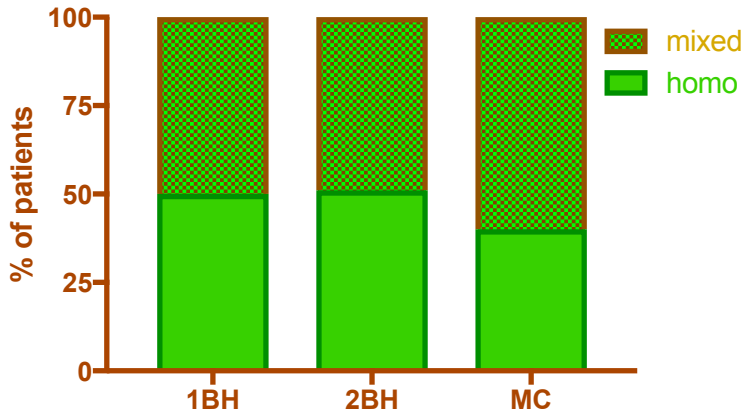

Figure 7.27; percentage of homogenous (homo) and mixed density CSDHs by operative technique. (1BH n = 8, 2BH = 131, MC = 33).

There were no differences in the mean density or percentage-hyperdense between CSDHs in patients with a favourable versus and unfavourable mRS at admission (data not shown), 3-month follow-up (Figure 7.28A&B) or 6-month follow-up (data not shown). However, there were significantly more mixed density CSDHs compared to homogenous CSDHs in patients with an unfavourable admission mRS (Fishers Exact Test, p = 0.0008, Figure 7.28C). This significance was lost at three months but there was still a trend towards unfavourable outcome in the mixed density CSDHs (Fishers Exact Test, p = 0.0697, Figure 7.28D), whilst there was no trend at six months (Fishers Exact Test, p = 0.2180). This suggests that mixed density CSDHs are more likely to present with a lower mRS score on admission and thus be neurologically worse off. This may in turn contribute to poorer outcome at 3-months, but is lost at 6-months.

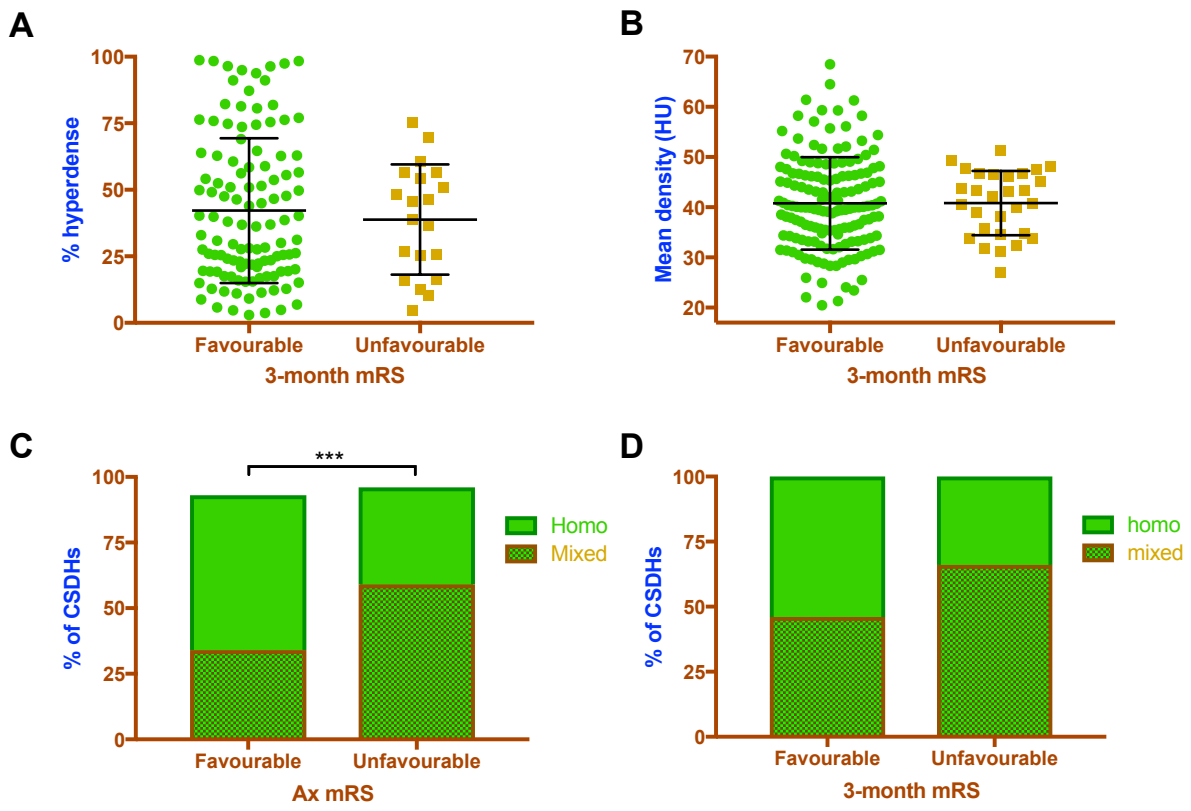

Figure 7.28; (A) 3-month mRS and percentage (%)-hyperdense, **(B)** 3-month mRS and mean density, line (mean) and bars (S.D.), **(C)** admission (Ax) mRS in mixed and homogenous (homo) CSDHs, **(D)** 3-month mRS in mixed and homogenous CSDHs. Statistically significant differences denoted as p<0.001(***).

As previously shown, larger CSDHs are also more likely to have an unfavourable mRS on admission and follow-up (Figure 7.16). Therefore, the volume of different density (mixed or

homogenous) CSDHs was assessed, and indeed found to be significantly higher in mixed density CSDHs (Mann-Whitney, p = 0.0061) (Figure 7.29A). As age was also a component in unfavourable outcome, this was also compared between density groups and was significantly higher in mixed density CSDHs (Mann-Whitney, p = 0.0012) (Figure 7.29B). In summary, mixed density CSDHs tend to be larger and found in older patients, therefore the combination of all these factors is likely to lead to an unfavourable mRS on admission and follow-up.

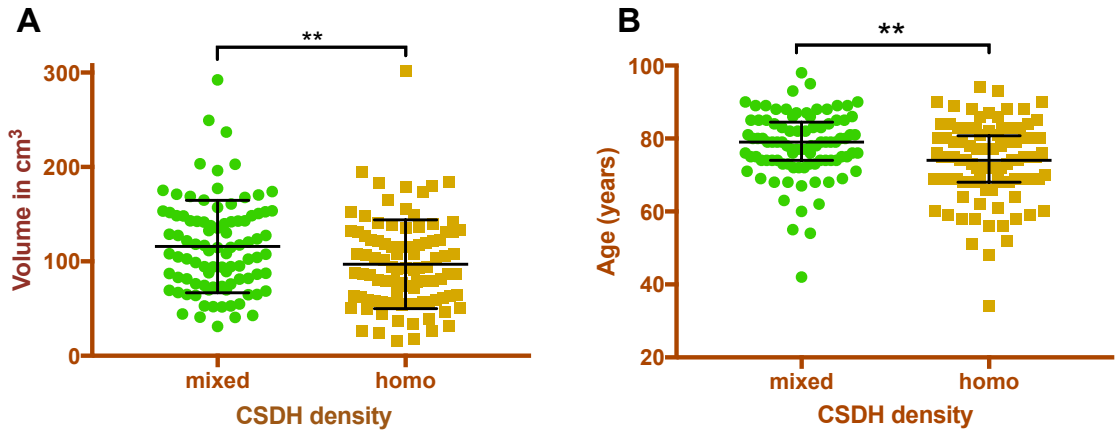

Figure 7.29; (A) volume in mixed density and homogenous (homo) CSDHs, (**B)** age in mixed density and homogenous CSDHs. Line (Median), bars (IQR), statistically significant differences denoted as P≤0.01 (**).

7.5 Post-operative imaging

There are conflicting opinions on the value of routine post-operative imaging in the management of CSDH. Some surgeons consider it a necessity to get a baseline confirmation of adequate CSDH evacuation; although what is considered adequate is hard to evaluate. Others consider that post-operative imaging results in unnecessary radiation and leads to no change in management unless there were clinical indications for the scan in the first place. There is some evidence in the literature that post-operative scans have some value in predicting outcome in terms of recurrence (Dudoit, Labeyrie, Deryckere, Emery, & Gaberel, 2016; Stanisic & Pripp, 2017; Yan et al., 2018; You & Zheng, 2018). Therefore, this section aims to explore what post-operative imaging can tell us about the evolution and resolution of CSDH in the post-operative period, with the hypothesis that excess post-operative air (pneumocephalus) may predispose to CSDH recurrence.

Post-operative imaging was available for 100 CSDHs in 86 patients; 72 patients with unilateral CSDH and 14 patients with bilateral CSDH. All post-operative imaging was taken within one week of surgery, at a median of two days (see Table 7.2).

Table 7.2: day post-operative imaging was performed

Post-operative day	Number of CSDHs
0 (same day as op)	4
1	26
2	35
3	23
4	6
5	3
6	2
7	1
TOTAL:	100

7.5.1 Residual fluid and air on post-operative imaging

Post-operative imaging usually contains a combination of fluid and air within the subdural cavity. The volume of both were measured using the watershed programme described in the methods and reported as; residual volume of fluid (RVF) and residual volume of air (RVA)

(see Figure 7.30). The combined volume of RVF and RVA was reported as the total residual volume (TRV).

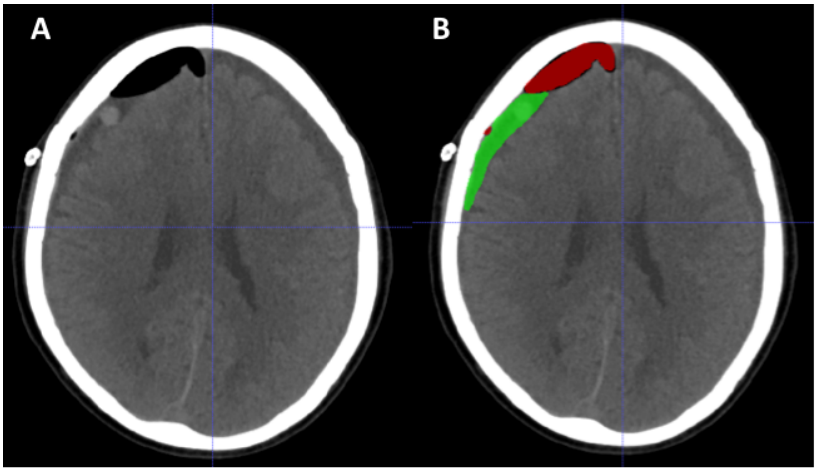

Figure 7.30; post-operative CSDH imaging showing: **(A)** pattern of residual air and fluid, **(B)** air (red) and fluid (green) highlighted by watershed programme to calculate volumes.

The post-operative volumes were compared to the paired pre-operative CSDH, which was available for 97/100 CSDHs; three patients had pre-operative scans with movement/artefact not allowing accurate volume calculations. The patterns in pre- and post-operative volumes can be seen in Figure 7.31A, with a significant reduction from total pre-operative volume to TRV (paired T-test, $p < 0.0001$). There were also significantly higher volumes of fluid than air across all the post-operative scans (unpaired T-test, $p < 0.0001$). This can be seen in more detail when the TRV was divided into percentage of fluid and air, which shows a median of 81.5% fluid to 18.5% air, but ranges from TRVs of 100% fluid to those that were 95% air (Figure 7.31B).

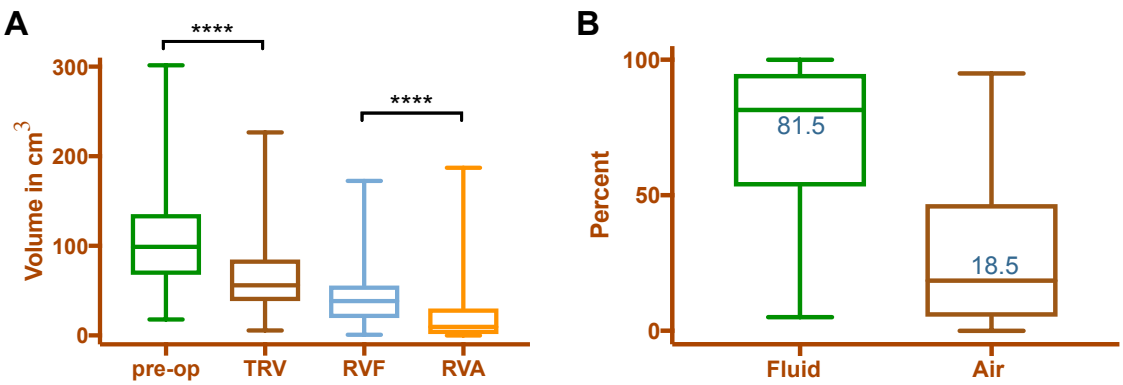

Figure 7.31; (A) CSDH volumes from pre-operative (pre-op, n = 97), to post-operative total residual volume (TVR, n = 100), **(B)** percentage of TRV that is fluid compared to air, line (median), box (IQR), whiskers (range). Statistically significant differences denoted as P≤0.0001 (****), (RVF = residual volume of fluid, RVA = residual volume of air).

The day the post-operative imaging was performed (as per Table 7.2), showed no clear relationship to the RVF, suggesting any residual fluid remains relatively unchanged within the first week of surgery (Figure 7.32).

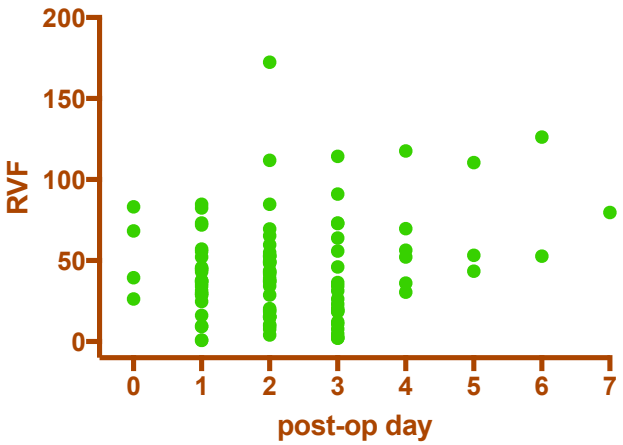

Figure 7.32; correlation between day of post-operative (post-op) imaging and residual volume of fluid (RVF).

When assessing each CSDH separately, bilateral CSDHs (n = 28) were smaller than the unilateral CSDHs (n = 72), as discussed previously. Therefore, it was unsurprising that the post-operative RVF was also significantly lower for bilateral CSDHs, with a similar decrease to that seen with the unilateral CSDHs from pre- to post-operation (Figure 7.33). Due to the significant differences in volume between bilateral and unilateral CSDHs, they are considered separately for some parts of the subsequent analysis, where appropriate.

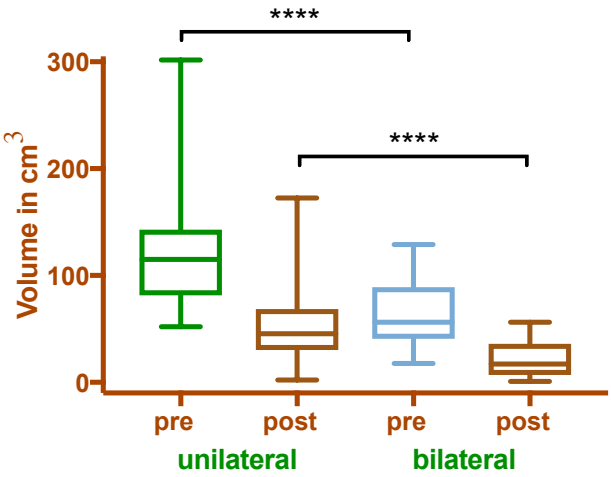

Figure 7.33; comparison of pre-operative (pre) and post-operative (post) volumes of fluid in unilateral and bilateral CSDHs, line = median, box = IQR, whiskers = range, statistically significant differences denoted as P≤0.0001 (****).

During the operation, a drain is usually placed for up to 48 hrs post-operatively to help prevent recurrence of the CSDH. A drain was placed for 95/100 CSDHs imaged post-operatively; three unilateral and one bilateral CSDH had no drains. Rapid brain re-expansion resulting in a lack of space for drain placement was reported in all cases with no drain. The numbers are too few for statistical analysis but the results shown in Figure 7.34 show that the five CSDHs with no drain placement tended to have small TRVs, suggesting there may not have been space for a drain. Whilst some of the CSDHs in the drain group have equally low volumes, this may be a result of the action of the drain.

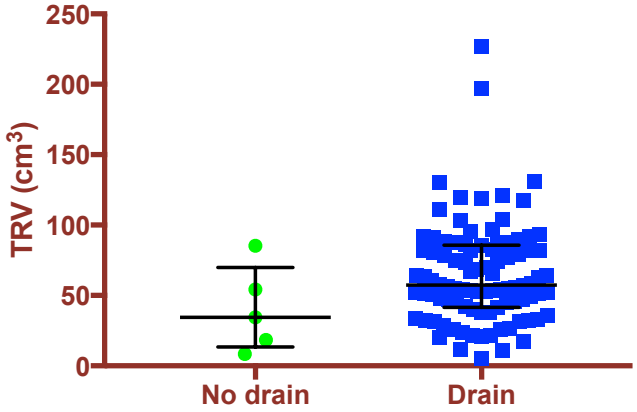

Figure 7.34; total residual volumes (TRV) in patients with no drain (n = 5) or a drain (n = 95) placed intra-operatively, line (median), bars (IQR).

Patients who were imaged before 48 hrs may have still had a drain *in situ*, to understand whether having the drain still *in situ* affected the RVF or RVA seen on the CT imaging at the time, all 95 CSDHs were divided into "drain *in situ*" or "no drain *in situ*".

There was a total of 29 images performed with a drain *in situ* (imaged at a median and mean of one day post-operatively) compared to 66 images performed with no drain *in situ* (imaged at a median and mean of three days post-operatively). The post-operative volumes were divided into unilateral and bilateral CSDHs, with 36% (25/69) of unilateral and 15% (4/26) of bilateral images performed with a drain *in situ*. There were no significant differences in the TRVs for unilateral or bilateral CSDHs, regardless of whether the drain was still *in situ* (Figure 7.35), and this was the case for both the RFV and RAV when considered separately (data not shown). This suggests that the process of removing the drain does not allow significant accumulation of air or fluid.

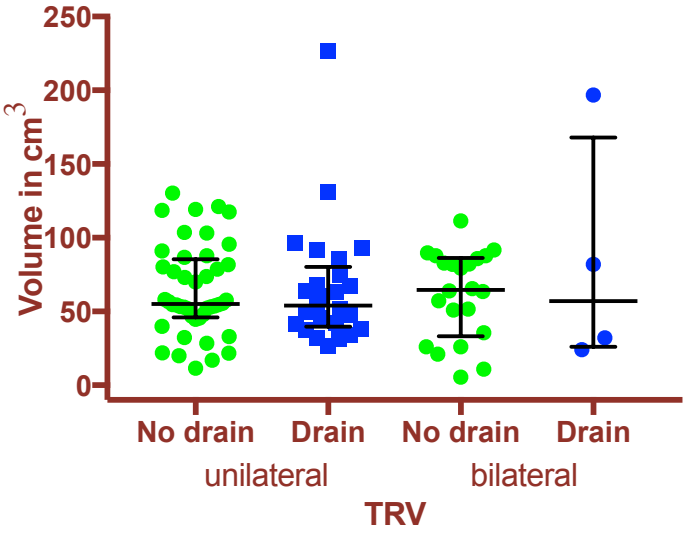

Figure 7.35; total residual volume (TRV) in unilateral and bilateral CSDHs with and without a drain *in situ*: unilateral (no drain n = 44, drain n = 25), Bilateral (no drain n = 22, drain n = 4), line (median), bars (IQR).

The patterns in the residual volumes of air (RVA) on post-operative imaging were reviewed. Unlike fluid, the volume of air appears to reduce dramatically the later imaging is performed after surgery, and Figure 7.36 suggests that most air is absorbed within seven days of surgery. This suggests that perhaps air does not have the persistent effect on limiting the ability of the

brain to re-expand, thus causing recurrence, as originally hypothesised. This is discussed further in section 7.3.3.2.

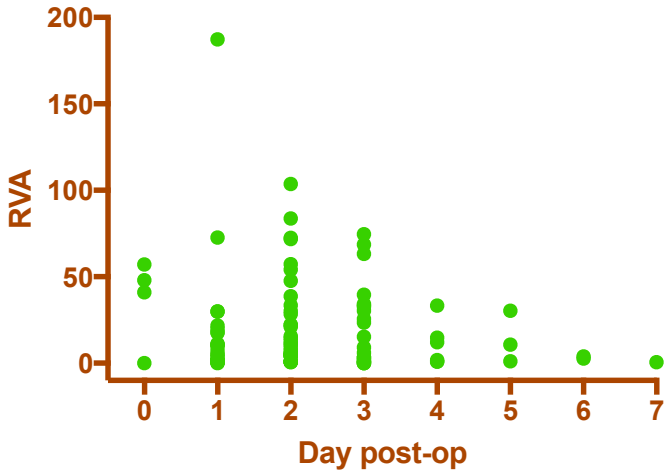

Figure 7.36; residual volume of air (RVA) by day imaging performed post-operatively (post-op), n = 100.

To assess the extent of residual fluid as a function of the original CSDH volume, the "percentage evacuated" (%-evac) was calculated as;

[Pre-operative CSDH volume - RVF]/ Pre-operative volume x 100.

All patients with a pre- and post-operative scan (n = 98) were analysed for %-evac, with a mean of 56.7%. There was no correlation between the %-evac and the pre-operative CSDH volume (Pearsons r = 0.028, p = 0.7824) (Figure 7.37A), suggesting that smaller CSDHs are no more likely to be well evacuated than larger ones. Although it is evident that some of the smaller CSDHs (<100cm^3) increased in fluid volume (thus have a negative % evacuation), suggesting the operation led to expansion instead of reduction of the subdural space. Bilateral CSDHs (n=26) had a significantly higher %-evac than unilateral CSDHs (n = 72) (unpaired T test, p = 0.0263) (Figure 7.37B). The reasons for this are not clear but the physiology of brain re-expansion may be different in bilateral cases where there is no opportunity for the brain to shift to the contra-lateral side.

