THE UNIVERSITY
of EDINBURGH

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Roanna Jane Hall

Doctor of Philosophy

The University of Edinburgh

2016

To John, Lucy and Tom

Contents

Declaration

The research described in this thesis was the unaided work of the author, except where acknowledgement is made by reference. No part of this work has previously been accepted for any other degree, nor is any part of it being concurrently submitted in candidature for another degree.

Roanna Jane Hall

January 2016

Acknowledgements

I would like to thank my supervisors Professor Alasdair MacLullich and Professor Jonathan Seckl for their help and support. I thank the staff of the Clinical Research Facility in the New Royal Infirmary of Edinburgh for their help in sample collection and processing, and follow-up visits. I thank Dr. Alison Green and Mrs Mary Andrews for performing assays of S100B and tau in cerebrospinal fluid, and I thank Mrs. Jill Harrison and Mr Neil Johnston for help and advice with ELISA assays and Mr Graham Harold, Dr Pamela Brown and Mrs Linda Ferguson for help and advice with the Luminex assays. I benefited greatly from conversations with Dr. Dan Davis, Dr Colm Cunningham and Dr Zoë Tieges. I thank Prof Ian Deary for his advice on the analysis of the follow-up cognitive test battery. I thank Dr Dan Davis and Dr Mike Allerhand for performing the statistical analyses described in Chapters 7 and 9. I thank Dr Leiv Otto Watne, Dr Torgeir Bruun Wyller and Dr Vibeke Juliebø from the University of Oslo who have been gracious collaborators. I am very grateful for the secretarial and administrative help of Mrs. Maureen Harding, Mrs. Elaine Smith and Mrs. Sharon Moncrieff. I would also very much like to thank the staff of the Orthopaedic Trauma Unit and Theatres at the New Royal Infirmary of help in recruitment and sample collection.

My collaborators and I acknowledge the financial support of AgeUK (Research into Ageing) and The British Geriatrics Society, the Medical Research Council. The Centre for Cognitive Ageing and Cognitive Epidemiology acknowledges the financial support of the BBSRC, ESRC and MRC as part of the Lifelong Health and Well-being initiative.

List of abbreviations

5-HIAA: 5-Hydroxyindoleacetic Acid

11β-HSD: 11β-Hydroxysteroid Dehydrogenase

AChE: Acetylcholinesterase

ACTH: Adrenocorticotrophic Hormone

ADL: Activities of Daily Living

AF: Atrial Fibrillation

ANCOVA: Analysis of Covariance

ANOVA: Analysis of Variance

APACHE: Age, Physiology and Chronic Health Evaluation

AST: Attentional Set-shifting Task

BBB: Blood Brain Barrier

BDNF: Brain-Derived Neurotrophic Factor

BIS: Bispectral Index

BLI: β-Endorphin-Like Immunoreactivity

CABG: Coronary Artery Bypass Grafting

CAM: Confusion Assessment Method

CAM-ICU: Confusion Assessment Method for the Intensive Care Unit

CBG: Cortisol Binding Globulin

CCI: Charlson Comorbidity Index

CCL: Chemokine (C-C motif) ligand

CDHIP: Cortisol and Delirium after Hip fracture

CI: Confidence Interval

CNS: Central Nervous System

COX II: Cyclooxygenase II

CRF: Clinical Research Facility

CRP: C-Reactive Protein

CSF: Cerebrospinal fluid

CT: Computed Tomography

C.V.: Coefficient of Variation

CXCL: Chemokine (C-X-C motif) ligand

DHEA: Dehydroepiandrosterone

DHEAS: Dehydroepiandrosterone Sulphate

DRS-R98: Delirium Rating Scale Revised ‘98

DSM: Diagnostic and Statistical Manual

DSST: Digit Symbol Substitution Test

ED: Emergency Department

EDTB-1: Edinburgh Delirium Test Box 1

EGF: Epidermal Growth Factor

ELISA: Enzyme-Linked Immunosorbent Assay

EQ-5D: European Quality of life scale with 5 components

EQ-VAS: EQ-5D Visual Analogue Scale

ESR: Erythrocyte Sedimentation Rate

FAS: Letters F, A and S total score in 60 seconds in verbal fluency

FGF: Fibroblast Growth Factor

Flt-3L: FMS-like tyrosine kinase 3 ligand

G-CSF: Granulocyte Colony Stimulating Factor

GCS: Glasgow Coma Scale

GFAP: Glial Fibrillary Acidic Protein

GM-CSF: Granulocyte Macrophage Colony Stimulating Factor

GP: General Practitioner

GR: Glucocorticoid Receptor

HELP: Hospital Elder Life Program

HPLC: High Performance Liquid Chromatography

hsCRP: high sensitivity –C-Reactive Protein

HPA: Hypothalamic-Pituitatary-Adrenal axis

HVA: Homovanillic Acid

HVLT-R: Hopkins Verbal Learning Test-Revised

IADL: Instrumental Activities of Daily Living

ICD: International Classification of Diseases

ICU: Intensive Care Unit

IFN: Interferon

IGF-1: Insulin-like Growth Factor-1

IL: Interleukin

IL-1ra: Interleukin-1 receptor antagonist

IPCC: In-Patient Continuing Care

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly

IQR: Interquartile Range

ISD: Information Services Division

IV: Intravenous

K-W: Kruskal-Wallis test

LIF: Leukaemia Inhibitor Factor

LP: Lumbar Puncture

LPS: Lipopolysaccharide

LTCI: Long-Term Cognitive Impairment

MCI: Mild Cognitive Impairment

MCP: Monocyte Chemotactic Protein

MDAS: Memorial Delirium Assessment Scale

MDC: Macrophage Derived Chemokine

MDD: Minimum Detectable Dose

MHPG: 3-methoxy-4-hydroxypheylglycol

MIF: Macrophage migration inhibitory factor

MIP: Macrophage Inflammatory Protein

MMP: Matrix Metalloproteinase

MMSE: Mini-Mental State Examination

MOOSE: Meta-analysis Of Observational Studies in Epidemiology

MR: Mineralocorticoid Receptor

MRI: Magnetic Resonance Imaging

MWU: Mann-Whitney U test

NART: National Adult Reading Test

NHFD: National Hip Fracture Database

NHS: National Health Service

NICE: National Institute of Clinical Excellence

NMDA: N-methyl-D-aspartate

NSAID: Non-Steroidal Anti-Inflammatory Drug

NSE: Neuron-Specific Enolase

OOT: Oslo Orthogeriatric Trial

OR: Odds Ratio

OSLA: Observational Scale of Level of Alertness

PADL: Personal Activities of Daily Living

PDGF: Platelet-Derived Growth Factor

POCD: Post-Operative Cognitive Dysfunction

RAGE: Receptor for Advanced Glycation Endproducts

RANTES