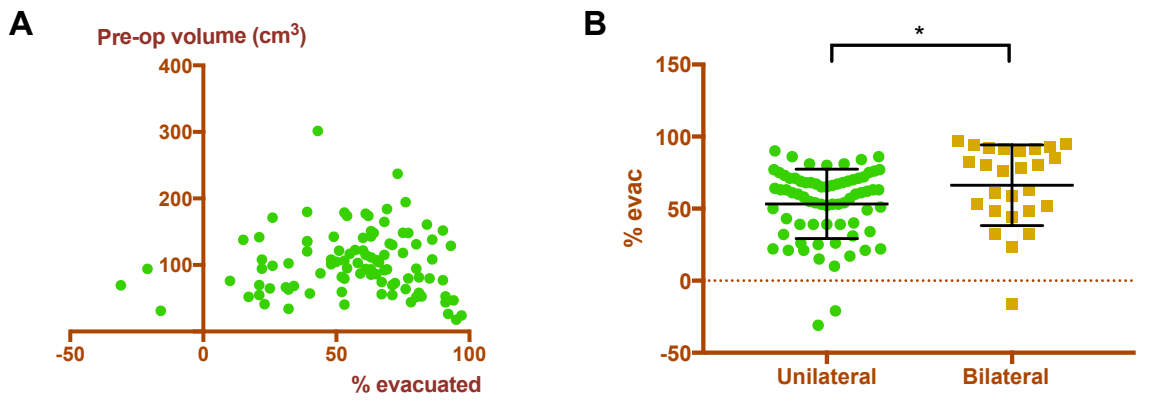

Figure 7.37; (A) correlation between pre-operative (pre-op) CSDH volume and %-evacuated, **(B)** comparison of unilateral (n = 72) and bilateral (n = 26) CSDHs for %-evacuated (evac), line (mean), bars (S.D.), statistically significant differences denoted as P≤0.05 (*).

The true volume of original CSDH evacuated is likely to be higher than the mean of 56.7% suggests, as the RVF is not necessarily all residual CSDH, but instead an amalgamation of residual CSDH, saline wash used intra-operatively and any acute haemorrhage which occurred intra- or post-operatively. Observing the change in density of fluid from pre- to post-operative imaging can help understand this further. In some patients there is a decrease in mean density as iso-, hyper- or mixed-density CSDH blood is replaced with more hypodense saline (Figure 7.38A), whereas other cases will have an increase in density when a hypodense CSDH is replaced with some hyperdense fresh bleeding (Figure 7.38B).

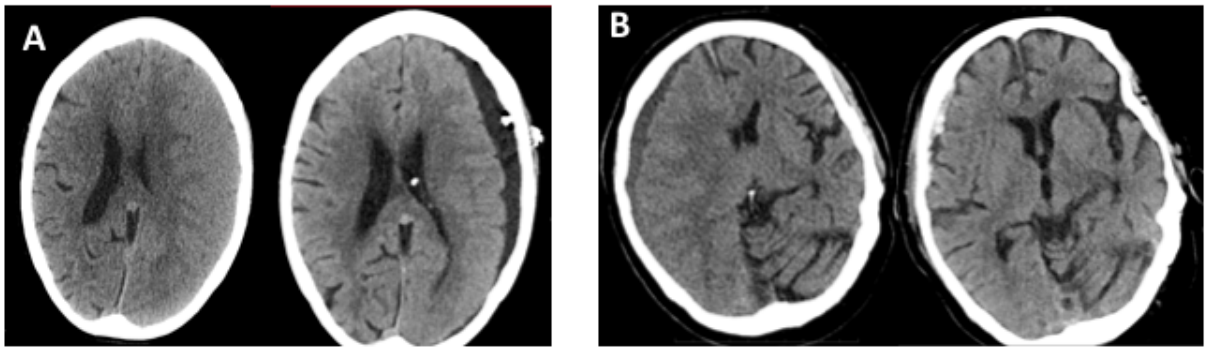

Figure 7.38; (A) pre-operative isodense CSDH (left) replaced post-operatively with hypodense saline (right), **(B)** pre-operative hypodense CSDH (left) replaced post-operatively with hyperdense acute haemorrhage (right).

The mean densities from all pre-operative and post-operative scans were 40.15 HU and 36.79 HU respectively, resulting in a significant decreased (paired T test, p = 0.0353) (Figure

7.39A). This suggests the majority of CSDHs are replaced with more hypodense fluid (i.e. saline) than with more hyperdense fluid (i.e. acute blood). There is also a weak negative correlation between pre- and post-operative density (Pearson r = -0.2465, p = 0.0144) such that the higher the density pre-operatively, the more likely it is to be lower post-operatively (due to washout and/or replacement with saline), whereas lower density CSDHs pre-operatively are more likely to increase in density, as only a tiny volume of acute bleeding would be needed to shift the density positively. However, the correlation is very weak because many CSDHs show a random pattern on the change in density (Figure 7.39B).

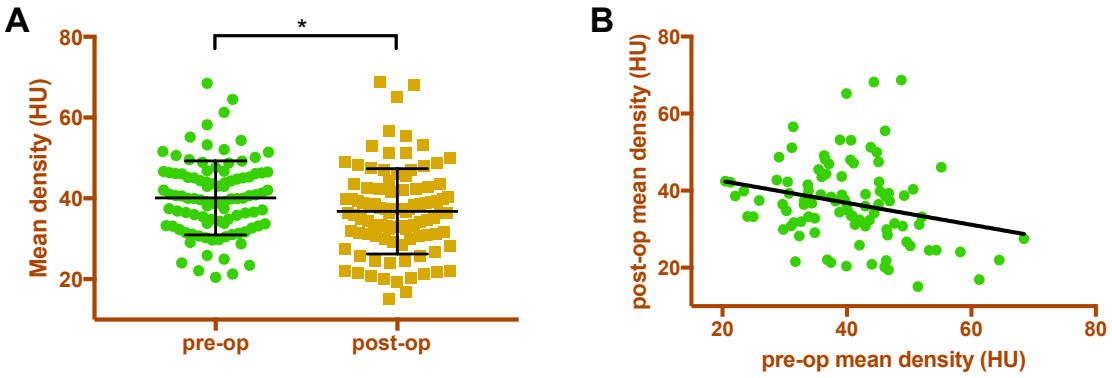

Figure 7.39; (A) change in mean densities from pre- to post-operative of CSDH, **(B)** correlation of pre- and post-operative densities, statistically significant differences denoted as P≤0.05 (*).

The TRV was significantly higher in patients who had 1BH (n = 5) in comparison to 2BH (n = 69) (Mann-Whitney, p = 0.0403), although the numbers are very small. There was no difference between 2BH and MC (Figure 7.40).

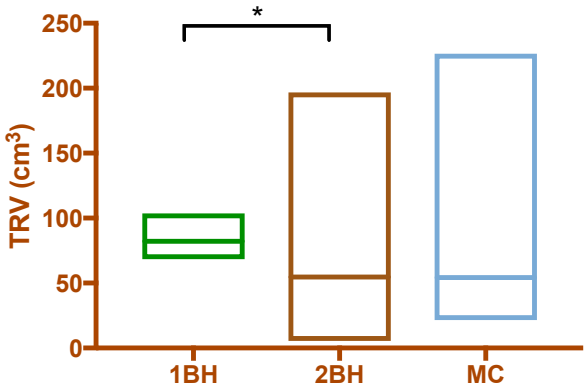

Figure 7.40; comparison of operative techniques and total residual volume (TRV). 1 burr hole (BH) n = 5, 2BH n = 69, mini-craniotomy (MC) n = 26, line (median), box (range), statistically significant differences denoted as P≤0.05 (*).

7.5.2 Post-operative imaging and recurrence risk

Significantly higher TRV was seen in the patients who went on to develop recurrence compared to those that did not (Mann-Whitney, $p = 0.0011$) (Figure 7.41A). When this is investigated further by assessing the RVF and RVA, it is clear that it is an increase in residual fluid (Mann-Whitney, $p < 0.0001$) that is the driver of this, and not air, which is actually decreased in those that go on to recur (Figure 7.41B).

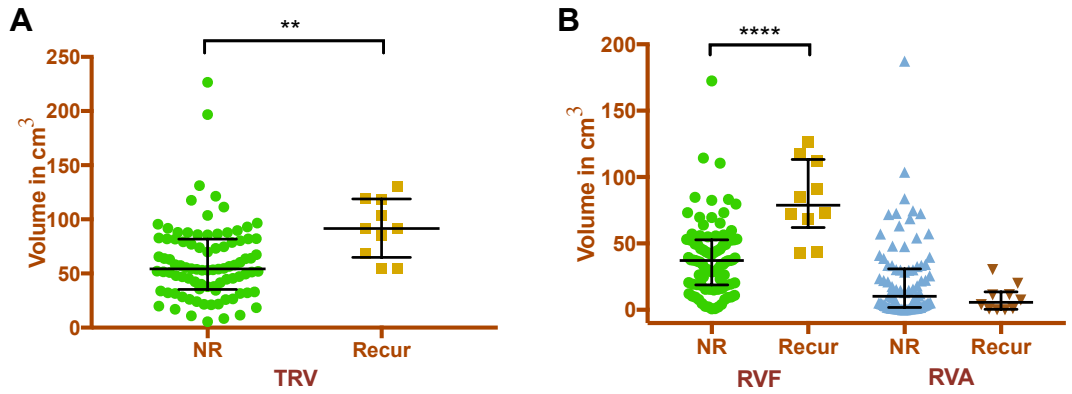

Figure 7.41; (A) total residual volume (TRV) in non-recurrent (NR) and recurrent (recur) CSDH, **(B)** residual volume of fluid (RVF) and residual volume of air (RVA) between NR and recurrent CSDH, NR n = 90, recur n = 10, line (median), bars (IQR), statistically significant differences denoted as $p < 0.01$ = **, $p < 0.0001$ = ****.

Some of the current literature contradicts this finding, suggesting that pneumocephalus is the driver of recurrence rather than fluid (Dudoit et al., 2016; You & Zheng, 2018). However, both of these studies have irregular findings with one reporting an extremely high recurrence rate of 32.6% in patients with pneumocepalus and still 17.7% in those without (You & Zheng, 2018), and the other only reporting a 4.4% recurrence rate and comparing these 15 recurrence patients with 30 selected matched controls (Dudoit et al., 2016). The latter paper also made a subjective assessment of air, only including "compressive pneumocephalus" if the cortex appeared flattened by the air. Some studies concur with my findings, that higher post-operative residual volumes of fluid are correlated to recurrence (Stanisic & Pripp, 2017; Yan et al., 2018).

As it has already been identified that most air is rapidly absorbed (Figure 7.36), it is logical that residual fluid is more likely to drive recurrence. Although, rather than drive recurrence per se, it may just be a marker of poor brain re-expansion or indeed inadequate evacuation

that contributes to recurrence. This latter point is exemplified by the significantly lower %-evac in recurrent CSDH (unpaired T-test, p <0.0001), suggesting it is not just the stand-alone residual volume that predisposes to recurrence but how this correlates to the pre-operative volume that is important (Figure 7.42).

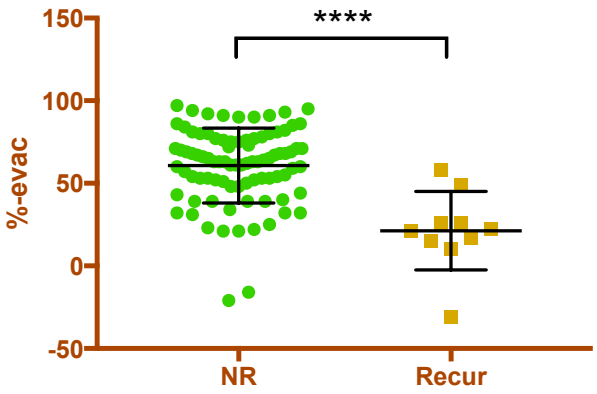

Figure 7.42; percentage (%)-evacuated of original CSDH in relation to subsequent non-recurrence (NR, n = 88) or recurrence (recur, n = 10), line (mean), bars (S.D.), statistically significant differences denoted as p < 0.0001 = ****.

Finally, when assessing the mean density of the RVF, there was a trend towards higher mean density in cases that went on to recur compared to those that did not (unpaired T test, p = 0.0968) (Figure 7.43). However, all the recurrence patients had mean densities over 34 HU, suggesting they were made up mostly of CSDH or ASDH, whilst the minimum density in the non-recurrent group was 15 HU, more in-keeping with low-density saline. This may indicate if there is a large RVF on a post-op scan but it appears very hypodense, consistent with saline, then recurrence is less likely than if there is persistent or new blood.

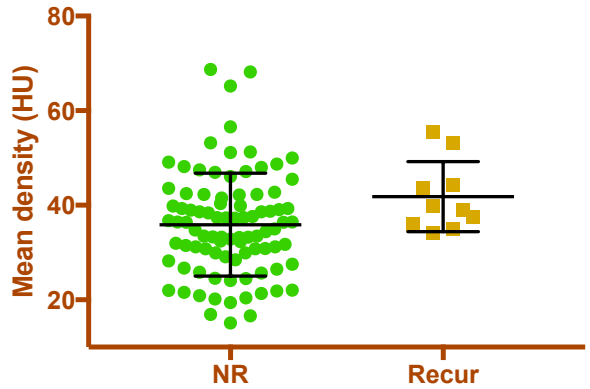

Figure 7.43; mean density of post-operative residual volume of fluid in CSDHs that are non-recurrent (NR n = 90) or recurrent (Recur n = 10).

7.6 Clinical correlations to imaging

7.6.1 Demographics and background

The mean age of the 189 patients in the imaging sub-study was 76 years with a range from 34-98 years. There was a very weak but significant correlation between age and volume of CSDH (Spearman r = 0.2674, p = 0.0002), (Figure 7.44). This suggests that older patients are slightly more inclined to have larger CSDHs, which would fit with increased atrophy in the elderly allowing more compensation for continued CSDH growth before presenting with clinical symptoms. No correlation was found between age and CSDH mean density (data not shown).

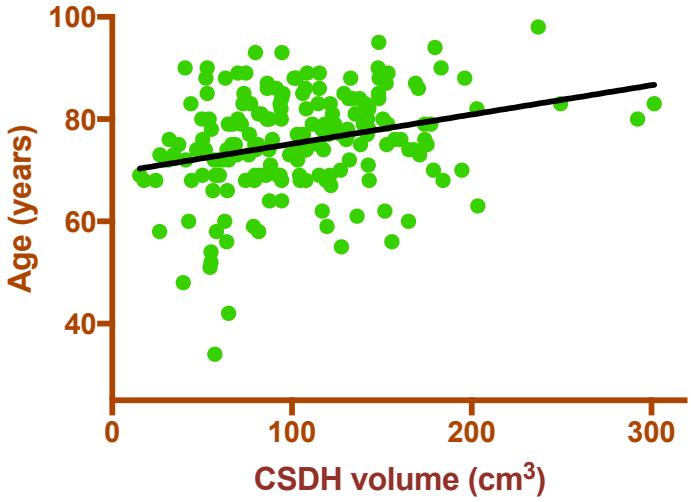

Figure 7.44; correlation between patient age and CSDH volume, spearman r = 0.2674, p = 0.0002, linear regression line y = 69.47 + 0.0572x. N = 189.

In 48/189 (25%) CSDHs the patient was female, reflecting the well-known predilection for CSDH in men. There was no significant difference in the CSDH volume or mean density between genders (data not shown). There was also no significant difference in the volume or mean density between patients who had been on anti-aggregants (AA), anti-coagulants (AC) or neither (data not shown). This is surprising as you might expect more bleeding and hence higher density in patients on AA/AC. The absence of a relationship suggests that although these medications may increase the risk of a patient getting a CSDH in the first instance, they do not appear to affect the final size or density of the CSDH on imaging.

7.6.2 Relationship with traumatic injury

A recent traumatic injury was reported in 134/189 (71%) of CSDHs with imaging available for analysis, of which 108 (81%) were unilateral and 26 (19%) bilateral. Of the 55 CSDHs with no reported history of trauma, 24 (44%) were bilateral. This high number of bilateral cases in the non-traumatic cases may be the reason why there is a small but significant decrease in volume compared to traumatic CSDHs (Mann-Whitney p = 0.0429) (Figure 7.45A). As it has already been established that individual bilateral CSDHs are significantly smaller in volume than unilateral CSDHs (Figure 7.13A). This data also appears to validate the theory in chapter two, whereby CSDHs that transform from ASDHs (CSDH-AT), and hence are traumatic, are less likely to be bilateral than those that form de novo (and thus have no history of trauma). There was no difference in the mean density between traumatic and non-traumatic CSDHs, suggesting that trauma does not influence the degree of haemorrhage within the final CSDH (Figure 7.45B).

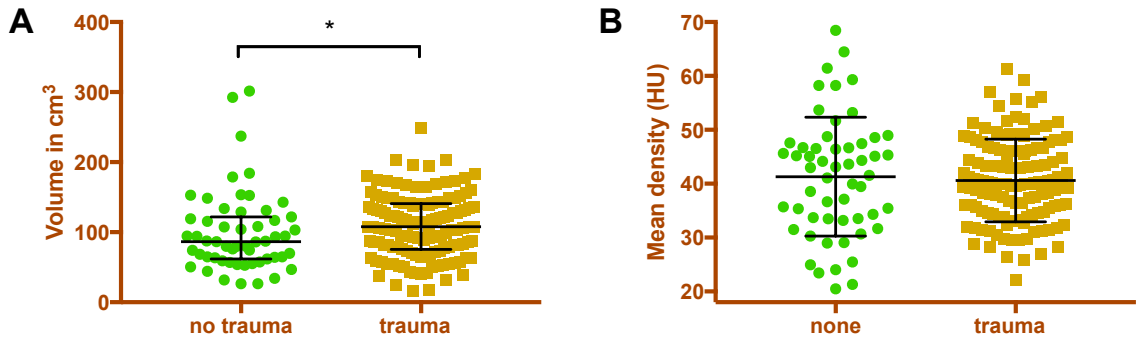

Figure 7.45; (A) volume in CSDHs where the patient reported trauma or no-trauma, line (median), bars (IQR), **(B)** mean density in CSDHs where the patient reported trauma or no-trauma, line (mean), bars (S.D.). No trauma n = 55, trauma n = 134, statistically significant differences denoted as $p<0.05$ = *.

For patients where the time of trauma was known (n = 124), they were sub-grouped into <4 weeks, 4-8 weeks and >8 weeks (as per chapter six). No significant differences were found in the volume or mean densities between time periods, although there did appear to be slightly lower mean densities in CSDHs presenting >8 weeks after trauma (Figure 7.46).

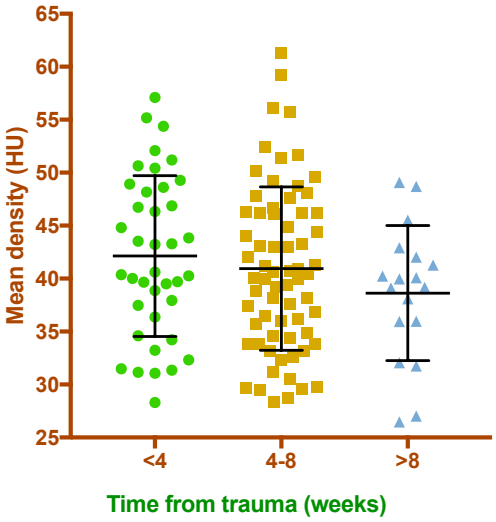

Figure 7.46; time from trauma in relation to mean density of CSDH, line (mean), bars (S.D.)

7.6.3 Inflammatory profile

Of the 189 imaged CSDHs, 65 had a neurochemistry sample collected which allowed comparison of the inflammatory profile within the CSDH to the imaging.

No significant correlations were found between any of the inflammatory markers in the CSDH fluid and either the volume, percentage-hyperdense, mean density or Nakaguchi classification of the CSDH (see examples for IL-6 in Figure 7.47). There was also no difference in any of the inflammatory markers between homogenous and mixed density CSDHs. The fact that the final volume of the CSDH does not directly correlated to the underlying inflammatory profile is probably due to the multiple confounders and variation in both CSDH size and inflammatory response in each patient. For example; some patients may have small CSDHs, because of limited atrophy or young age, but still have a highly active inflammatory response, whilst another patient with a small CSDH may be in the earlier stages of CSDH development and have lower inflammatory markers. Density would be expected to be more related to the inflammatory profile, as membranes are the source for inflammatory markers and haemorrhage, therefore these may be likely to co-exist in similar concentrations. However, it was already shown in chapter 3 that blood breakdown (as measured with MetHb concentration) was not related to inflammation, therefore bleeding and inflammation to appear to be relatively independent processes in CSDH expansion.

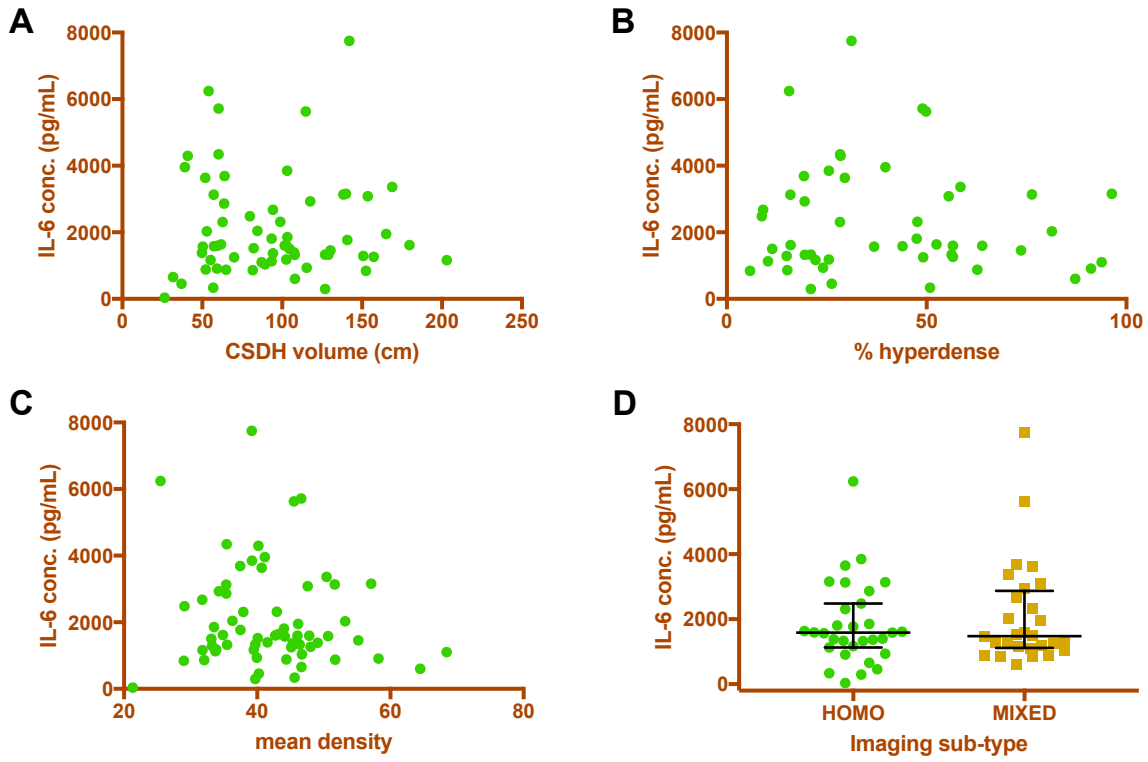

Figure 7.47; correlation of IL-6 concentration with CSDH: **(A)** volume, **(B)** mean density, **(C)** percentage-hyperdense, **(D)** imaging sub-type (homogenous = homo), line (median), bars (IQR), n = 64, 1 outlier with extremely high IL-6 excluded for easier graph representation.

7.6.4 Dexamethasone and recurrence

Of the 14 surgically treated patients who had a recurrent CSDH (one bilateral CSDH and one patient with two further surgeries), five received dexamethasone therapy (36%). However only 2/5 patients had been compliant with the medication with one patient withdrawn on day three for confusion, one on day seven for vomiting and one patient was given no dexamethasone on day two (missing 16mg in total). The remaining two patients did complete the full course of 154mg over 14 days.

Of the 135 patients treated surgically who had no recurrence, 68 (50%) were on dexamethasone and the remainder on placebo. Therefore, there is a trend to higher recurrence in placebo patients; nine recurrences out of 76 placebo patients (12%) compared to five recurrences out of 73 dexamethasone patients (7%). Although not significant, this may provide some early evidence for the role of dexamethasone in preventing recurrence (Figure

7.48). The volume between treatment groups was also comparable, so this did not influence the recurrence risk (data not shown).

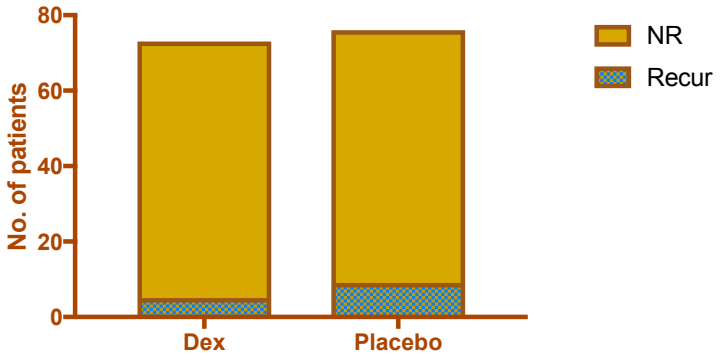

Figure 7.48; number of patients with CSDH recurrence (Recur) and no recurrence (NR) by treatment groups, line (median), bars (IQR), (dex = dexamethasone).

A previous study has suggested that the earlier the dexamethasone is started pre-operatively, the greater the impact on recurrence risk (Sun, Boet, & Poon, 2005). Therefore, the start date of the dexamethasone course was assessed, as it was permitted to start pre- or post-operatively in study patients (Table 7.3). Of the five dexamethasone-treated patients with recurrence, three patients started the course pre-operatively and the remaining two post-operatively, whereas in the non-recurrent CSDH group, more patients stated the course post-operatively (43/68). Therefore, there is no clear evidence from this data to suggest that starting the medication earlier is protective against recurrence.

Table 7.3; number of days of dexamethasone treatment pre- or post-operatively in patients with recurrent or non-recurrent CSDH.

Dexamethasone started	**Recurrent CSDH**	**Non-recurrent CSDH**
Pre-operative; total n	3/5	25/68
1 day	2	11
2 days	1	5
3 days	0	5
4 or more days	0	4
Post-operative; total n	2/5	43/68
1 day	2	36
2 days	0	7

Of the 15 patients (two with bilateral CSDH) that were managed conservatively, three failed and required subsequent surgery. Two of which were on placebo and one bilateral CSDH patient was on dexamethasone. Interestingly the patient on dexamethasone who failed conservative treatment showed a large reduction in volume on both sides of the bilateral CSDH by 45% and 39% on the pre-operative follow-up imaging (Figure 7.49). Therefore, in hindsight it could be argued this patient was showing a therapeutic effect and could have continued with conservative management if clinically stable. The two patients on placebo both showed increases in the size of the CSDH on follow-up imaging.

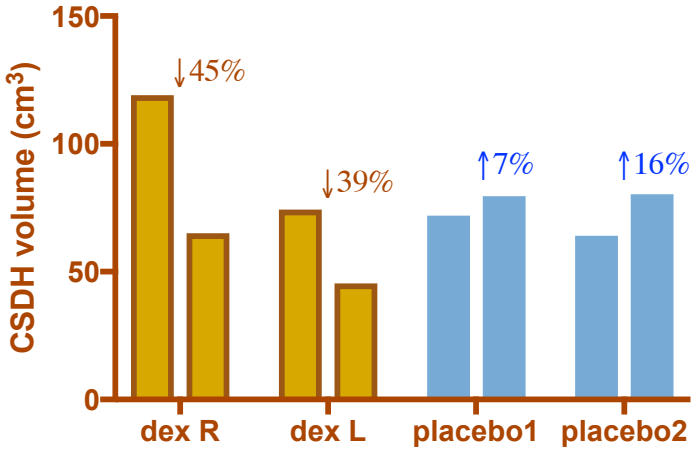

Figure 7.49; volume of CSDH at original and follow-up imaging in CSDHs that failed conservative management, with percentage change in volume shown, (dex = dexamethasone treated patient, R = right, L = left).

All three patients who failed conservative management had a pre-morbid mRS of 0; two out of three had a 3-month mRS of 0, whilst one placebo patient had a 3-month mRS of 3, this was the patient with the largest increase in CSDH size and may reflect that conservative management of CSDHs that are continuing to grow risks worsened neurological outcome. This advocates early follow-up imaging for any conservatively managed CSDHs, with careful assessment of the change in volume of the CSDH.

Of the 12 patients with 13 CSDHs (one bilateral) who were successfully managed conservatively, 7/13 (54%) were on dexamethasone and the remaining 46% on placebo. Although conservative CSDHs have already been shown to be significantly smaller in volume than those treated surgically, there was no difference in volume between the treatment groups within the conservative cohort (Figure 7.50). The fact that almost the same

number of patients were successfully treated with placebo as dexamethasone suggests that perhaps these patients had CSDHs that were small enough to resolve without treatment anyway, hence why the surgeon opted for conservative management in the first place. The only clear exception is an outlier in the dexamethasone group with a CSDH of 250cm^3, which one would not expect to resolve as part of the natural history of a large CSDH, suggesting in this patient the dexamethasone had an important therapeutic action leading to resolution of this large CSDH.

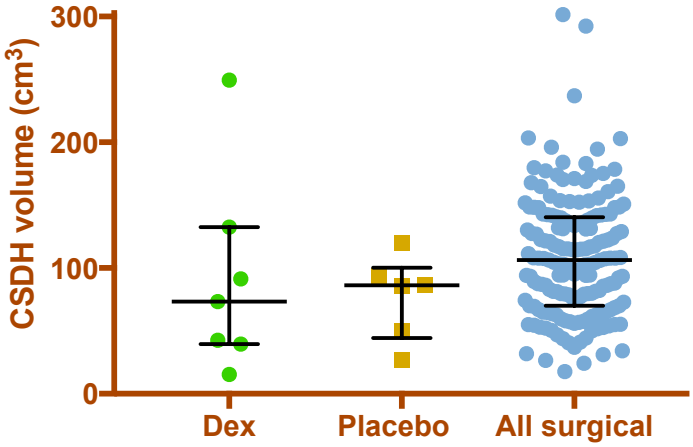

Figure 7.50; CSDH volume in successfully managed conservative patients on dexamethasone (dex) or placebo compared to all surgically treated patients (all surgical), line (median), bars (IQR).

7.7 Conclusions

A mean volume of 103 cm^3 was found across 189 CSDHs analysed in this study, with a significant but weak correlation between CSDH volume and age, suggested to some extent older patients have larger CSDHs. This is likely to relate to increased cerebral atrophy and hence more space for CSDH expansion, in older patients. This also helps explain why midline shift was found to be a good correlate for CSDH volume only in younger patients (<65), where presumably cerebral atrophy is less of a confounder.

Unilateral CSDHs were significantly larger than the individual sides of bilateral CSDHs, which were present in 25/164 (15%) patients. CSDHs in patients who did not report a history of trauma were also smaller in volume, which may also relate to the fact that more of them were bilateral. CSDHs that were selected for conservative management were also significantly smaller in volume than surgical cases, with 4/17 (24%) failing conservative management and converting to surgical treatment.

Subjective assessment of density, using the Nakaguchi classification, fitted well with mean densities and percentage-hyperdense calculated on objective computational analysis for the three homogenous sub-types. The mixed density sub-types could not be discriminated by mean density but the separated sub-type had the highest percentage-hyperdense. Volume and mean density of CSDH were not correlated and only hypodense CSDHs on the Nakaguchi classification were significantly smaller than the separated sub-type, with all other categories in between.

Neither volume or density showed any correlation to the inflammatory marker profiles. Suggesting that imaging cannot be used as an assessment of the level of underlying inflammation occurring.

Post-operative residual volumes were composed of significantly more fluid than air, with a median of 81.5% fluid, but with a wide range. Air also appears to be mostly absorbed within seven days of surgery, although few patients had post-operative imaging that late. 95% of patients had a drain placed intra-operatively, and it was only omitted if the space was considered too small for drain placement following brain re-expansion. The data here within and from previous studies supports the role of drains in preventing recurrence, therefore it should be placed wherever possible.

A mean of 56.7% of pre-operative CSDH volume was evacuated but the original size of the CSDH did not affect efficiency of evacuation, although bilateral CSDHs showed higher evacuation percentages than unilateral ones. Most CSDHs reduced in density from pre- to post-operative imaging, likely due to replacement of CSDH with hypodense saline.

Recurrence and outcome

Pre-operative CSDH volume and midline shift alone do not appear to be risk factors for CSDH recurrence, despite previous reports suggesting this in the literature. However, volume was significantly greater in patients with an unfavourable mRS on admission and at 3-month and 6month follow-up. Assuming that CSDHs grow over time, this leaves room to hypothesis that earlier diagnosis of CSDHs could potentially improve outcome by preventing neurological deterioration in relation to CSDH growth. However, age is likely to also have some influence here with older patients being more likely to have an unfavourable outcome and also more likely to have a larger CSDH.

Although previous studies suggest recurrence is higher in bilateral CSDH, we found the opposite with 1/25 (4%) patients with bilateral operative CSDHs experiencing recurrence, compared to 13/126 (10%) unilateral operative CSDHs.

Only mixed density (particularly trabecular) CSDHs had a trend towards increased recurrence. Significantly more mixed density CSDHs had an unfavorable mRS at admission, with the same trend at 3-months but none at 6-months, no difference was seen with mean density or percentage-hyperdense. As mixed density CSDH also had a significantly higher volume and older age, these three factors are likely to all contribute to some degree to the cause of poorer outcome. The complexity of the Nakaguchi classification with conflicting results in the literature and the finding that mean density calculations are not predictive of recurrence suggests that a new classification is needed. This could involve grouping CSDH appearance simply into “membranous” or “non-membranous” and is an important area for future research.

Significantly higher total residual volumes were found on post-operative imaging in patients that experienced subsequent recurrence compared to CSDHs that did not recur. Contrary to the original hypothesis, this was primarily due to residual fluid, not air, and may be the driver

for recurrence, particularly if the fluid is hyperdense, suggesting perhaps either acute bleeding or residual membranes are the driving force.

Higher recurrence in placebo compared to dexamethasone patients (12% versus 7%), with an overall rate of 9.4%, was found, although this difference was not significant. This data is comparable to recent UK literature showing a 9% recurrence rate amongst 1205 CSDH patients (Brennan et al., 2017). Whether the dexamethasone was started pre-operatively or immediately post-operatively appeared to make no difference. For patients treated conservatively, close assessment of CSDH volume on imaging may help determine whether a therapeutic effect is being seen. The growing use of dexamethasone may prove essential in both conservative CSDH treatment and prevention of recurrence in surgical patients, but the final trial results on this are still awaited.

7.8 References

Almenawer, S. A., Farrokhyar, F., Hong, C., Alhazzani, W., Manoranjan, B., Yarascavitch, B., . . . Singh, S. (2014). Chronic subdural hematoma management: a systematic review and meta-analysis of 34,829 patients. *Ann Surg, 259*(3), 449-457. doi:10.1097/SLA.0000000000000255

Bartek, J., Jr., Sjavik, K., Kristiansson, H., Stahl, F., Fornebo, I., Forander, P., & Jakola, A. S. (2017). Predictors of Recurrence and Complications After Chronic Subdural Hematoma Surgery: A Population-Based Study. *World Neurosurg, 106*, 609-614. doi:10.1016/j.wneu.2017.07.044

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet, 1*(8476), 307-310.

Brennan, P. M., Kolias, A. G., Joannides, A. J., Shapey, J., Marcus, H. J., Gregson, B. A., . . . Coulter, I. C. (2017). The management and outcome for patients with chronic subdural hematoma: a prospective, multicenter, observational cohort study in the United Kingdom. *J Neurosurg*, 1-8. doi:10.3171/2016.8.JNS16134.test

Dudoit, T., Labeyrie, P. E., Deryckere, S., Emery, E., & Gaberel, T. (2016). Is systematic post-operative CT scan indicated after chronic subdural hematoma surgery? A case-control study. *Acta Neurochir (Wien), 158*(7), 1241-1246. doi:10.1007/s00701-016-2820-6

Han, M. H., Ryu, J. I., Kim, C. H., Kim, J. M., Cheong, J. H., & Yi, H. J. (2017). Predictive factors for recurrence and clinical outcomes in patients with chronic subdural hematoma. *J Neurosurg, 127*(5), 1117-1125. doi:10.3171/2016.8.Jns16867

Huang, Y. H., Lin, W. C., Lu, C. H., & Chen, W. F. (2014). Volume of chronic subdural haematoma: is it one of the radiographic factors related to recurrence? *Injury, 45*(9), 1327-1331. doi:10.1016/j.injury.2014.02.023

Jack, A., O'Kelly, C., McDougall, C., & Findlay, J. M. (2015). Predicting recurrence after chronic subdural haematoma drainage. *Can J Neurol Sci, 42*(1), 34-39. doi:10.1017/cjn.2014.122

Kim, J., Moon, J., Kim, T., Ahn, S., Hwang, G., Bang, J., . . . Oh, C. W. (2015). Risk Factor Analysis for the Recurrence of Chronic Subdural Hematoma: A Review of 368 Consecutive Surgical Cases. *Korean J Neurotrauma, 11*(2), 63-69. doi:10.13004/kjnt.2015.11.2.63

Kuhn, E. N., Erwood, M. S., Oster, R. A., Davis, M. C., Zeiger, H. E., Pittman, B. C., & Fisher, W. S., 3rd. (2018). Outcomes of Subdural Hematoma in the Elderly with a

History of Minor or No Previous Trauma. *World Neurosurg, 119*, e374-e382. doi:10.1016/j.wneu.2018.07.168

Kwon, C. S., Al-Awar, O., Richards, O., Izu, A., & Lengvenis, G. (2018). Predicting Prognosis of Patients with Chronic Subdural Hematoma: A New Scoring System. *World Neurosurg, 109*, e707-e714. doi:10.1016/j.wneu.2017.10.058

Nakaguchi, H., Tanishima, T., & Yoshimasu, N. (2001). Factors in the natural history of chronic subdural hematomas that influence their postoperative recurrence. *J Neurosurg, 95*(2), 256-262. doi:10.3171/jns.2001.95.2.0256

Ohba, S., Kinoshita, Y., Nakagawa, T., & Murakami, H. (2013). The risk factors for recurrence of chronic subdural hematoma. *Neurosurg Rev, 36*(1), 145-149; discussion 149-150. doi:10.1007/s10143-012-0396-z

Santarius, T., & Hutchinson, P. J. (2009). Chronic subdural haematoma: time to rationalize treatment? *British Journal of Neurosurgery, 18*(4), 328-332. doi:10.1080/02688690400004845

Schwarz, F., Loos, F., Dunisch, P., Sakr, Y., Safatli, D. A., Kalff, R., & Ewald, C. (2015). Risk factors for reoperation after initial burr hole trephination in chronic subdural hematomas. *Clin Neurol Neurosurg, 138*, 66-71. doi:10.1016/j.clineuro.2015.08.002

Soleman, J., Lutz, K., Schaedelin, S., Mariani, L., & Fandino, J. (2016). Use of Subperiosteal Drain Versus Subdural Drain in Chronic Subdural Hematomas Treated With Burr-Hole Trepanation: Study Protocol for a Randomized Controlled Trial. *JMIR Res Protoc, 5*(2), e38. doi:10.2196/resprot.5339

Stanisic, M., & Pripp, A. H. (2017). A Reliable Grading System for Prediction of Chronic Subdural Hematoma Recurrence Requiring Reoperation After Initial Burr-Hole Surgery. *Neurosurgery, 81*(5), 752-760. doi:10.1093/neuros/nyx090

Sucu, H. K., Gokmen, M., & Gelal, F. (2005). The value of XYZ/2 technique compared with computer-assisted volumetric analysis to estimate the volume of chronic subdural hematoma. *Stroke, 36*(5), 998-1000. doi:10.1161/01.STR.0000162714.46038.0f

Sun, T. F., Boet, R., & Poon, W. S. (2005). Non-surgical primary treatment of chronic subdural haematoma: Preliminary results of using dexamethasone. *Br J Neurosurg, 19*(4), 327-333. doi:10.1080/02688690500305332

Torihashi, K., Sadamasa, N., Yoshida, K., Narumi, O., Chin, M., & Yamagata, S. (2008). Independent predictors for recurrence of chronic subdural hematoma: a review of 343 consecutive surgical cases. *Neurosurgery, 63*(6), 1125-1129; discussion 1129. doi:10.1227/01.Neu.0000335782.60059.17

Yamamoto, H., Hirashima, Y., Hamada, H., Hayashi, N., Origasa, H., & Endo, S. (2003). Independent predictors of recurrence of chronic subdural hematoma: results of multivariate analysis performed using a logistic regression model. *J Neurosurg, 98*(6), 1217-1221. doi:10.3171/jns.2003.98.6.1217

Yan, C., Yang, M. F., & Huang, Y. W. (2018). A Reliable Nomogram Model to Predict the Recurrence of Chronic Subdural Hematoma After Burr Hole Surgery. *World Neurosurg, 118*, e356-e366. doi:10.1016/j.wneu.2018.06.191

You, C. G., & Zheng, X. S. (2018). Postoperative pneumocephalus increases the recurrence rate of chronic subdural hematoma. *Clin Neurol Neurosurg, 166*, 56-60. doi:10.1016/j.clineuro.2018.01.029

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., & Gerig, G. (2006). User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage, 31*(3), 1116-1128. doi:10.1016/j.neuroimage.2006.01.015

Chapter 8: Trial design, recruitment and interim data

8.1 Introduction

Randomised controlled trials (RCTs) are considered the most rigorous way of determining the efficacy of an intervention (Dechartres et al., 2017), and represent the gold standard within clinical evidence based medicine. However, method design, particularly randomisation and blinding must be robust and clearly reported to avoid bias (Dechartres et al., 2017).

Neurosurgeons have reported a clear preference for data collected from an RCT (Barker, 2016), indicating that such data may be more likely to alter their clinical practice. However, there are several factors which can cause concern to clinicians when conducting an RCT, such as randomising to treatments perceived as inferior, or indeed any alternative treatment to a well-established standard, and the high time and cost investments required (Sibbald & Roland, 1998).

Several studies have published evidence that steroids, with their potent anti-inflammatory effect, can be used as a primary or adjunctive treatment for CSDH (Bender, 1974; Berghauser Pont, Dammers, Schouten, Lingsma, & Dirven, 2012; Berghauser Pont, Dirven, Dippel, Verweij, & Dammers, 2012; Delgado-Lopez et al., 2009; Qian, Yang, Sun, & Sun, 2017; Sun, Boet, & Poon, 2005; Thotakura & Marabathina, 2015). However, none are RCTs and equipoise in this area has led to inconsistent prescribing of steroids for CSDH patients (Santarius, Lawton, Kirkpatrick, & Hutchinson, 2008). Thus, it was determined that an RCT would be an appropriate method of providing definitive evidence on whether steroids are an efficacious treatment for CSDH. The Dex-CSDH (Dexamethasone for CSDH) trial was designed by a consortium of clinicians as a randomised, double-blind, placebo-controlled trial of dexamethasone for adult patients with symptomatic CSDH.

The original intention of the trial was that all patients admitted to a neurosurgical unit (NSU) with a CSDH would be treated with the trial drug (investigational medicinal product; IMP) as first-line management. If successful, this could negate the risks associated with any surgical intervention. However, although dexamethasone has a rapid onset of action, its effect on

reducing the size of a CSDH would take time, as the blood and fluid would still need to be broken down and reabsorbed. Thus, patients who are admitted *in extremis* with significant mass effect from a CSDH would still need urgent surgery. It was therefore determined that trial patients would have the IMP in addition to their standard care as determined by the treating neurosurgeon. This meant that patients requiring urgent surgery could start the IMP before or after surgical intervention, with the aim of the IMP being to prevent recurrence and thus the need for further surgery. For more stable patients the IMP could be started as first-line treatment and the patient potentially avoid surgery altogether if they improved. However, as is discussed in the data section, the majority of patients had combination treatment with surgery and IMP, with very few patients managed conservatively with the IMP alone.

The Consolidation Standards of Reporting Trials (CONSORT) group have written a statement on the minimum requirements for reporting of trials, most recently updated in 2010 (Kenneth F Schulz, Altman, & Moher, 2010). This defines what needs to be included in a trial report to facilitate transparency on how the trial was run. The Dex-CSDH trial protocol was designed in accordance with these recommendations; I was not involved in the original study design but helped finalise the protocol details and publication (Kolias et al., 2018).

This trial was funded by the National Institute for Health Research Health Technology Assessment (NIHR-HTA) scheme. As the Dex-CSDH is a Clinical Trial of an Investigational Medicinal Product (CTIMP), it is subject to The Medicines for Human Use (Clinical Trials) Regulations 2004 (UK Parliament, 2004). This legislation outlines essential processes in clinical trial conduct including Research and Ethics Committee (REC) approval, Clinical Trials Authorisation (CTA), Good Clinical Practice (GCP), pharmacovigilance and manufacture of the IMP. In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) are responsible for CTA and provided authorisation for the Dex-CSDH trial. This is considered a phase IV trial, as the IMP is already licensed, but being investigated for a new use (Medicine and Healthcase products Regulatory Agency, 2014). All trials should be registered before commencement, and the Dex-CSDH trial was registered on the European Clinical Trials Database (EudraCT 2014-004948-35) and the UK Clinical Trials Gateway (ISRCTN80782810).

A Trial Steering Committee (TSC) was set up to enable supervision of the trial on behalf of the sponsors (University of Cambridge and Cambridge University NHS Healthcare Trust) and meets biannually to review trial progress. They are supplied with reports from the Independent Data Monitoring and Ethics Committee (IDMEC), who are an independent group of clinicians who have access to unblinded trial data. The IDMEC review safety reports 3-monthly and full trial data reports 6-monthly, in order to determine whether it is safe and ethical to continue the trial. Further to this, a trial management group (TMG), including myself, the trial principal investigator (PI), a statistician and the data manager, meet weekly to discuss general administration, recruitment and any working issues.

The Dex-CSDH trial has completed recruitment at the time of submitting this thesis but follow-up is on-going, therefore final unblinded results are not available. This chapter will explore specific aspects of the trial design, the recruitment planning and blinded results from the internal pilot and interim data analysis. The trial is anticipated to complete in by June 2019, following which the final results analysis will be performed.

8.2 Trial design

Historically, neurosurgery has published fewer RCTs than almost any other medical or surgical specialty (Barker, 2016). RCTs in neurotrauma (which CSDH is considered to fall under) are particularly rare, comprising only two out of 61 RCTs published between 2000-2014 (Mansouri, Cooper, Shin, & Kondziolka, 2016). This may be due in part to the constraints an emergency situation places on time and consent opportunities for clinical trials. This was recognised as an issue by the UK clinical trials directive in 2006, leading to an amendment to the Medicines for Human Use (Clinical Trials) Regulations such that patients could be enrolled into emergency trials even if they are lacking capacity and there is no next-of-kin (NOK) to provide assent (UK Parliament, 2006). Relevant REC approval would be required, but this would significantly broaden the opportunities to recruit patients into trials on emergency interventions, particularly relevant to neurosurgery.

A public opinion survey in 2013 reported that 91% of respondents would be happy for an independent doctor to assent for patient inclusion in a severe traumatic brain injury trial (Clark et al., 2013). This has helped support the implementation of Independent Healthcare Professional (IHP) consent, where a trained trial team member and an independent doctor caring for the patient, can co-sign to enrol the patient into an approved clinical trial. As patients with CSDH commonly have cognitive impairment and treatment is urgent, during which time a NOK may not be able to attend, IHP consent was included in the study protocol to maximise the opportunities for enrolment.

With a mean age range of 68-77 years in CSDH patients, the Dex-CSDH trial needed to recruit almost exclusively from the elderly population (Baechli, Nordmann, Bucher, & Gratzl, 2004; Gelabert-Gonzalez, Iglesias-Pais, Garcia-Allut, & Martinez-Rumbo, 2005; Goto et al., 2015; Santarius & Hutchinson, 2009; Wada et al., 2014). Older patients are often under-represented in medical research, even for pathologies or medicines most relevant to their age group (Aapro, Kohne, Cohen, & Extermann, 2005; Konrat et al., 2012; McMurdo, Witham, & Gillespie, 2005). Indeed, it is recognised that elderly patients can be perceived by clinicians as being vulnerable and needing "protection from researchers", despite their desire to engage in trials (McMurdo et al., 2005). Members from a local public involvement research group (INsPIRE) were involved in the trial design to help ensure it would be acceptable for our elderly patient group.

Evidence that elderly patients are more likely to participate if follow-up if it is done from home led to the design of remote trial follow-up for the Dex-CSDH trial (Watts, 2012). Easy to follow questionnaires were designed to be completed by post or over the phone. This would negate any issues with poor mobility, lack of transport or other confining medical conditions. In the case of cognitive, visual or hearing problems, the questionnaires could be completed over the phone or with the NOK where appropriate.

8.2.1 Drug regimen

Simple drug regimens also aid compliance with elderly patients (Aapro et al., 2005). Steroids require weaning before stopping, therefore reducing doses would be required. To try and simplify this, a medication diary with pictures of the tables to be taken each day was designed by the trial team (Figure 8.1).

A randomised, double blind, placebo-controlled trial of a two-week course of dexamethasone for adult patients with a symptomatic chronic Subdural Haematoma (Dex-CSDH study)

Medication Diary

In the event of missing a dose of medication, these can be taken when remembered, but only up to the time of the next planned dose on the same day. If a dose is missed, please write MD (for missed dose) in the 'Number of Capsules Taken' column

Tick or cross the circles to indicate that each capsule was taken

		AM - Morning	PM- Lunchtime	Number of capsules taken	If feeling unwell, please give details
On days 1-3, take 4 capsules in the morning and 4 at lunchtime	Day 1 _____ Start date	○ ○ ○ ○	○ ○ ○ ○	am _____ pm _____	
	Day 2 _____ date	○ ○ ○ ○	○ ○ ○ ○	am _____ pm _____	
	Day 3 _____ date	○ ○ ○ ○	○ ○ ○ ○	am _____ pm _____	
On days 4-6, take 3 capsules in the morning and 3 at lunchtime	Day 4 _____ date	○ ○ ○	○ ○ ○	am _____ pm _____	
	Day 5 _____ date	○ ○ ○	○ ○ ○	am _____ pm _____	
	Day 6 _____ date	○ ○ ○	○ ○ ○	am _____ pm _____	*[Please turn over page to continue]*

PATIENT IDENTIFICATION NUMBER..... PATIENT INITIALS.....

Patient Medication diary v1.0 dated 12Feb15 Page 1 of 2

Figure 8.1; medication diary

Balancing the efficacy of the drug regimen against side effects and tolerability was also essential. Particularly as concerns on the use of steroids in head injury were raised following an increased 2-week and 6-month mortality reported in the "Corticosteroid Randomisation After Significant Head injury" (CRASH) trial (Edwards et al., 2005; Roberts et al., 2004).

Elderly patients also frequently have pre-existing co-morbidities such as diabetes, which can increase complications from high dose steroids (Caughey, Preiss, Vitry, Gilbert, & Roughead, 2013). Use of steroids in general have been linked with higher rates of sepsis, venous thromboembolism and fractures (Waljee et al., 2017). However, steroids are commonly prescribed for many conditions in the elderly including polymyalgia rheumatica, temporal arteritis and COPD exacerbations (Buttgereit, Dejaco, Matteson, & Dasgupta, 2016; Vondracek & Hemstreet, 2006; Walters, Tan, White, & Wood-Baker, 2018). They are also widely used in neurosurgery for cerebral oedema in brain tumour patients, where their efficacy was first proven in the 1960's (Maxwell RE, 1972). The peak age group for development of glioblastoma multiforme (GBM), the most common type of primary brain tumour, is 65-75 years, thus is a similar population to those affected by CSDH (Brodbelt et al., 2015). The side effects of steroids are dose and duration dependent, which must also be considered (Vecht, Hovestadt, Verbiest, van Vliet, & van Putten, 1994; Vondracek & Hemstreet, 2006).

The trial drug regimen was determined by the trial team, including a pharmacology specialist, and pragmatic clinical experience of prescribing steroids to neurosurgical patients. A review of the current literature on steroid dosing in CSDH can be seen in Table 8.1. The regimen in the Dex-CSDH trial starts with a high dose (16mg/day) and tapers down quickly to stop over 14 days, providing an average weekly dose of 62mg dexamethasone. This is comparable to the average steroid doses reported in previous CSDH studies, and is at the lower end of course duration, to minimise complications from prolonged use and optimise compliance.

Table 8.1; review of dexamethasone dosing schedules, adverse events and outcomes in the CSDH literature compared to the Dex-CSDH trial.

Paper (year)	**Patient number (follow-up)**	**Dex dosing schedule (average weekly dose and no. of weeks given)**	**Adverse events**	**Outcome**
Dex-CSDH trial	750 dex/placebo (6 months)	8mg BD for 3D, 6mg BD for 3D, 4mg BD for 3D, 2mg BD for 3D, 2mg OD for 2D (**62mg for 2 weeks)**	See Tables 8.9 & 8.10	Primary outcome will be mRS at 6 months.
Bender (1974)	37 (mean 2.5 years)	60-120mg prednisolone for average of 21 days **(Equivalent 70-140mg for 3 weeks)**	None reported.	Reduced bed rest & hospitalisation. 71% patients avoided surgery.
Sun (2005)	26 dex 69 dex & surgery 13 surgery	Dex alone: 16mg daily for approx. 21 days. **(112mg for 2 weeks)**	2/4 DM patients needed additional insulin, resolved on stopping treatment.	84% favourable outcome in dex alone. Recurrence 4% with dex versus 15% without.
Delgado-Lopez (2009)	101 (median 6 months)	12mg daily, tapering by 1mg every 3 days **(46.8mg for 5 weeks)**	Hyperglycaemia (14.8%) and infections (9%), 1 gastric ulcer (<1%).	78.2% dex patients avoided surgery. 96% favourable outcome with dex.
Berghauser Pont (2012)	496 (3 months)	dex 16mg daily starting median of 4 days pre-op and then weaning (**unspecified**).	Empyema 2.8% DVT/PE 1.8% Hyperglycaemia only whilst on dex.	Longer pre-operative dex dose associated with lower recurrence and no increased morbidity.
Berghauser Pont (2012)	Metanalysis of 5 studies with **total 520 patients**	Study 1-3 as per Bender, Delgado-Lopez, Sun. Study 4: 16mg/day tapering over 8wks. Study 5: 0.5mg/kg pred = 6mg dex/day for 4 wks.	Infections 9% GI bleed <1% (2/520) Hyperglycaemia 7.7-14.8% (higher with long-term use)	Good outcome in 83-100% with steroids and 64-92% surgery alone **Recurrence**: 4-27.8% with steroids and 15-26.3% surgery alone.
Emich (2014)	820 dex/placebo (24 weeks)	6 day course of dex from 16mg/day to, 4mg/day. **(68mg for 1 week)**	Trial on-going since 2014: no safety issues reported	Primary outcome will be re-operation within 12 weeks.
Chan (2015)	122 dex & surgery 126 surgery alone	16mg for 4D, 6mg for 3D, 2mg for 3D **(61.6mg for 1.5 weeks).**	No increase in adverse events with dex	6.6% recurrence dex & surgery, 13.5% surgery only. 83-85% favourable outcome in both groups.
Thotakura (2015)	26 (mean 16.5 months)	12mg/day for 3D, then tapered over 4 weeks **(27.5mg for 4 weeks)**	1 hyperglycaemia 1 gastritis.	42% avoided surgery
Prudhomme (2016)	20 dex/placebo (6 months)	12mg/day for 21D,tapered over 7D (**70.25mg for 4 weeks)**	4 hyperglycaemia, 5 other SAEs	Trial halted due to high SAE rate.
Qian (2017)	75 dex 167 no dex	4.5mg TDS for 4D, weaned every 4D (**155.13mg for 3.5 weeks)**	5/13 DM patients with hyperglycaemia.	Recurrence 8% with dex, 19.8% without dex.

(BD = twice a day, D = day, dex = dexamethasone, DM = Diabetes Mellitus, GI = Gastrointestinal, OD = Once a Day, SAE = Serious Adverse Event, TDS = Three times a Day)

8.2.2 Trial outcomes

The modified Rankin Scale (mRS) is a core instrument for measuring the degree of disability or dependence in daily activities of living (Figure 8.2). It is one of the two most common global functional outcome measures used in CSDH studies, with the other being Glasgow outcome scale (GOS) (Chari et al., 2016). However, the mRS was originally designed for, and continues to be used in stroke studies, particularly RCTs, whereas the GOS was designed for evaluating outcome after severe traumatic brain injury (TBI) (Banks & Marotta, 2007; Jennett & Bond, 1975; Sulter, Steen, & De Keyser, 1999; van Swieten, Koudstaal, Visser, Schouten, & van Gijn, 1988). The severe TBI population is usually young and patients suffer profound cognitive impairment in the long term (Shukla, Devi, & Agrawal, 2011). CSDH was considered more akin to the stroke population, with similar mean ages and clinical manifestations such as limb weakness, speech and cognitive disturbance (Santarius & Hutchinson, 2009; stroke association). Thus, the mRS was selected as the primary outcome measure in this trial.

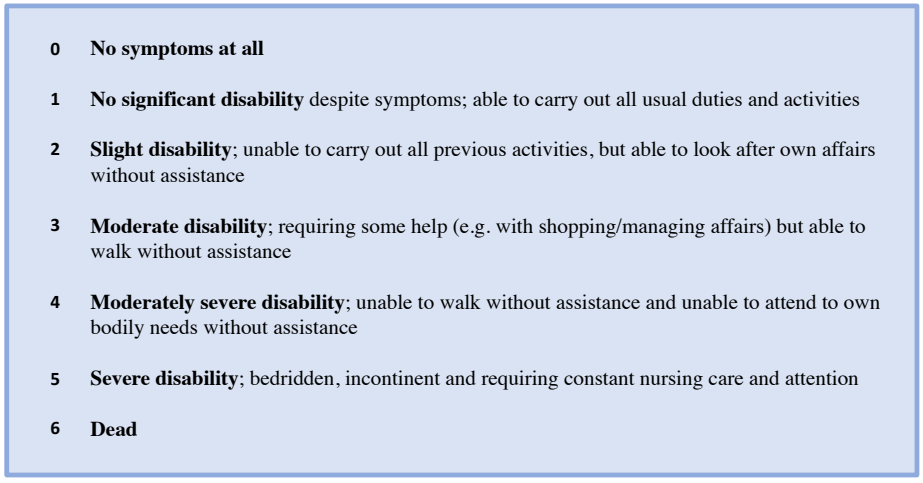

Figure 8.2; modified Rankin Scale, category 6 added (van Swieten et al., 1988)

Although the mRS is an ordinal scale, dichotomised values have been used in previous studies to optimise the statistical power to demonstrate change (Manickam, Marshman, & Johnston, 2016; Santarius & Hutchinson, 2009). Therefore, outcome would be recorded as either favourable (mRS of 0-3) or unfavourable (mRS of 4-6). A recent UK multi-centre audit on 798 CSDH patients showed that unfavourable pre-operative mRS, lack of a drain placement at operation, higher age and post-operative bed rest were all associated with a poorer outcome (mRS 4-6) on discharge (Brennan et al., 2017). Unfavourable admission and

discharge mRS scores have also been correlated with higher mortality at 6-months and one year (Manickam et al., 2016; Santarius & Hutchinson, 2009).

As it is hypothesised that dexamethasone leads to reduced CSDH recurrence and the need for surgical intervention, it was important to allow time for the effect of any surgical event on outcome. Although recurrence can occur up to one year later, the majority occur in the first four weeks, and nearly all by four months (Schmidt, Gortz, Wohlfahrt, Melbye, & Munch, 2015). Therefore, assessing outcome at six months was considered appropriate to capture the effect of surgical recurrence and subsequent recovery. The primary outcome measure in the Dex-CSDH trial was therefore determined as the modified Rankin Scale (mRS) at 6 months' post-randomisation, dichotomised into favourable (mRS 0-3) and unfavourable (mRS 4-6) outcome. A validated version of the mRS which can be used in postal questionnaires was adopted (Bruno et al., 2011). Secondary outcome measures are listed in Table 8.2 and include assessment of treatments given in addition to the IMP, additional function outcomes such as Barthel Index and EQ-5D as well as cost-effectiveness of the IMP.

Table 8.2; secondary outcome measures.

Purpose of outcome	**Outcome measure** (time point)
Assess CSDH treatment in addition to IMP	Number of CSDH surgical interventions in first admission and subsequent admissions during follow-up
Assess functional outcome	**mRS** (discharge from NSU and 3 months)
	Barthel Index (discharge from NSU, 3 months and 6 months)
	EQ-5D (discharge from NSU, 3 months and 6 months)
	Glasgow Coma Scale (discharge from NSU and at 6 months)
Other assessments of outcome	**Mortality** (30 days and 6 months)
	Adverse events (first 30 days)
Assess cost effectiveness of IMP	**Length of stay in NSU and secondary care** (all admissions)
	Discharge destination from NSU (all admissions)
	Health economic analysis (all admissions and home care/support)
Assess action of IMP	**Exploratory outcome measures** (all admissions to NSU)

(IMP = investigational medicinal product, mRS = modified Rankin Scale, NSU = neurosurgical unit)

The Barthel Index (BI) is a disability scale, commonly used alongside the mRS as a validated tool for assessing functional recovery from stroke, including both self-care (feeding, grooming, bathing, dressing and continence) and mobility (ambulation, transfers and climbing stairs) assessments (Sulter et al., 1999). The EQ-5D was designed to provide a standard instrument for describing and evaluating quality of life, and has been widely used in a range of studies and disease states (Brooks, 2015). All the functional outcome measures were assessed in one consolidated trial questionnaire, which could be completed by the patient, NOK or blinded research assessor.

Cost-effectiveness is a critical factor when considering new treatment interventions for the National Health Service (NHS), and is determined by assessing the impact of the intervention on how long a patient will live multiplied by their quality of life in those years, the so-called quality-adjusted-life-years (QALY) (Ogden, 2017)**.** During the Dex-CSDH trial, costs to the NHS and personal social services will be estimated by looking at length of stay in neurosurgical, intensive care and rehabilitation units. Additionally, informal care given by family, friends and carers will also be assessed in the final follow-up questionnaire at 6 months. This will allow a final comparison between treatment groups and an incremental cost-effectiveness ratio associated with dexamethasone to be estimated in relation to cost-effectiveness thresholds. For example, £20,000 - £30,000 per QALY is recommended by the National Institute for Health and Care Excellence (NICE, 2013).

Exploratory outcome measures were also assessed during the trial and refer to the scientific data discussed throughout this thesis, such as the biological markers and imaging findings in CSDH. This data was all collected and analysed separately, without involvement of the main study team.

8.2.3 Randomisation, blinding and safety

Patients were randomly assigned by an interactive web-based system to the intervention group (dexamethasone) or control group (placebo). There was a 1:1 allocation as per a computer-generated randomisation schedule stratified by site using permuted blocks of random sizes.

To maintain blinding, dexamethasone was over-encapsulated so that it was visibly indistinguishable from placebo. The IMP was then supplied in identical, individually numbered patient bottles. There were some potential scenarios where unblinding could occur, such as;

i) Nasogastric administration

In some situations, CSDH patients were unable to take oral medication, due to cerebral compression resulting in drowsiness or swallowing dysfunction. To avoid exclusion of such patients it was determined that the IMP could also be given via the nasogastric (NG) route. This entailed opening capsules to dissolve the contents and deliver via an NG tube. Whilst opening the capsule was potentially unblinding, it was determined as acceptable as long as it was performed by a ward nurse (who were not trial study members) and the contents not disclosed to the patient and/or in any written record. This maintained the double blinding of patient/NOK and the research team/outcome assessors.

ii) Hypergylcaemia

Hypergylcaemia is the most common and well recognised side-effect of steroids, occurring in up to 48% of non-diabetic patients on high-dose steroids (Fong & Cheung, 2013). However, it is important to recognise that hyperglycaemia can occur in any acutely unwell patient admitted to hospital, reported in around 35% (Levetan, Passaro, Jablonski, Kass, & Ratner, 1998). To maintain safety, blood glucose was monitored during in-patient stay, and therefore higher measurements could have indicated but not proven that the patient was in the active treatment arm.

iii) Emergency unblinding

Finally, as with all studies, patients could undergo emergency un-blinding in any case where the clinical team felt that knowledge of the treatment was necessary in order to improve management of the patient. In such cases the patient was withdrawn from the study following unblinding.

Close monitoring for adverse events occurred for all patients during the first 30 days of the trial, which is in-keeping with reporting periods for other CSDH studies (Sun et al., 2005). Both serious adverse events (SAEs) and adverse events of special interest (AESIs) were

reported. The latter were adverse events the trial team pre-specified as expected in relation to steroid use, from clinical experience in neurosurgery and included; hyperglycaemia, new-onset diabetes, psychosis and gastric symptoms (e.g. dyspepsia, gastric ulcer). Some SAEs were classified as "expected" as they commonly occur in patients operated on for CSDH, and therefore were exempt from expedited reporting, but recorded on an SAE log instead (Table 8.3).

Table 8.3; adverse events of special interest and expected serious adverse events

Adverse Events of Special Interest	Expected Serious Adverse Events (non-reportable)
METABOLIC - Hyperglycaemia necessitating treatment or stopping of IMP - New onset diabetes necessitating on-going medical treatment at day 30 follow-up - Hyperosmolar hyperglycaemic state	PERI-OPERATIVE - Re-bleeding into cavity forming ASDH - Tension Pneumocephalus - Intracerebral Haemorrhage - Residual CSDH exerting mass effect - Seizures - Neurological worsening - Anaesthetic complications
PSYCHIATRIC - New onset psychosis	EARLY - Residual CSDH - Expansion of contralateral CSDH - Seizures
GASTRIC - Upper gastrointestinal side (e.g. heartburn, vomiting) - Peptic ulceration and gastro-intestinal bleeding	INTERMEDIATE and LATE - Recollection of CSDH - Wound complications - Surgical site infection and subdural empyema - Epilepsy

8.2.4 Patient eligibility

CSDH is such a heterogeneous condition, ranging from very small collections which exert no mass effect on the underlying brain and resolve spontaneously, to large haematomas resulting in significant neurology and even coma. To try and target an appropriate population, only patients with a CSDH severe enough to warrant admission to an NSU were eligible for the trial. This excluded patients being managed in the community with very small CSDHs which were unlikely to ever require any treatment.

The eligibility criteria were planned in a way that would maximise participation and thus support eventual translation of findings to as broad a CSDH population as possible (Table 8.4).

Table 8.4; Dex-CSDH trial inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Adult patients (aged 18 and older)	Patients with conditions where steroids are clearly contra-indicated
Symptomatic CSDH confirmed on cranial imaging (e.g. CT/MRI).	Patients who are on (or within 1 month) of regular PO or IV glucocorticoid steroids
Informed consent or IHP authorisation	Time interval from time to admission to NSU to first dose of trial medication exceeds 72 hours
	Previous enrolment in this trial for a prior episode or concurrent enrolment in any other trial of an IMP
	Patients with CSF shunt or history of psychotic disorders
	Severe lactose intolerance or known hypersensitivity to dexamethasone or other IMP excipients, or desire to avoid gelatin

(CSF = Cerebrospinal Fluid, CT = Computerised Tomography, IMP = Investigational Medicinal Product, NSU = Neurosurgical Unit, IHP = Independent Healthcare Provider).

8.3 Sample size and recruitment

8.3.1 Sample size calculation

The Dex-CSDH trial aimed to detect an 8% increase in the rate of favourable outcome (mRS 0-3) with dexamethasone versus placebo at 6 months, which was considered to represent a clinically relevant treatment effect. A favourable outcome was estimated to occur in 80-85% of the control group based on studies investigating treatment effects in the CSDH population (Santarius & Hutchinson, 2009; Sun et al., 2005). Using a 2-sided test at the 5% significance level, a sample of 750 patients (allowing 15% loss to follow-up) would detect an absolute difference of 8% with a power of 81-92%. The study would be analysed on an intention-to-treat basis.

Given the large sample size, an internal pilot was performed to ensure feasibility before committing resource to the substantive trial. Pilot trials normally conduct the RCT on a smaller scale to try and answer whether the trial can be done, should be done and if so, how (Eldridge et al., 2016). Thus, we designed the Dex-CSDH pilot trial to act as an internal pilot due to the well-recognised benefit of data collected in the pilot contributing to the final trial analyses (Avery et al., 2017). Reviewing the results of pilot trials is helpful to inform the design of future pilot trials and their progression to substantive trials and therefore the recruitment and blinded outcome data is discussed later in this chapter (Avery et al., 2017).

The Dex-CSDH pilot recruited patients from 5-10 NSUs ranging in catchment population, research experience and resources, to reflect a realistic picture of multi-centre recruitment (Avery et al., 2017). Hospital episode statistics indicated that a medium sized NSU admits 60-80 CSDH patients per year. Setting a conservative estimate, a recruitment rate of two patients per month for each NSU was assumed, with an overall target of 100 patients to be recruited within 12 months for the pilot study. Pre-determined criteria had to be met to progress to the substantive phase of the study (Figure 8.3).

1. The target recruitment rate is 2 patients per site per month. If there is a >30% shortfall from the recruitment target (i.e. less than 70 patients have been recruited by month 12 of stage 1) without an identifiable and correctable reason it would not be feasible to progress to the main trial.
2. If the loss to follow-up (primary endpoint) exceeds 15% without an identifiable and correctable reason it would not be feasible to progress to the main trial (stage 2) without substantial changes in the study design.
3. No ethical or safety concerns raised by the IDMEC.

Figure 8.3; progression criteria for Dex-CSDH pilot trial.

8.3.2 Recruitment strategies

Recruiting to target is a challenge for every clinical trial and multi-centre trials often require an extension to meet their original target (Treweek et al., 2013). Of 64 neurosurgical RCTs registered between 2000-2012, 26.6% were discontinued early, mainly because of insufficient patient recruitment (Jamjoom, Gane, & Demetriades, 2017). Poor recruitment also contributes to the median time of 7.6 years taken from trial start to publication for neurosurgical trials (Jamjoom et al., 2017). These issues with recruitment to neurosurgical trials are likely to be due to a combination of limited research experience and infrastructure within NSUs, challenging patient populations often lacking capacity and the time-poor clinical neurosurgeon. To try and overcome some of these issues, the Dex-CSDH trial was performed in collaboration with the British Neurosurgical Trainee Collaborative (BNTRC) (Chari et al., 2018). The BNTRC identified an interested local neurosurgical trainee in each unit to act as a "Co-PI" (co-principal investigator) and aid trial set-up and recruitment.

A recent review on strategies to improve recruitment to RCTs suggested that only open-label studies and telephone reminders have been shown to increase recruitment [31]. As neither of these strategies were appropriate for this blinded trial in an acute neurosurgical population, we considered that efforts would be best directed towards promoting site engagement and incentivising investigators at each site to screen and enrol patients. Specific strategies to do this are listed in Table 8.5.

Table 8.5; recruitment strategies for site engagement

Strategies to promote initial site engagement	Strategies to promote continued recruitment at sites
Trainee co-PI at all sites; to support PI with site set-up administration and encourage local recruitment	**Monthly screening logs**; to monitor screening and allow early identification of any institutional reasons for screen failures
Face-to-face initiation; to engage maximum number of people in clinical team and answer questions/concerns before starting the trial	**Trial website and regular newsletters** with local and national recruitment figures and trial updates
Promotion of trial at regular neurosurgical meetings	**Annual investigators day** - all local PIs, co-PIs and research nurses invited.
	Top recruiting site every six months invited to attend academic neurosurgical meeting

(PI = principal investigator)

I was responsible for organising and running the annual investigators day and involvement of real trial patient in a simulated consent session gave research team members the opportunity to understand the patient perspective and experience. Patient involvement in study set-up and running has become recognised as the gold standard in modern trial design and management (Gray-Burrows et al., 2018). Often funding cannot be gained without appropriate public and patient involvement (PPI) from the early stages. Early patient-researcher interactions also allow recruiters to gain insight into the barriers and drivers for patients to be involved in trials and feedback from trial members was that patient training promotes confidence in recruiting.

8.3.3 Recruitment results

The trial opened to recruitment on August 12th 2015, six months later than anticipated due to delays in approvals. The internal pilot was completed by seven NSUs by May 2016, in nine months instead of 12, but still behind the original target recruitment line due to the delayed start. The trial continued into the substantive stage and completed recruitment nine months behind target in November 2018 (Figure 8.4)

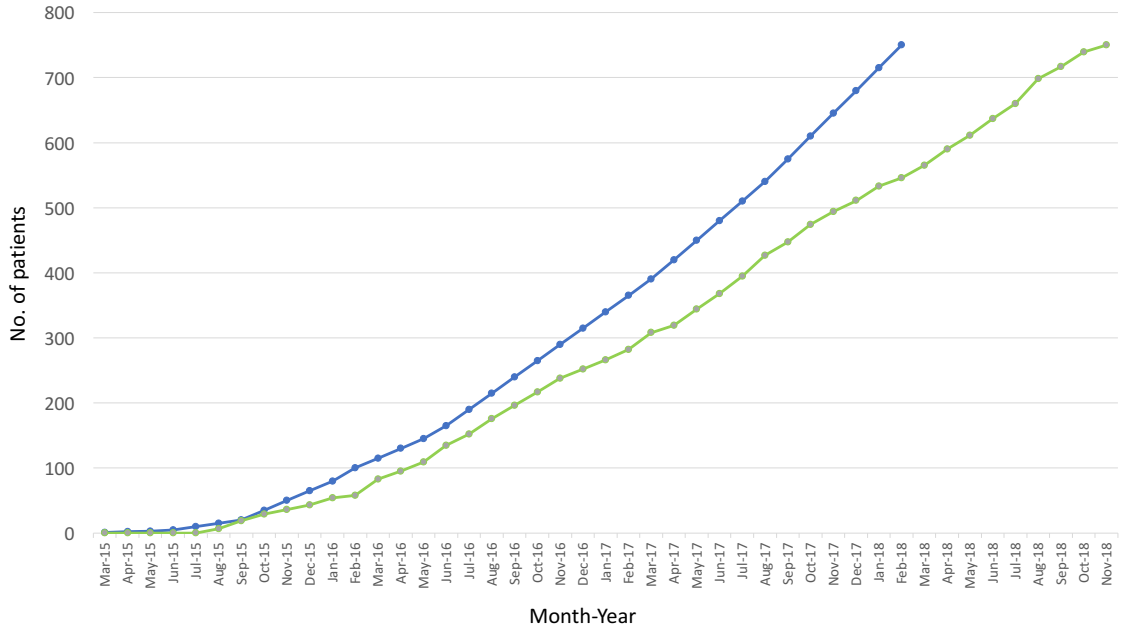

Figure 8.4; final recruitment graph with original target in blue and actual recruitment line in green.

The opening times and average recruitment rates per site during the pilot and substantive trial aid understanding of the recruitment patterns further and can be seen in Table 8.6.

Table 8.6; site opening timetable and recruitment in order of site openings.

Site	**R+D to site opening (months)**	**Date of site opening**	**Pilot:** All pts (n)	**Pilot:** mean pts/month	**ST:** All pts (n)	**ST:** mean pts/month
Cambridge (Lead site)	19	Aug 2015	75	**8**	172	**5.7***
Plymouth	7	Oct 2015	5	0.7	28	0.9
Imperial	5	Jan 2016	2	0.5	3	0.1
Southampton	4	Jan 2016	13	**3.3**	71	**2.4***
Middlesbrough	5	Mar 2016	2	1	21	0.7
Sheffield	5	Mar 2016	2	1	55	1.8
Birmingham	8	April 2016	1	1	28	0.9
Brighton	6	May 2016	N/A	N/A	26	0.9
Leeds	7	May 2016	N/A	N/A	59	**2.0***
Glasgow	7	May 2016	N/A	N/A	61	**2.0***
Stoke	9	June 2016	N/A	N/A	20	0.7
Preston	11	Aug 2016	N/A	N/A	8	0.3
Aberdeen	9	Sep 2016	N/A	N/A	10	0.4
Edinburgh	11	Oct 2016	N/A	N/A	15	0.6
Newcastle	12	Nov 2016	N/A	N/A	9	0.4
Dundee	8	Nov 2016	N/A	N/A	6	0.3
Hull	15	Mar 2017	N/A	N/A	15	0.7
Romford	9	May 2017	N/A	N/A	7	0.4
Cardiff	11	July 2017	N/A	N/A	1	0.06
RLH	6	Sept 2017	N/A	N/A	12	0.8
SGH	26	April 2018	N/A	N/A	15	**1.9***
Oxford	8	June 2018	N/A	N/A	7	1.2
Manchester	38	Nov 2018	N/A	N/A	1	1
Total (per site)	**227 exc. lead (10 months per site)**		**100**	**15.5 (2.2 per site)**	**650**	**26.2 (1.1 per site)**

*top 5 recruiting sites from substantive trial (ST).

The time from research and development (R+D) first contact to site opening was an average of 5.7 months in the pilot trial (excluding the sponsor site which required a more rigorous opening procedure), and increased to 10 months for the whole trial. Extensive administrative R+D approval processes can be a significant source of delayed recruitment for multi-site trials, such as is seen here and therefore early contact with all sites is necessary.

The average recruitment rate of 2.2 patients/month per site during the pilot trial exceeded the target of two patients/month per site. Therefore, the target recruitment plan was considered realistic and projected recruitment remained unchanged into the substantive trial. However, in hindsight, it would have been important to assess recruitment patterns in addition to the absolute numbers in order to understand recruitment feasibility and guide the recruitment projection for the substantive trial.

During the Dex-CSDH pilot trial the recruitment target was far exceeded in two of the sites (8/month in Cambridge and 3.3/month in Southampton), and was below the target in the remaining five sites. Of these five sites, two were small centres (Plymouth and Imperial) with limited populations to recruit from and the remaining three had only been open two months. However, there were clues that perhaps not all sites would attain the two patients/month target. Further to this, recruitment curves are traditionally exponential in design, but this often does not reflect the realities of trial recruitment, which after an initial take-off can remain constant. Recruitment fatigue can also mean that previously well-recruiting centres may decline over the course of the years it takes to complete a large trial. Many of the sites opened after the pilot phase recruited less well than those opened during the pilot phase, with three of the seven pilot sites being in the top five recruiting sites for the substantive trial. This may mean that strong sites were opened during the pilot period, or that the longer sites are open the better they are at recruiting. However, recruitment rates at these top centres have also remained stable or declined. Overall this led to a decline in recruitment from an average of 2.2 patients per site per month to 1.1, resulting in recruitment falling behind target in the substantive trial despite exceeding the pilot targets (Figure 8.4).

The earliest part of the recruitment process involved screening patients, and the rate of screening directly related to the subsequent recruitment rate. This was exemplified by the top two recruiting sites from the pilot, who also screened the highest number of patients (11-14

per site per month), whilst other sites only screened 2-4 patients per month (Table 8.7). Higher screening rates may have been related to the staffing at these sites, as both had a research fellow and nurse dedicated to trials. This enabled them to invest more time and effort into identifying, approaching and discussing the trial with potential patients. Most other centres were reliant on the clinical staff to screen and enrol patients, adding to their daily workload and therefore requiring significantly more motivation. There was also variable availability and support from local research nurses (RN), who often do not have a neuroscience background. It is self-evident that limited infrastructure and research staffing has an impact on the delivery of RCTs, as was seen with this trial.

Table 8.7; screening and recruitment rates at pilot sites, in order of sites opened.

NSU	All Patients screened (n)	Average screened per month (n)	Patients recruited (n)	Recruitment rate (%)
Cambridge	126	14	75	60%
Plymouth	25	4	5	20%
Imperial	9	2	2	22%
Southampton	43	11	13	30%
Middlesbrough	5	2.5	2	40%
Sheffield	2	2	2	100%
Birmingham	4	4	1	25%
Total:	**214 (6/site)**		**100**	**47%**

8.4 Blinded interim trial data

The following data is based upon a review of interim trial data analysed on the 19th October 2017. Data was available on 461 patients at this time point. Not all data points were available for all patients, but each section had no more than 10% missing data points. Percentages have been used throughout to avoid confusion about variations in the exact number of patients for each section of data.

8.4.1 Baseline data

It is well established that CSDH affects the elderly, therefore although the range of patients recruited to was from 21-95 years, the mean age was 74.1 and median 75, showing the majority are clustered in the expected age group. This age distribution is likely to affect the incidence of CSDH, which will undoubtedly increase as the elderly population expands. A recent report from the Office of National statistics showed an increase in the percentage of the population aged over 65 years from 14.2% in 1976 to 18% in 2016 and projected a rise to 24.7% by 2046 (Office for National Statistics, 2017).

In total, 73.3% of the patients recruited were male, which is similar to other large CSDH studies, reporting 64% - 82% male patients (Aspegren, Astrand, Lundgren, & Romner, 2013; Baechli et al., 2004; Brennan et al., 2017; Kanat, Kayaci, Yazar, Kazdal, & Terzi, 2010; Ro et al., 2016; Santarius & Hutchinson, 2009). It has been postulated that this gender bias relates to earlier and more significant brain atrophy occurring in men (Kanat et al., 2010). Although other studies have shown that atrophy, alongside other potential risk factors such as anti-coagulant and anti-aggregant use, trauma and alcohol abuse affect both genders equally (Aspegren et al., 2013; Marshman, Manickam, & Carter, 2015). Thus, the male preponderance remains a clinical conundrum.

Of the 461 patients analysed, 97.6% were Caucasian. Although the 2011 census revealed that 85.4% of UK residents were Caucasian, this increased to 95.3% when looking at those aged over 65 (Office for National Statistics, 2016). Therefore, although we wanted to include an ethnically diverse population, this is relatively representative of the elderly people living in the UK.

The use of anti-coagulants (AC) and anti-aggregants (AA) are often considered a risk factor for the development of CSDH, and their increasing use for conditions such as atrial fibrillation over recent years may also contribute to a growth in the number of CSDHs (Gonugunta & Buxton, 2001; Hansen et al., 2018). Interestingly, they may be most relevant to CSDH pathophysiology in patients who do not report a history of trauma (Aspegren et al., 2013). The rates of AC and AA use in the Dex-CSDH interim data can be seen in Table 8.8. Compared with previous literature on CSDH, there were higher rates of AC use (25% versus 14-16%) and similar rates of AA use (27.4% aspirin and clopidogrel versus 25-26%) (Aspegren et al., 2013; Marshman et al., 2015).

Table 8.8; anti-coagulant and anti-aggregant medication and reversal in Dex-CSDH patients

Anti-Coagulants (AC)	**Percentage of patients**
Warfarin	19.6%
Novel Oral AC: *(Apixiban, Dabigatran, Rivoroxaban, Edoxaban)*	4.2%
Low Molecular Weight Heparin *(Dalteparin, Enoxaparin, Tinzaparin)*	0.7%
Anti-Aggregants (AA)	
Aspirin	21%
Clopidogrel	6.4%
Other *(Dipyridamole, Ticagrelor)*	1.4%
All reversal treatments (can include more than one)	**26.3%**
Vitamin K	12.1%
Pro-thrombin Complex Concentrate	10%
Platelets	11.3%
Other (e.g fresh frozen plasma)	4.1%

Whether AC and AA increase the risk of CSDH recurrence is widely debated, with evidence on both sides (Aspegren et al., 2013; Fornebo et al., 2017; Gonugunta & Buxton, 2001; Wang et al., 2017). In the case where higher recurrence rates are reported in association with AAs, this can be negated by delaying surgery for at least 3 days to allow recovery of platelets (Wada et al., 2014). Often treatments are given to aid the reversal process, with Vitamin K and Pro-thrombin Complex Concentrate (PCC) used for warfarin reversal and platelets given to patients on AAs. Although approximately half the Dex-CSDH patients were on either AC

or AA, only 26.3% of patients were given any reversal, with the remainder simply given time for the medication effects to wear off. Vitamin K was the most common reversal treatment given, and was nearly always in combination with PCC, as the latter is recognised to lead to more rapid reversal (Cartmill, Dolan, Byrne, & Byrne, 2000; Woo et al., 2014). A comparison between recurrence rates and outcome for patients on AA/AC versus no treatment will be performed on trial completion to provide further definitive evidence regarding this.

Recent head trauma was reported in 69.4% of patients, which is similar to the literature rates of between 61-80% (Baechli et al., 2004; Gelabert-Gonzalez et al., 2005; Sambasivan, 1997; Santarius & Hutchinson, 2009; Stroobandt, Fransen, Thauvoy, & Menard, 1995). The timing of the trauma was known in 97% of these patients and most commonly occurred one to three months prior to CSDH diagnosis (Figure 8.5).

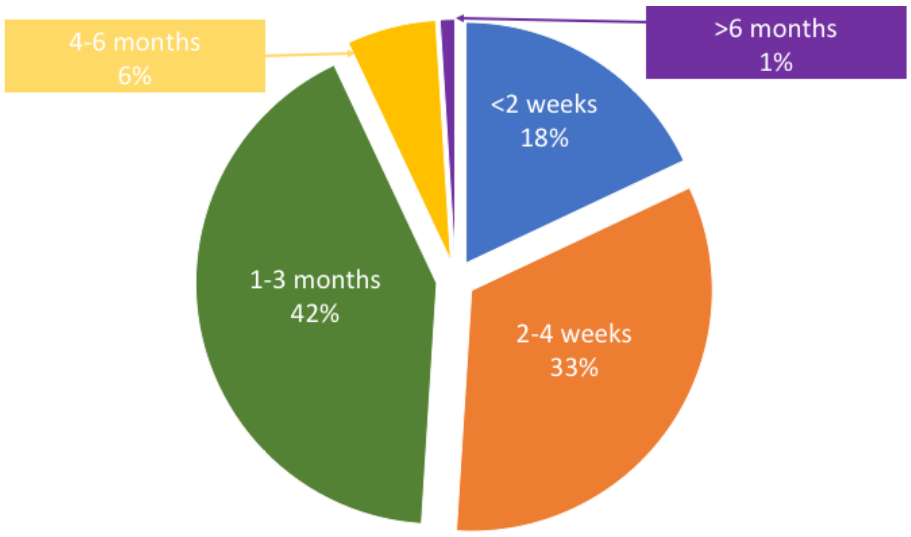

Figure 8.5; time from head trauma to CSDH diagnosis

The majority of patients (36%) had symptoms for less than a week prior to diagnosis of CSDH. A further 29% had symptoms for 1-2 weeks, 22% for 2-4 weeks, 7% 4-6 weeks and 6% for >6 weeks.

Throughout the trial approximately 10% of patients were managed conservatively with the trial IMP only, whilst the remaining 90% received surgery in addition to the trial IMP. Of those who received surgery, 84% had burr holes and 16% a mini-craniotomy. 84% of patients

also had a subdural drain inserted, which has become common practice following a recent trial showing that subdural drains reduce recurrence and mortality (Santarius & Hutchinson, 2009).

8.4.2 Adverse events

Adverse event data was available on 655 patients recruited up to July 2018. In this time period, 95 Serious Adverse Events (SAEs) occurred in 81 patients (12%). Of these, 10 patients in the pilot period and 71 in the substantive study experienced an SAE (Table 8.9). This resulted in similar SAE rates in the pilot (10%) and substantive trial (13%), suggesting pilot studies can be useful in providing preliminary data of the safety of an intervention.

The most common SAE classifications during the pilot also remained frequent in the substantive trial, and included; infections (most commonly pneumonia and not including wound infections as per Table 8.3), nervous system (most commonly stroke) and injuries (such as falls). Infections are common in hospitalised elderly patients, with reported rates ranging from 18.5 - 38.5%, increasing with intravenous and urinary catheterization (Hussain et al., 1996; Laurent et al., 2012). A meta-analysis in the stroke population, which affects a similar patient group to CSDH, also showed an overall infection risk of 30% (Westendorp, Nederkoorn, Vermeij, Dijkgraaf, & van de Beek, 2011). In this trial only 3% (20/655) of patients had an infection reported as an SAE, suggesting that CSDH patients are lower risk than most other elderly in patients. However, it may also be that most infections did not meet the benchmark to be reported as "serious", thus under-estimating the true infection rate. However, these findings still allay some of the concerns about steroid prescribing in the elderly predisposing patients to a much higher risk of serious infections. Vascular events such as deep vein thrombosis (DVT) and pulmonary embolism (PE), were the next most common SAEs occurring in 1.98% (13/655) of patients. This is within the reported range of 1.4 -4.1% DVTs and PEs in neurosurgical patients, and therefore doesn't suggest that dexamethasone increases the thrombosis risk (Chibbaro et al., 2018).

The SAEs that were considered "possibly", "probably" or "definitely" related to dexamethasone were termed serious adverse reactions (SAR). This accounted for only 8% of all SAEs and included five infective conditions (pneumonia, UTI, abscess and shingles), two

endocrine (hyperglycaemia and adrenal insufficiency) and one case of acute psychosis. As the determination of whether an SAE is "related" to the IMP is subjective, each recruiting doctor may classify this differently. For example, most episodes of pneumonia, a common surgical complication in elderly post-operative patients, were not classified as SARs, but two were. It can be argued that the classification of SAR has little value until the end of the trial when the treatment allocation is known.

Data was also collected on adverse events of special interest (AESIs) in the same 655 patients. These are adverse events anticipated to occur in this population of patients in relation to steroid use (see Table 8.3). An AESI was reported in 12 pilot patients and 36 substantive trial patients, making an overall rate of 7% (Table 8.10).

As previously discussed, hyperglycaemia is a common side-effect of dexamethasone, and was reported in six patients during the pilot (one patient had two episodes). An additional 10 patients experienced hyperglycaemia in the substantive trial, making an overall rate of 2.4% (16/655). This is significantly lower than the rates of up to 15% reported in the literature (Berghauser Pont, Dirven, et al., 2012; Fong & Cheung, 2013). This low rate may be for several reasons; firstly although diabetes is not an exclusion, due to the perceived high risk of hyperglycaemia with steroids, patients with diabetes or pre-existing hyperglycaemia of any cause may have been screened out from trial inclusion. Secondly the most unwell patients with multiple co-morbidities (who are probably also those at higher risk of hyperglycaemia) may also be screened out prior to recruitment if they are not seen as fit enough to take part in the trial or lack the ability to consent. Although IHP consent is available, we found very low uptake of this method of consent, suggesting that if the patient or NOK were not available to consent then the patient tended not to be recruited. Finally, the broad terminology used in the exclusion criteria of any patient in whom "steroids are clearly contraindicated" is open to interpretation and may be used as a reason for excluding diabetic or unwell patients. The only way to determine whether this rate of hyperglycaemia is higher than it would normally be in the CSDH population would be to take random blood sugar readings from all CSDH patients admitted and compare this with the trial cohort.

Table 8.9 - SAEs in pilot and substantive study grouped by systems.

System classification	N of events (total)	SAES during pilot	SAEs during substantive trial (* = SAR)
Cardiac	5	0	4 cardiac failure 1 acute myocardial infarction
Endocrine	3	0	1 hyperglycaemia* 1 adrenal insufficiency* 1 hyperparathyroidism
Gastrointestinal	6	1 large bowel perforation	1 vomiting 1 dysphagia 1 incarcerated hernia 1 small bowel obstruction 1 pseudo-obstruction
General	6	1 general health decline	2 deaths of unknown cause 1 general health decline 1 non-cardiac chest pain 1 pyrexia of unknown origin
Immune system	1	0	1 Anaphylactic reaction
Infections	23	1 pneumonia 1 urinary tract infection	11 pneumonia (2*) 3 c.difficile colitis/sepsis 2 Urinary tract infections (1*) 1 meninigitis 1 endocarditis 1 Influenza 1 axillary abscess* 1 shingles*
Injury	10	2 lacerations 1 fractured hip	5 falls 1 laceration 1 head injury
Metabolism and Nutrition	3		2 hyponatraemia 1 reduced oral intake
Musculoskeletal	1	0	1 intermittent back pain
Neoplasm	1		1 cholangiocarcinoma
Nervous system	14	2 acute subdural haematoma 1 stroke	4 stroke 2 acute subdural haematoma 1 intracerebral haemorrhage 1 transient ischaemic attack 1 brain oedema 1 dysphasia 1 headache and vomiting
Psychiatric	5		2 confusional state 1 acute psychosis* 1 senile dementia 1 alcohol withdrawal
Renal and urinary	1		1 acute kidney injury
Respiratory	1		1 pneumothorax
Skin and tissue	1		1 facial swelling
Vascular	14	1 deep vein thrombosis	5 pulmonary embolisms 3 deep vein thromboses 2 syncope 2 dizziness 1 intestinal infarction
Total no. of events	95	11	84
Total no. of patients	81/655 (12%)	10/100 (10%)	71/555 (13%)
SAR (% of events)	8/95 (8%)	0/11 (0%)	8/84 (10%)

(SAR = serious adverse event)

During the pilot period 11% (12/100) of patients experienced an AESI, with hyperglycaemia most common (Table 8.10). Two episodes of hyperglycaemia resolved without treatment, two resolved after stopping study medication and three required short-term treatment with either an increase in normal diabetes medication or addition of insulin. As the patients remain blinded to everyone apart from the IDMEC, we do not know whether the AESIs occurred in patients on dexamethasone or placebo. However, the current AESI rate in the substantive study is significantly lower at 7% (46/655) which is reassuring. All unblinded safety data was also regularly scrutinised by the IDMEC and there was no suggestion that the trial needed to be halted on safety grounds at any time point.

Table 8.10; Adverse events of special interest in the pilot and substantive trial.

System classification	N (total)	AESIs during internal pilot	AESIs during substantive trial
Hyperglycaemia requiring stopping of IMP	5	3	2
Hyperglycaemia requiring treatment	12	4	8
New onset Diabetes	2	1	1
Upper GI symptoms	14	3 gastric reflux	4 gastric reflux 4 nausea and vomiting 1 abdominal pain 1 instestinal obstruction 1 haematemesis (normal OGD)
Psychosis	13	1 Hallucinations	4 psychosis 3 hallucinations 2 deliruim 2 agitations 1 euphoria
Other	2		1 diarrhoea 1 constipation
Total no. of events	48	12	36
Total no. of patients (%)	46/655 (7%)	11/100 (11%)	35/555 (6%)

(OGD = oesophagoduodenoscopy).

8.4.3 Outcome data

Data on the primary outcome measure, mRS, was reviewed once all 100 pilot patients had completed their 6-month follow- up (at which point 242 patients had been recruited). A further interim analysis was performed when approximately 300 patients had completed 6-month follow-up (Table 8.11). Trial retention was excellent during the pilot with only 2/110

(1.8%) patients withdrawn and 2/110 (1.8%) lost to follow-up (LTFU) at six months, resulting in a 96.4% follow-up rate for the primary outcome. This translated into the main trial (for the first 293 patients) with a slightly higher patient withdrawal rate (4.8%), and similar rate LTFU at six months (1.7%), resulting in an overall follow-up rate of 93.5% in the interim review. This small difference of just under 3% in the final follow-up rate suggests that pilot trials are also relatively reliable in estimating follow-up rates for large trials.

Data was considered transiently missing (TM) if it could not be collected at three months, and was often due to difficult tracking patients who may still be in a care or rehabilitation facility. By six months nearly all patients had returned home or to a permanent new location known to the General Practitioner (GP), enabling improved follow-up at this final time point. Patients were only considered lost to follow-up (LTFU) if data could not be collected at six months.

Table 8.11; primary outcome measure collected during pilot and substantive trial (includes pilot data)

	patients completed time point, n	**Withdrawn** n (%)	**TM/LTFU** n (%)	**Data received** n (%)
PILOT	**242 recruited**			
Pre-morbid mRS				175
Admission mRS				170
Discharge data		11		157
3-month follow-up	160	6 (3.75%)	22 (13.75%)	132 (82.5%)
6-month follow-up	110	2 (1.8%)	2 (1.8%)	106 (96.4%)
MAIN TRIAL	**461 recruited**			
Pre-morbid mRS				385
Admission mRS				378
Discharge data		16		389
3-month follow-up	373	16 (4.3%)	27 (7.2%)	330 (88.5%)
6-month follow-up	293	14 (4.8%)	5 (1.7%)	274 (93.5%)

(LFTU = lost to follow-up, TM = transiently missing)

After completion of the pilot generous follow-up windows of -4/+8 weeks were added for the follow-up time points. This was because it was clear that there is little change in mRS scores between three and six months (Figure 8.7) and we wanted to maximise inclusion of all data received which would often trickle in between three and six months and later.

The three and six-month follow-up rates during the pilot were representative of that experienced in the main trial. This is impressive considering the number of trial sites

increased from seven to 21 in the main trial and extended from England into Scotland and Wales, which could make tracking patients more challenging. The follow-up rate at three months improved due to a decline in TM data (from 13.75% to 7.2%), this was achieved through changes to the questionnaire assessment of mRS. Originally the mRS was assessed with a validated questionnaire (often completed by the patient or carer), which involved instructions tracking which questions to go complete, although if not followed correctly then the final mRS could not be calculated (Bruno et al., 2011), see Figure 8.6A. This was simplified and the instructions removed so that all questions were answered, leading to a large reduction in missing data (Figure 8.6B). An adjudication process was also introduced so that all questionnaires where checked by a clinician before sending to the data entry team, allowing repeat patient contact within the time window if needed. These processes reduced the errors in collection of mRS data and highlight the importance of testing questionnaires in the target research population prior to use, even when previously validated. Follow-up rates were also improved by implementing a dedicated research nurse to track and do all follow-ups, instead of the administrative team who do not have clinical experience.

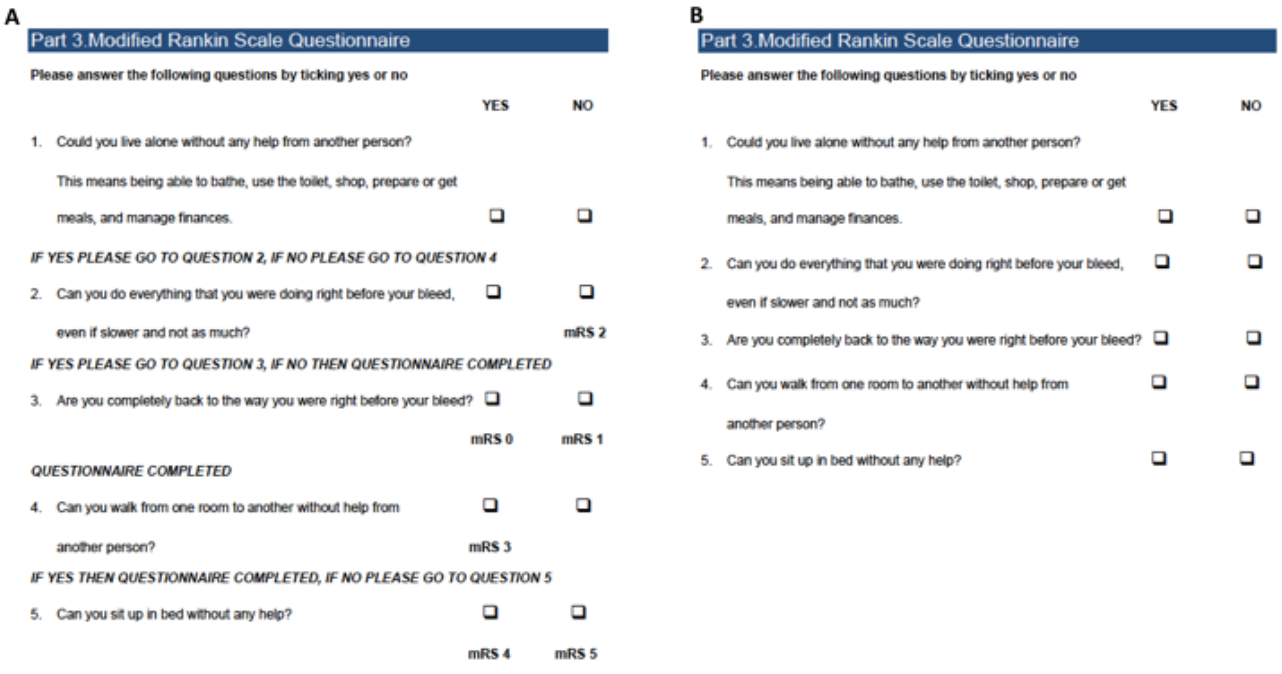

Figure 8.6; (A) original mRS questionnaire with instructions, (B) updated mRS questionnaire without instructions.

The very low rate of LTFU is likely to reflect the trial design aspects intended to maximise engagement from the target elderly population. Such that follow-up was remote and could be done by NOK/carer or a blinded assessor over the phone. A review of strategies to improve retention in randomised trials reported that money incentives improve return of postal and electronic questionnaires, however this was not considered appropriate in an NHS setting (Brueton et al., 2014). Brueton et al. also suggested that reminders have no significant effect on follow-up, however we found follow-up telephone reminders very helpful. This may be specific to our patient cohort who may suffer from cognitive and physical impairments that make filling in and returning a written questionnaire challenging. Some patients reported preferring to answer the questionnaires over the phone, therefore this is an important alternative.

Withdrawal rates have been suggested to minimise bias to trial results if maintained under 5% (K. F. Schulz & Grimes, 2002). Therefore, the current rate of 4.8% withdrawal at six months is just about acceptable and has been closely scrutinised to understand how we can minimise it. Most withdrawals relate to patients who experience an adverse event soon after starting the IMP, represented by a mean withdrawal time of 3.7 days from starting IMP (range 0-19 days). Even if the adverse event is considered unrelated to the trial IMP, experiencing such an event can change the patient's perspective and desire to participate in a trial. Wherever possible, patients who withdraw from the IMP are still encouraged to remain in the trial for follow-up to minimise the bias of missing data (Ye et al., 2011). The final study results will be analysed on an intention-to-treat basis but data on IMP non-compliance is collected so that a further analysis can be done in relation to different levels of medication compliance.

Other causes of withdrawal include patients or NOK who changed their mind or in rare cases where a clinician determined the need for withdrawal or unblinding. Unblinded data was reviewed by the IDMEC every three months throughout the trial and no ethical or safety issues were raised.

The pooled (dexamethasone and placebo patients) mRS scores from the pilot and interim substantive trial data are displayed in Figure 8.7. This includes the time points; pre-morbid (prior to onset of CSDH symptoms), admission to NSU (also termed enrolment), discharge from NSU, three month and six month follow-up.

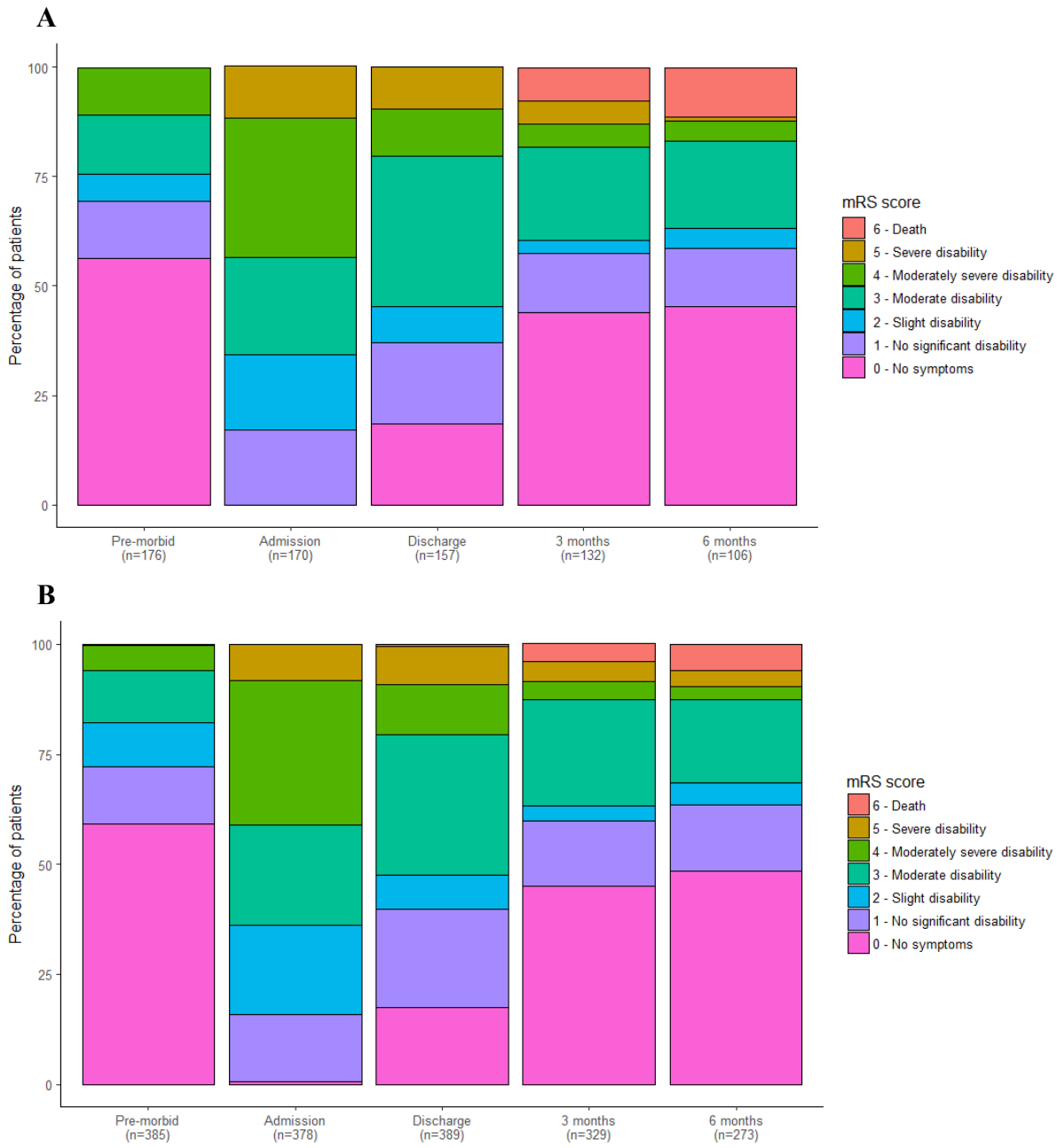

Figure 8.7; (A) pooled mRS scores from all pilot study patients (n = 106 at 6 months), **(B)** pooled mRS scores from interim data review (n = 273 at 6 months). X-axis; chronological outcome assessments (enrolment refers to admission to NSU), Y-axis; percentage of patients.

The pooled change in mRS scores from pre-morbid to six months are remarkably similar in the pilot and interim substantive trial results, suggesting the pilot was helpful in determining expected outcomes. Pilot data is often used to confirm the sample size of a larger trial for this reason. However, there has been a small shift in the pooled favourable outcome (mRS 0-3) rate at six months from 83% in the pilot study to 87% in the substantive trial to date. This

could be a sufficient difference to alter the sample size needed, and hence a secondary sample size calculation was performed after 500 patients reached six months to ensure a sufficient recruitment target. This secondary analysis suggested that 750 patients would be sufficient.

Interestingly there is little change in the rate of favourable outcome from three to six months, only that those with a poor mRS (4 or 5) at three months are more likely to be dead (mRS 6) at six months. The biggest change in mRS score from unfavourable to favourable occurs from admission to discharge, exemplifying the immediate and successful results from in-patient CSDH treatment (surgery in most cases). However, there is also a substantial increase in the number of favourable mRS scores from discharge to three months, which appears to be a more important time of recovery than from three to six months. To understand the patterns of change in mRS more fully, an ordinal analysis will be performed in addition to the primary dichotamised analysis.

The results from other functional secondary outcome measures can be seen in Table 8.12. The EQ-5D has been broken down into two parts; the visual analogue scale (VAS) and the Utility Index (UI). The VAS comprises of a vertical scale from 0 to 100 and asks the patient to grade their health from "worst possible" (0) to "best possible" (100). This method of data collection is well understood by the majority of patients leading to high rates of completion and is a good overall measure of the patients own perception of their health (Feng, Parkin, & Devlin, 2014). The UI incorporates scores from all the answers on the EQ-5D (excluding the VAS) into one overall score representing the "health state", where one is equivalent to full health, zero is death, and a negative score is when living is considered worse than death (Devlin, Shah, Feng, Mulhern, & van Hout, 2018). The BI is represented as the total score (maximum 100) of function in all the different activities of daily living and mobility.

Table 8.12; secondary outcomes measure results.

	Discharge	**3 month**	**6 month**
EQ-5D mean VAS (SD)	72.8 (18.2)	77.1 (20)	81.3 (17.9)
range	*0-100*	*3-100*	*5-100*
EQ-5D UI (SD)	0.788 (0.23)	0.844 (0.219)	0.87 (0.214)
BI mean score (SD)	79.1 (29)	87.9 (22.1)	89 (21.7)
range	*0-100*	*0-100*	*0-100*

(BI = Barthel Index, SD = standard deviation, UI = utility index, VAS = visual analogue scale)

The high rates of favourable outcome as assessed with the mRS is corroborated by the high mean BI and EQ-5D scores (Table 8.12), which also showed the most improvement from discharge to three months and little change thereafter. The high mean BI scores show that many patients have good independent function across a spectrum of daily activities, although the lowest score of 0 does mean that some patients are dependent for all care even at six months. Equally, the EQ-5D scores suggest high overall patient satisfaction with their quality of life, but again the very lowest limit in the VAS score (3/100 at three months) shows some patients perceive their health as close to the "worst possible". There is also a relatively wide standard deviation for both the secondary outcome scales showing variability in functionality and quality of life. The difficulty with analysing these outcomes is that there is no "pre-morbid" scoring to compare to, therefore those patients with the worst perceived health and functionality may have been like this prior to their CSDH. A small number of patients did have an mRS of four (moderately severe disability) pre-morbidly, and therefore were unable to mobilise independently and would have been dependent for care. Further to this some patients sustained further injuries, falls and medical illnesses during their follow-up time which were not necessarily directly related to the CSDH but severely affected their quality of life. This is to be expected in a cohort of elderly individuals with multiple co-morbidities, therefore the mean outcome scores (which are high) are more valuable than any extreme outliers.

The only facet of outcome which was not sufficiently assessed during follow-up was cognitive function. Originally the Montreal cognitive assessment (MOCA) tool was going to be used to assess cognition in all patients on discharge from the NSU and at follow-up but it requires face-to-face assessment and most patients did not have clinic follow-up. Therefore. this tool was excluded due to extremely high missing data. This does make it difficult to make any inferences on the impact CSDH has on long-term cognitive function and dementia risk, as discussed in chapter two. This is an area for future research.

8.5 Conclusions

The Dex-CSDH trial is the first randomised control trial to assess the role of dexamethasone in treating patients with CSDH. Whilst recruitment is on-going, scrutiny of the design and methodology of the trial is important as the final results are likely to significantly impact the clinical practice of CSDH treatment worldwide.

Recruitment feasibility was assessed as adequate during the internal pilot period, but closer review of the recruiting patterns may have signified that this was skewed by data from the lead site, and the recruitment target was unlikely to be met at all centres. Strategies were implemented to attempt to maximise recruitment but the clinical trials community should review the common practice of exponential recruitment projections, which are often unattainable.

With regards to trial design, the intention was to meet the needs of the elderly population as much as possible and to maximise participation and engagement with follow-up. This appears to have been successful, reflected in the excellent follow-up rates with an extremely low rate of LTFU (1.7%) and an acceptable withdrawal rate (4.8%). The drug regimen also appears to have been well tolerated with an SAE rate of only 12% overall and most events as expected with this population. The AESI rate was also only 7% and therefore is lower than one might expect when treating elderly patients with a course of steroids. True causal relationships between these adverse events and dexamethasone can only be determined once the data in unblinded, but the IDMEC have raised no safety or ethical concerns to date.

Finally, the outcome measures appear to have been successfully collected via postal and telephone questionnaire and patients have engaged well with this. The results from secondary outcomes such as BI and EQ-5D also corroborate the findings from the mRS scores of a high rate of favourable outcome at three months and little change up to six months. The pooled rate of favourable outcome measured with the mRS is within the range anticipated in the original sample size calculation, and no changes to the sample size have been required. This suggests that patients recruited to the Dex-CSDH trial have similar outcomes to the CSDH population in the literature and therefore hopefully the results will be widely applicable.

8.6 References

Aapro, M. S., Kohne, C. H., Cohen, H. J., & Extermann, M. (2005). Never too old? Age should not be a barrier to enrollment in cancer clinical trials. *Oncologist, 10*(3), 198-204. doi:10.1634/theoncologist.10-3-198

Aspegren, O. P., Astrand, R., Lundgren, M. I., & Romner, B. (2013). Anticoagulation therapy a risk factor for the development of chronic subdural hematoma. *Clin Neurol Neurosurg, 115*(7), 981-984. doi:10.1016/j.clineuro.2012.10.008

Avery, K. N., Williamson, P. R., Gamble, C., O'Connell Francischetto, E., Metcalfe, C., Davidson, P., . . . Blazeby, J. M. (2017). Informing efficient randomised controlled trials: exploration of challenges in developing progression criteria for internal pilot studies. *BMJ Open, 7*(2), e013537. doi:10.1136/bmjopen-2016-013537

Baechli, H., Nordmann, A., Bucher, H. C., & Gratzl, O. (2004). Demographics and prevalent risk factors of chronic subdural haematoma: results of a large single-center cohort study. *Neurosurg Rev, 27*(4), 263-266. doi:10.1007/s10143-004-0337-6

Banks, J. L., & Marotta, C. A. (2007). Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. *Stroke, 38*(3), 1091-1096. doi:10.1161/01.STR.0000258355.23810.c6

Barker, F. G., 2nd. (2016). Editorial: Randomized clinical trials and neurosurgery. *J Neurosurg, 124*(2), 552-556; discussion 556-557. doi:10.3171/2015.2.Jns142960

Bender. (1974). Nonsurgical Treatment of Subdural Hematomas. *Arch Neurol, 31*.

Berghauser Pont, L. M., Dammers, R., Schouten, J. W., Lingsma, H. F., & Dirven, C. M. (2012). Clinical factors associated with outcome in chronic subdural hematoma: a retrospective cohort study of patients on preoperative corticosteroid therapy. *Neurosurgery, 70*(4), 873-880; discussion 880. doi:10.1227/NEU.0b013e31823672ad

Berghauser Pont, L. M., Dirven, C. M., Dippel, D. W., Verweij, B. H., & Dammers, R. (2012). The role of corticosteroids in the management of chronic subdural hematoma: a systematic review. *Eur J Neurol, 19*(11), 1397-1403. doi:10.1111/j.1468-1331.2012.03768.x

Brennan, P. M., Kolias, A. G., Joannides, A. J., Shapey, J., Marcus, H. J., Gregson, B. A., . . . Coulter, I. C. (2017). The management and outcome for patients with chronic subdural hematoma: a prospective, multicenter, observational cohort study in the United Kingdom. *J Neurosurg*, 1-8. doi:10.3171/2016.8.JNS16134.test

Brodbelt, A., Greenberg, D., Winters, T., Williams, M., Vernon, S., & Collins, V. P. (2015). Glioblastoma in England: 2007-2011. *Eur J Cancer, 51*(4), 533-542. doi:10.1016/j.ejca.2014.12.014

Brooks, R. G. (2015). 28 Years of the EuroQol Group: an overview. *EuroQol Working Paper Series, EuroQol Group*.

Brueton, V. C., Tierney, J. F., Stenning, S., Meredith, S., Harding, S., Nazareth, I., & Rait, G. (2014). Strategies to improve retention in randomised trials: a Cochrane systematic review and meta-analysis. *BMJ Open, 4*(2), e003821. doi:10.1136/bmjopen-2013-003821

Bruno, A., Akinwuntan, A. E., Lin, C., Close, B., Davis, K., Baute, V., . . . Nichols, F. T. (2011). Simplified modified rankin scale questionnaire: reproducibility over the telephone and validation with quality of life. *Stroke, 42*(8), 2276-2279. doi:10.1161/strokeaha.111.613273

Buttgereit, F., Dejaco, C., Matteson, E. L., & Dasgupta, B. (2016). Polymyalgia Rheumatica and Giant Cell Arteritis: A Systematic Review. *Jama, 315*(22), 2442-2458. doi:10.1001/jama.2016.5444

Cartmill, M., Dolan, G., Byrne, J. L., & Byrne, P. O. (2000). Prothrombin complex concentrate for oral anticoagulant reversal in neurosurgical emergencies. *Br J Neurosurg, 14*(5), 458-461.

Caughey, G. E., Preiss, A. K., Vitry, A. I., Gilbert, A. L., & Roughead, E. E. (2013). Comorbid diabetes and COPD: impact of corticosteroid use on diabetes complications. *Diabetes Care, 36*(10), 3009-3014. doi:10.2337/dc12-2197

Chari, A., Hocking, K. C., Broughton, E., Turner, C., Santarius, T., Hutchinson, P. J., & Kolias, A. G. (2016). Core Outcomes and Common Data Elements in Chronic Subdural Hematoma: A Systematic Review of the Literature Focusing on Reported Outcomes. *J Neurotrauma, 33*(13), 1212-1219. doi:10.1089/neu.2015.3983

Chari, A., Jamjoom, A. A., Edlmann, E., Ahmed, A. I., Coulter, I. C., Ma, R., . . . Kolias, A. G. (2018). The British Neurosurgical Trainee Research Collaborative: Five years on. *Acta Neurochir (Wien), 160*(1), 23-28. doi:10.1007/s00701-017-3351-5

Chibbaro, S., Cebula, H., Todeschi, J., Fricia, M., Vigouroux, D., Abid, H., . . . Ganau, M. (2018). Evolution of Prophylaxis Protocols for Venous Thromboembolism in Neurosurgery: Results from a Prospective Comparative Study on Low-Molecular-Weight Heparin, Elastic Stockings, and Intermittent Pneumatic Compression Devices. *World Neurosurg, 109*, e510-e516. doi:10.1016/j.wneu.2017.10.012

Clark, D. J., Kolias, A. G., Corteen, E. A., Ingham, S. C., Piercy, J., Crick, S. J., . . . Hutchinson, P. J. (2013). Community consultation in emergency neurotrauma research: results from a pre-protocol survey. *Acta Neurochir (Wien), 155*(7), 1329-1334; discussion 1334. doi:10.1007/s00701-013-1748-3

Dechartres, A., Trinquart, L., Atal, I., Moher, D., Dickersin, K., Boutron, I., . . . Ravaud, P. (2017). Evolution of poor reporting and inadequate methods over time in 20 920 randomised controlled trials included in Cochrane reviews: research on research study. *Bmj, 357*, j2490. doi:10.1136/bmj.j2490

Delgado-Lopez, P. D., Martin-Velasco, V., Castilla-Diez, J. M., Rodriguez-Salazar, A., Galacho-Harriero, A. M., & Fernandez-Arconada, O. (2009). Dexamethasone treatment in chronic subdural haematoma. *Neurocirugia (Astur), 20*(4), 346-359.

Devlin, N. J., Shah, K. K., Feng, Y., Mulhern, B., & van Hout, B. (2018). Valuing health-related quality of life: An EQ-5D-5L value set for England. *Health Econ, 27*(1), 7-22. doi:10.1002/hec.3564

Edwards, P., Arango, M., Balica, L., Cottingham, R., El-Sayed, H., Farrell, B., . . . Yutthakasemsunt, S. (2005). Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months. *Lancet, 365*(9475), 1957-1959. doi:10.1016/s0140-6736(05)66552-x

Eldridge, S. M., Chan, C. L., Campbell, M. J., Bond, C. M., Hopewell, S., Thabane, L., & Lancaster, G. A. (2016). CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *Bmj, 355*, i5239. doi:10.1136/bmj.i5239

Feng, Y., Parkin, D., & Devlin, N. J. (2014). Assessing the performance of the EQ-VAS in the NHS PROMs programme. *Qual Life Res, 23*(3), 977-989. doi:10.1007/s11136-013-0537-z

Fong, A. C., & Cheung, N. W. (2013). The high incidence of steroid-induced hyperglycaemia in hospital. *Diabetes Res Clin Pract, 99*(3), 277-280. doi:10.1016/j.diabres.2012.12.023

Fornebo, I., Sjavik, K., Alibeck, M., Kristiansson, H., Stahl, F., Forander, P., . . . Bartek, J., Jr. (2017). Role of antithrombotic therapy in the risk of hematoma recurrence and thromboembolism after chronic subdural hematoma evacuation: a population-based consecutive cohort study. *Acta Neurochir (Wien), 159*(11), 2045-2052. doi:10.1007/s00701-017-3330-x

Gelabert-Gonzalez, M., Iglesias-Pais, M., Garcia-Allut, A., & Martinez-Rumbo, R. (2005). Chronic subdural haematoma: surgical treatment and outcome in 1000 cases. *Clin Neurol Neurosurg, 107*(3), 223-229. doi:10.1016/j.clineuro.2004.09.015

Gonugunta, V., & Buxton, N. (2001). Warfarin and chronic subdural haematomas. *Br J Neurosurg, 15*(6), 514-517.

Goto, H., Ishikawa, O., Nomura, M., Tanaka, K., Nomura, S., & Maeda, K. (2015). Magnetic resonance imaging findings predict the recurrence of chronic subdural hematoma. *Neurol Med Chir (Tokyo), 55*(2), 173-178. doi:10.2176/nmc.oa.2013-0390

Gray-Burrows, K. A., Willis, T. A., Foy, R., Rathfelder, M., Bland, P., Chin, A., . . . McEachan, R. R. C. (2018). Role of patient and public involvement in implementation research: a consensus study. *BMJ Quality & Safety, 27*(10), 858-864. doi:10.1136/bmjqs-2017-006954

Hansen, P. W., Sehested, T. S. G., Fosbol, E. L., Torp-Pedersen, C., Kober, L., Andersson, C., & Gislason, G. H. (2018). Trends in warfarin use and its associations with thromboembolic and bleeding rates in a population with atrial fibrillation between 1996 and 2011. *PLoS One, 13*(3), e0194295. doi:10.1371/journal.pone.0194295

Hussain, M., Oppenheim, B. A., O'Neill, P., Trembath, C., Morris, J., & Horan, M. A. (1996). Prospective survey of the incidence, risk factors and outcome of hospital-acquired infections in the elderly. *J Hosp Infect, 32*(2), 117-126.

Jamjoom, A. A. B., Gane, A. B., & Demetriades, A. K. (2017). Randomized controlled trials in neurosurgery: an observational analysis of trial discontinuation and publication outcome. *J Neurosurg, 127*(4), 857-866. doi:10.3171/2016.8.Jns16765

Jennett, B., & Bond, M. (1975). Assessment of outcome after severe brain damage. *Lancet, 1*(7905), 480-484.

Kanat, A., Kayaci, S., Yazar, U., Kazdal, H., & Terzi, Y. (2010). Chronic subdural hematoma in adults: why does it occur more often in males than females? Influence of patient's sexual gender on occurrence. *J Neurosurg Sci, 54*(3), 99-103.

Kolias, A. G., Edlmann, E., Thelin, E. P., Bulters, D., Holton, P., Suttner, N., . . . Hutchinson, P. J. (2018). Dexamethasone for adult patients with a symptomatic chronic subdural haematoma (Dex-CSDH) trial: study protocol for a randomised controlled trial. *Trials, 19*(1), 670. doi:10.1186/s13063-018-3050-4

Konrat, C., Boutron, I., Trinquart, L., Auleley, G. R., Ricordeau, P., & Ravaud, P. (2012). Underrepresentation of elderly people in randomised controlled trials. The example of

trials of 4 widely prescribed drugs. *PLoS One, 7*(3), e33559. doi:10.1371/journal.pone.0033559

Laurent, M., Bories, P. N., Le Thuaut, A., Liuu, E., Ledudal, K., Bastuji-Garin, S., & Paillaud, E. (2012). Impact of comorbidities on hospital-acquired infections in a geriatric rehabilitation unit: prospective study of 252 patients. *J Am Med Dir Assoc, 13*(8), 760.e767-712. doi:10.1016/j.jamda.2012.07.002

Levetan, C. S., Passaro, M., Jablonski, K., Kass, M., & Ratner, R. E. (1998). Unrecognized diabetes among hospitalized patients. *Diabetes Care, 21*(2), 246-249.

Manickam, A., Marshman, L. A., & Johnston, R. (2016). Long-term survival after chronic subdural haematoma. *J Clin Neurosci, 34*, 100-104. doi:10.1016/j.jocn.2016.05.026

Mansouri, A., Cooper, B., Shin, S. M., & Kondziolka, D. (2016). Randomized controlled trials and neurosurgery: the ideal fit or should alternative methodologies be considered? *J Neurosurg, 124*(2), 558-568. doi:10.3171/2014.12.Jns142465

Marshman, L. A., Manickam, A., & Carter, D. (2015). Risk factors for chronic subdural haematoma formation do not account for the established male bias. *Clin Neurol Neurosurg, 131*, 1-4. doi:10.1016/j.clineuro.2015.01.009

Maxwell RE, L. D., French LA. . (1972). *The clinical effects of synthetic glucocorticoid used for brain edema in the practice of neurosurgery* (S. K. Reulen HJ Ed.). Berlin: Springer-Verlag.

McMurdo, M. E., Witham, M. D., & Gillespie, N. D. (2005). Including older people in clinical research. *Bmj, 331*(7524), 1036-1037. doi:10.1136/bmj.331.7524.1036

Medicine and Healthcase products Regulatory Agency. (2014). *Clinical trials for medicines*. Gov.uk Retrieved from https://www.gov.uk/guidance/clinical-trials-for-medicines-apply-for-authorisation-in-the-uk.

NICE. (2013). Guide to the methods of technology appraisal 2013. *National Institute of Health and Clinical Excellence publications*.

Office for National Statistics. (2016). *CT0570_2011 Census - Sex by age by IMD2004 by ethnic group*. Retrieved from https://www.ons.gov.uk/peoplepopulationandcommunity/culturalidentity/ethnicity/adhocs/005378ct05702011censussexbyagebyimd2004byethnicgroup.

Office for National Statistics. (2017). *Overview of the UK population: July 2017*.

Ogden, J. (2017). QALYs and their role in the NICE decision-making process. *Prescriber, 28*(4), 41-43. doi:doi:10.1002/psb.1562

Qian, Z., Yang, D., Sun, F., & Sun, Z. (2017). Risk factors for recurrence of chronic subdural hematoma after burr hole surgery: potential protective role of dexamethasone. *Br J Neurosurg, 31*(1), 84-88. doi:10.1080/02688697.2016.1260686

Ro, H. W., Park, S. K., Jang, D. K., Yoon, W. S., Jang, K. S., & Han, Y. M. (2016). Preoperative predictive factors for surgical and functional outcomes in chronic subdural hematoma. *Acta Neurochir (Wien), 158*(1), 135-139. doi:10.1007/s00701-015-2625-z

Roberts, I., Yates, D., Sandercock, P., Farrell, B., Wasserberg, J., Lomas, G., . . . Edwards, P. (2004). Effect of intravenous corticosteroids on death within 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *Lancet, 364*(9442), 1321-1328. doi:10.1016/s0140-6736(04)17188-2

Sambasivan, M. (1997). An overview of chronic subdural hematoma: experience with 2300 cases. *Surg Neurol, 47*(5), 418-422.

Santarius, T., & Hutchinson, P. J. (2009). Chronic subdural haematoma: time to rationalize treatment? *British Journal of Neurosurgery, 18*(4), 328-332. doi:10.1080/02688690400004845

Santarius, T., Lawton, R., Kirkpatrick, P. J., & Hutchinson, P. J. (2008). The management of primary chronic subdural haematoma: a questionnaire survey of practice in the United Kingdom and the Republic of Ireland. *Br J Neurosurg, 22*(4), 529-534. doi:10.1080/02688690802195381

Schmidt, L., Gortz, S., Wohlfahrt, J., Melbye, M., & Munch, T. N. (2015). Recurrence of Subdural Haematoma in a Population-Based Cohort - Risks and Predictive Factors. *PLoS One, 10*(10), e0140450. doi:10.1371/journal.pone.0140450

Schulz, K. F., Altman, D. G., & Moher, D. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Bmj, 340*, c332. doi:10.1136/bmj.c332

Schulz, K. F., & Grimes, D. A. (2002). Sample size slippages in randomised trials: exclusions and the lost and wayward. *Lancet, 359*(9308), 781-785. doi:10.1016/s0140-6736(02)07882-0

Shukla, D., Devi, B. I., & Agrawal, A. (2011). Outcome measures for traumatic brain injury. *Clin Neurol Neurosurg, 113*(6), 435-441. doi:10.1016/j.clineuro.2011.02.013

Sibbald, B., & Roland, M. (1998). Understanding controlled trials. Why are randomised controlled trials important? *Bmj, 316*(7126), 201.

stroke association. What are the symptoms of stroke? Retrieved from https://www.stroke.org.uk/what-is-stroke/what-are-the-symptoms-of-stroke

Stroobandt, G., Fransen, P., Thauvoy, C., & Menard, E. (1995). Pathogenetic factors in chronic subdural haematoma and causes of recurrence after drainage. *Acta Neurochir (Wien), 137*(1-2), 6-14.

Sulter, G., Steen, C., & De Keyser, J. (1999). Use of the Barthel index and modified Rankin scale in acute stroke trials. *Stroke, 30*(8), 1538-1541.

Sun, T. F., Boet, R., & Poon, W. S. (2005). Non-surgical primary treatment of chronic subdural haematoma: Preliminary results of using dexamethasone. *Br J Neurosurg, 19*(4), 327-333. doi:10.1080/02688690500305332

Thotakura, A. K., & Marabathina, N. R. (2015). Nonsurgical Treatment of Chronic Subdural Hematoma with Steroids. *World Neurosurg, 84*(6), 1968-1972. doi:10.1016/j.wneu.2015.08.044

Treweek, S., Lockhart, P., Pitkethly, M., Cook, J. A., Kjeldstrom, M., Johansen, M., . . . Mitchell, E. D. (2013). Methods to improve recruitment to randomised controlled trials: Cochrane systematic review and meta-analysis. *BMJ Open, 3*(2). doi:10.1136/bmjopen-2012-002360

UK Parliament. (2004). *The Medicines for Human Use (Clinical Trials) Regulations 2004*. (No. 1031). The National Archive Retrieved from http://www.legislation.gov.uk/uksi/2004/1031/contents/made.

UK Parliament. (2006). *The Medicines for Human Use (Clinical Trials) Amendment (No.2) Regulations 2006*.

van Swieten, J. C., Koudstaal, P. J., Visser, M. C., Schouten, H. J., & van Gijn, J. (1988). Interobserver agreement for the assessment of handicap in stroke patients. *Stroke, 19*(5), 604-607.

Vecht, C. J., Hovestadt, A., Verbiest, H. B., van Vliet, J. J., & van Putten, W. L. (1994). Dose-effect relationship of dexamethasone on Karnofsky performance in metastatic brain tumors: a randomized study of doses of 4, 8, and 16 mg per day. *Neurology, 44*(4), 675-680.

Vondracek, S. F., & Hemstreet, B. A. (2006). Is there an optimal corticosteroid regimen for the management of an acute exacerbation of chronic obstructive pulmonary disease? *Pharmacotherapy, 26*(4), 522-532. doi:10.1592/phco.26.4.522

Wada, M., Yamakami, I., Higuchi, Y., Tanaka, M., Suda, S., Ono, J., & Saeki, N. (2014). Influence of antiplatelet therapy on postoperative recurrence of chronic subdural

hematoma: a multicenter retrospective study in 719 patients. *Clin Neurol Neurosurg, 120*, 49-54. doi:10.1016/j.clineuro.2014.02.007

Waljee, A. K., Rogers, M. A., Lin, P., Singal, A. G., Stein, J. D., Marks, R. M., . . . Nallamothu, B. K. (2017). Short term use of oral corticosteroids and related harms among adults in the United States: population based cohort study. *Bmj, 357*, j1415. doi:10.1136/bmj.j1415

Walters, J. A., Tan, D. J., White, C. J., & Wood-Baker, R. (2018). Different durations of corticosteroid therapy for exacerbations of chronic obstructive pulmonary disease. *Cochrane Database Syst Rev, 3*, Cd006897. doi:10.1002/14651858.CD006897.pub4

Wang, Y., Zhou, J., Fan, C., Wang, D., Jiao, F., Liu, B., & Zhang, Q. (2017). Influence of antithrombotic agents on the recurrence of chronic subdural hematomas and the quest about the recommencement of antithrombotic agents: A meta-analysis. *J Clin Neurosci, 38*, 79-83. doi:10.1016/j.jocn.2016.12.001

Watts, G. (2012). Why the exclusion of older people from clinical research must stop. *Bmj, 344*, e3445. doi:10.1136/bmj.e3445

Westendorp, W. F., Nederkoorn, P. J., Vermeij, J. D., Dijkgraaf, M. G., & van de Beek, D. (2011). Post-stroke infection: a systematic review and meta-analysis. *BMC Neurol, 11*, 110. doi:10.1186/1471-2377-11-110

Woo, C. H., Patel, N., Conell, C., Rao, V. A., Faigeles, B. S., Patel, M. C., . . . Flint, A. C. (2014). Rapid Warfarin reversal in the setting of intracranial hemorrhage: a comparison of plasma, recombinant activated factor VII, and prothrombin complex concentrate. *World Neurosurg, 81*(1), 110-115. doi:10.1016/j.wneu.2012.12.002

Ye, C., Giangregorio, L., Holbrook, A., Pullenayegum, E., Goldsmith, C. H., & Thabane, L. (2011). Data withdrawal in randomized controlled trials: Defining the problem and proposing solutions: a commentary. *Contemp Clin Trials, 32*(3), 318-322. doi:10.1016/j.cct.2011.01.016

Chapter 9: Conclusions and future work

9.1 Pathological origins of CSDH

The review of 41 patients who had baseline imaging prior to their CSDH diagnosis, demonstrated two different pathological origins of CSDH; De-Novo formation from a normal scan (CSDH-DN), and acute subdural haematoma that transforms (CSDH-AT). Perhaps surprisingly, CSDH-DN was more common in 26/41 (63%) of CSDH patients. This challenges the general perception that CSDH always progresses from acute haemorrhage and wider understanding of this in the medical community is necessary so that elderly patients with a "normal" scan immediately following trauma are not assumed to have experience no injury. This also highlights the importance of on-going clinical assessment in elderly patients following trauma, with a low threshold for re-imaging in the case of new or progressive symptoms. Even if symptoms progress long after the trauma this may indicate related pathology, as the mean time of diagnosis of CSDH-DN was 56 days (eight weeks) from original trauma imaging, and occurred as late as 145 days (approximately five months). Ideally, one would be able to predict the patients who will develop a CSDH following trauma, enabling earlier diagnosis and treatment. One possible route of doing this is better identification of patients with subdural hygroma, as this is likely to be involved in the pathophysiological chain of events forming a CSDH-DN, and was present in 14/25 (56%) baseline trauma images. It is more commonly apparent at scans delayed from trauma, so it is particularly important to assess for this in any imaging performed later. However, as the incidence of subdural hygromas that resolve is unknown, this may still have limited predictive value for CSDH. Overall, greater awareness of this pathophysiological mechanism should lead to closer follow-up and research on this patient group to improve early diagnosis and allow possible preventative treatment options. This is particularly relevant in the era of the new application of pharmacological therapies, which, if proven to work, could conceivably be applied early to prevent the progression from subdural hygroma to CSDH entirely.

The mean time from ASDH to CSDH-AT diagnosis was 16 days, suggesting 10-14 days would be a good time delay for re-imaging conservatively managed ASDHs to diagnose those developing CSDHs needing treatment.

Importantly the pathological origin of CSDH did not influence the outcome as measured with GCS at discharge or mRS at three and six months. However longer and more detailed follow-up studies with greater patient numbers would be valuable in confirming that this. There is a growing body of research interested in the long-term impact of head injury and cerebral atrophy, as this may also have an impact on dementia risk. This is highly relevant to the CSDH population, who are already largely elderly and often have established, and in some cases significant, cerebral atrophy. Therefore, studies with follow-up times exceeding the normal 6-12 months should be planned in the future, with a focus on long-term imaging changes as well as detailed cognitive as well as functional assessment.

9.2 CSDH composition, inflammation and the impact of dexamethasone

This work has demonstrated that CSDHs contain significant amounts of haemorrhage, with an average red cell density approximately 1/3 of that measured in paired venous blood. This haemorrhage is of differing ages, which can be measured to some extent by evaluating the methaemoblobin content by UV-Vis spectroscopy. CSDHs that are low density on imaging are likely to contain more methaemoglobin and be larger in volume compared to those which are more hyperdense, containing more acute haemorrhage. Importantly, this latter group have a higher recurrence risk, therefore perhaps clinicians should try and either avoid operating on CSDHs whilst they contain higher concentrations of acute haemorrhage/hyperdensity if the patient is well and/or engage alternative treatments such as dexamethasone, if shown to be beneficial in the final Dex-CSDH trial results, to avoid recurrence.

All CSDHs were found to contain an abundance of inflammatory markers, including the novel markers IL-1α, MIP-1α and MIP-1β which contribute to the pro-inflammatory environment in CSDH. VEGF was the marker with highest concentrations in comparison to peripheral plasma and therefore likely to be critical in CSDH development, with a role in both inflammation and angiogenesis. The next highest concentrations were seen with MCP-1, IL-6, IL-8 and IP-10, all pro-inflammatory markers, several of which were correlated with VEGF or one another, supporting the theory of an interactive inflammatory cascade. Despite the belief that CSDHs are all independently contained collections, patients with bilateral CSDHs showed congruity between the inflammatory profiles on each side in most cases, suggesting either communication or co-ordination of the response in each patient.

Many of the inflammatory markers were higher in the recurrent samples compared to primary samples, suggesting on-going escalating inflammation as a cause of recurrence. The markers in primary CSDH were not predictive of recurrence risk, apart from IL-10 which was significantly lower in primary CSDHs that went on to recur. This may suggest that a lack of an appropriate anti-inflammatory response contributes to recurrence. MMP-9 may also be important for driving recurrence as it was more commonly lower in CSDH than plasma in most primary CSDHs, whereas the opposite pattern was observed for most recurrent CSDHs. This may implicate MMP-9 in the early development of CSDH membranes, which is then re-activated by surgery, particularly as very high concentrations were seen in post-operative drain samples (mean 1557% increase at 41-65 hrs). Interestingly, most of the markers escalated in the post-operative drain samples, suggesting there is a reactive increase in the first few days rather than an obliteration of inflammation from "washing out" during surgery; MIP-1, MMP-9 and IL-1β escalated to the highest levels post-operatively. However, those patients treated with dexamethasone did appear to show reduced peaks in inflammatory markers post-operatively, although this only reached statistical significance for VEGF at 25-40hrs. Conversely, the intra-operative samples had higher concentrations of inflammatory markers, including VEGF, in the dexamethasone treated group. This either suggests that dexamethasone only works post-operatively, or that VEFG (and some other markers) are stimulated in the initial stages of "repair" and resolution of CSDH.

Dexamethasone is rapidly metabolised within the peripheral circulation and it is unclear whether it can pass freely into the subdural space, but it does not accumulate there. Its relationship with the inflammatory markers is clearly complex, and many more samples in dexamethasone treated patients are needed to understand this fully. Unfortunately, post-operative concentrations of inflammatory markers cannot be measured for longer than 48 hrs in most cases as the drains are removed, therefore it is impossible to know what on-going pattern of inflammation occurs between primary and recurrent CSDH. It was hoped that the inflammatory markers may correlate to patterns on imaging, so that this could be used as an alternative measure of inflammation, but no such correlations were found. This may be due to the fact that the inflammatory marker profiles were also unrelated to the degree of acute haemorrhage found within each CSDH sample, suggesting that although they must interact, rates of haemorrhage and inflammation are relatively independent processes during CSDH

growth. Imaging is much better aligned to measure the haemorrhage, rather than inflammation, thus the only way to measure the outcome of any effective anti-inflammatory treatment is by the final end-point; CSDH resolution.

9.3 Clinical outcome and the role of imaging in CSDH

Overall, clinical outcome in good for most patients who suffer with CSDH, with 87% of patients showing a favourable mRS score at six months. There is also complimentary data showing high mean scores with functional and quality of life assessments (BI and EQ-5D) at three and six months. However, it is evident that some patients still have a poor outcome, and significant morbidity or even mortality in relation to CSDH, and therefore an opportunity to improve this.

The imaging results from this study have shown that larger and mixed density CSDHs have the highest potential for a poor outcome, but not necessarily recurrence. This appears to relate to two issues; the correlation with older age and more significant neurological deficit on admission. It is logical that patients whose initial neurology is worse have a lower potential for recovery and this supports the need for earlier diagnosis and treatment of CSDH, before severe neurological deterioration occurs. There is a lot of interest in classifying the density of CSDH, but the results in the literature are conflicting on how and whether this can predict recurrence. The very poor inter-rater reliability shown with the most common density classification used (Nakaguchi, Tanishima, & Yoshimasu, 2001) may explain why data is conflicting and this advocates a simpler and more user-friendly approach. Quantative evaluation of density in this thesis has not shown to be predictive either, and I suggest that a new approach to assessing just the "membranous" pattern of the CSDH may yield more useful information in the future.

The widespread implementation of subdural drain placement during CSDH surgery has reduced recurrence rates in the last decade, and is advocated in the literature. Despite this there is an on-going recurrence rate of around 9% and potential to improve this. Post-operative imaging to assess residual volumes of fluid, particularly when hyperdense, may help predict those patients at the highest risk of recurrence, indicating closer follow-up. Such patients may also be eligible for targeted adjuvant treatments such as post-operative

dexamethasone. The Dex-CSDH trial is aimed at answering whether dexamethasone significantly improves the functional outcome (as measured with mRS) in all CSDH patients. If shown to be efficacious then it is likely to be widely adopted into clinical practice, however if the trial does not show significance then it may be that dexamethasone needs to be more selective applied rather than completely disregarded (i.e. given only to those patients at greatest risk of recurrence). The cost-effectiveness of the treatment will also be assessed in the trial, with the anticipation that preventing operative intervention or re-intervention will allow a significant cost saving in terms of the surgery, in-patient stay and associated morbidly. However, for patients in whom urgent surgery is indicated anyway, the addition of dexamethasone for all cases may be an unnecessary cost and risk, when it could be targeted at only those patients suspected to be at high risk of recurrence and/or poor outcome.

9.4 Future CSDH research

Widespread identification of CSDH as DN or AT will help determine whether this pathophysiological division is useful clinically. Prospective radiological and clinical review of elderly patients with "normal" post-trauma CT imaging may help identify markers (i.e. subdural hygroma) than can lead to earlier diagnosis of CSDH-DN before clinical deterioration and surgery is required.

Longer-term follow-up studies on CSDH patients will aid our understanding of whether this condition increases the risk of cerebral atrophy and/or accelerated cognitive decline and dementia. An area of critical importance in the ever-aging population, particularly if adjuvant therapies such as dexamethasone, not only reduce recurrence but improve cognitive recovery in general. This, as always, must be finely balanced against the risks and side-effects of such therapies, although these have shown to be acceptable in the pilot data presented here.

The small number of patients who started their dexamethasone therapy prior to surgery has limited the data available for analysis on the mechanism of action of dexamethasone. Therefore, further work is needed, ideally with large numbers of samples from dexamethasone-treated patients. The availability and value of such data will depend almost entirely on the success or failure of the Dex-CSDH trial. If dexamethasone is adopted into clinical practice following trial results then this will provide the opportunity to collect and

interrogate samples further. Information gained here on the varying patterns of inflammatory profiles with different areas of CSDH should promote multiple-sampling were possible. If the Dex-CSDH trial is neutral or negative then further study on its mechanism will be more challenging. However, assessment of the inflammatory profiles seen in CSDH fluid and how they change over time and in response to medical therapies is clearly an area for on-going research. It may be that a more appropriate treatment solution for CSDH is targeted anti-VEGF treatment, which is already being applied to angiogenic diseases such as macular degeneration (Pieramici & Rabena, 2008).

There is individual case-based data within this thesis to suggest that dexamethasone does reduce recurrence post-operatively, but also that it can aid conservative management of CSDH. As the Dex-CSDH trial has mainly recruited surgically-treated CSDH patients, a further study focused on early, conservative treatment of CSDH with dexamethasone is likely to be indicated. Such a study could also provide more information about the natural course of CSDH, as surprisingly some CSDHs just over 100cm^3 in volume resolved despite receiving only placebo treatment. There is a fine balance to be met between early CSDH treatment to prevent deterioration and over-treating CSDHs which would otherwise have resolved.

Finally, relatively little has been explored on the role of anti-platelet and anti-coagulant drugs in the pathophysiology of CSDH development and recurrence. This was due to the limited and heterogenous data available, with varying time points from stopping and reversing these medications. However, as hyperdensity, and increased recent haemorrhage is clearly implicated in CSDH growth and recurrence, these drugs are likely to be important. Therefore, prospective clinical data needs to be collected and amalgamated with imaging and surgical findings to understand this further.

Clearly there is still much to learn about the pathophysiology and treatment algorithms for this interesting and complex condition. Hopefully the findings of this thesis have improved our understanding in some areas and can be used to guide future research and therapy.

9.5 References

Nakaguchi, H., Tanishima, T., & Yoshimasu, N. (2001). Factors in the natural history of chronic subdural hematomas that influence their postoperative recurrence. *J Neurosurg, 95*(2), 256-262. doi:10.3171/jns.2001.95.2.0256

Pieramici, D. J., & Rabena, M. D. (2008). Anti-VEGF therapy: comparison of current and future agents. *Eye (Lond), 22*(10), 1330-1336. doi:10.1038/eye.2008.88

Publications and presentations

Original Article Publications

1. **Edlmann E**, Giorgi-Coll S, Whitfield PC, Carpenter KLH, Hutchinson PJ. Pathophysiology of chronic subdural haematoma: inflammation, angiogenesis and implications for pharmacotherapy. J Neuroinflammation. 2017 May 30;14(1):108.
2. Kolias AG (*joint 1st author), **Edlmann E** (*joint 1st author), Thelin EP, Bulters D, Holton P, Suttner N, Owusu-Agyemang K, Al-Tamimi YZ, Gatt D, Thomson S, Anderson IA, Richards O, Whitfield P, Gherle M, Caldwell K, Davis-Wilkie C, Tarantino S, Barton G, Marcus HJ, Chari A, Brennan P, Belli A, Bond S, Turner C, Whitehead L, Wilkinson I, Hutchinson PJ; British Neurosurgical Trainee Research Collaborative (BNTRC) and Dex-CSDH Trial Collaborators. Dexamethasone for adult patients with a symptomatic chronic subdural haematoma (Dex-CSDH) trial: study protocol for a randomised controlled trial. Trials. 2018 Dec 4;19(1):670
3. **Edlmann E**, Thelin EP, Caldwell K, Turner C, Whitfield P, Bulters D, Holton P, Suttner N, Owusu-Agyemang K, Al-Tamimi YZ, Gatt D, Thomson S, Anderson IA, Richards O, Gherle M, Toman E, Nandi D, Kane P, Pantaleo B, Davis-Wilkie C, Tarantino S, Barton G, Marcus HJ, Chari A, Belli A, Bond S, Gafoor R, Dawson S, Whitehead L, Brennan P, Wilkinson I, Kolias AG, Hutchinson PJ, Dex-CSDH trial collaborative and BNTRC collaborative. Dex-CSDH randomised, placebo-controlled trial of dexamethasone for chronic subdural haematoma: report of the internal pilot phase. *Accepted by Scientific reports Jan 2019*.

Manuscripts in preparation

1. Two pathophysiological origins of CSDH: De novo and acute transformed. *Submission to Neurosurgery*.
2. The inflammatory profile of CSDH fluid in primary and recurrent CSDH and the role of dexamethasone.
3. Assessing the composition and acute haemorrhage rate in CSDH with UV-Vis spectroscopy
4. HPLC method development for detecting dexamethasone in CSDH fluid.
5. Validation of a semi-automated method for volume and density assessment in CT imaging of CSDH.

6. CT imaging analysis in 189 CSDHs; predicting recurrence and outcome

Abstract publications

1. **Edlmann E,** Tarantino S, Caldwell K, Suttner N, Owusu-Agyemang K, Bulters D, Holton P, Whitfield P, Eglington C, Kolias A, Turner C, Davis-Wilkie C, Pantaleo B, Allison A, Bond S, Hutchinson PJ, Dex-CSDH collaborative group, BNTRC. Recruitment, safety and progress; over half-way in the Dex-CSDH trial. In: Proceedings of the 2018 Spring Meeting of the Society of British Neurological Surgeons. Br J Neurosurg. 2018 Jun;32(3):312-351.
2. **Edlmann E,** Caldwell K, Suttner N, Kane P, Anderson I, Bulters D, Kolias A, Davis-Wilkie C, Turner CL, Bond S, Hutchinson PJ. First 100 patients reach final follow-up in the Dex-CSDH Trial: a randomised, placebo controlled trial of Dexamethasone in Chronic Subdural Haematoma. In: Proceedings of the 2017 Spring Meeting of the Society of British Neurological Surgeons. *British Journal of Neurosurgery*, April 2017; 31(2) 119-158.
3. **E. Edlmann**, A. Kolias, C. Turner, A. Belli, H. Marcus, A. Chari, P. Brennan, P. Mitchell, P. Myint, D. Nandi, E. Warburton, G. Barton, S. Bond, I. Wilkinson, C. Brayne, A.T. King, D. Bulters, P. J. Hutchinson, British Neurotrauma Group, British Neurosurgical Trainee Research Collaborative, Dex-CSDH Collaborative Group. Randomised, double blind, placebo-controlled trial of a 2-week course of Dexamethasone for adult patients with a symptomatic Chronic Subdural Haematoma (Dex-CSDH trial) – a progress update. In: Proceedings of the 2016 Spring Meeting of the Society of British Neurological Surgeons. *British Journal of Neurosurgery,* April 2016; 30(2): 130-186.
4. **E. Broughton**, A. Kolias, C. Turner, A. Belli, H. Marcus, A. Chari, P. Brennan, P. Mitchell, P. Myint, D. Nandi, E. Warburton, G. Barton, S. Bond, I. Wilkinson, C. Brayne, A.T. King, D. Bulters, P. J. Hutchinson, British Neurotrauma Group, British Neurosurgical Trainee Research Collaborative, Dex-CSDH Collaborative Group. Dex-CSDH trial: a randomised, double blind, placebo-controlled trial of a two-week course of dexamethasone for adult patients with a symptomatic Chronic Subdural Haematoma. In: Proceedings of the 2015 Spring Meeting of the Society of British Neurological Surgeons jointly with, as invited guests, the German Society of Neurosurgery (DGNC – Deutche Gessellschaft für Neurochirurgie). *British Journal of Neurosurgery*, April 2015; 29(2): 124–163

Oral presentations

1. Final phase of recruitment and statistics analysis plan for Dex-CSDH trial. Society of British Neurological Surgeons, September 2018, London.
2. Translational research on CSDH pathophysiology. European Association of Neurological Surgeons, October 2018, Brussels, Belgium.
3. Drivers of inflammation and recurrence in chronic subdural haematoma. European Association of Neurological Surgeons, October 2018, Brussels, Belgium.
4. Recruitment, safety and progress; over half-way in the Dex-CSDH trial. Society of British Neurological Surgeons, April 2018, Torquay, UK. *Winner prize for highest scoring oral presentation*.
5. Mechanisms of inflammation and the role of dexamethasone in treating chronic subdural haematoma. British Neurosurgical Research Group, March 2017, Birmingham, UK.
6. First 100 patients reach final follow-up in the Dex-CSDH Trial: a randomised, placebo controlled trial of Dexamethasone in Chronic Subdural Haematoma. Society of British Neurological Surgeons, March 2017, Oxford, UK.
7. Project update: 1 year of recruitment to the Dex-CSDH trial. National Research Collaborative Meeting, Dec 2016, Sheffield.
8. Dex-CSDH Trial update. British Neurosurgical Research Group, Cambridge, UK, March 2016.
9. The Dex-CSDH trial. A neurosurgical collaborative. National Research collaborative meeting, Sheffield, UK, Dec 2015.
10. Dex-CSDH trial: a randomised, double blind, placebo-controlled trial of a two-week course of dexamethasone for adult patients with a symptomatic Chronic Subdural Haematoma. Society of British Neurological Surgeons, April 2015, Southampton, UK.
11. Dex-CSDH trial: a randomised, double blind, placebo-controlled trial of a short course of dexamethasone for adult patients with a symptomatic Chronic Subdural Haematoma. British Neurosurgical Research Group, March 2015, Cardiff, UK

Poster presentations

1. **E Edlmann**, A Kolias, E Thelin, S Tarantino, K Caldwell, C. Turner, C Davis-Wilkie, B Pantaleo, A Allison, S. Bond, D Bulters, P Holton, N Suttner, K Owusu-Agyemang, Y Al-Tamimi, P. J. Hutchinson, Dex-CSDH Collaborative Group, and BNTRC.

Dexamethasone in Chronic Subdural Haematoma (Dex-CSDH) Trial Update. EANS October 2018, Brussels, Belgium.

2. **E Edlmann,** S Giorgi-Coll, PC Whitfield, PJ Hutchinson, KLH Carpenter**.** Mechanisms of inflammation and the role of dexamethasone in treating chronic subdural haematoma. British Neurological Association, April 2017.
3. **E Edlmann,** K Caldwell, A Kolias, C Davis-Wilkie, C Turner, P Hutchinson, Dex-CSDH Collaborative, BNTRC. Trial update on a randomised, double-blind, placebo-controlled trial of a two-week course of dexamethasone for adult patients with a symptomatic Chronic Subdural Haematoma (Dex-CSDH trial). European Association of Neurological Surgeons, Oct 2016, Athens, Greece.
4. E Edlmann. Dex-CSDH: A randomised, double blind, placebo controlled trial on Dexamethasone in Chronic Subdural Haematoma. Neurotrauma, October 2015, London, UK. *Won prize for best poster presentation*.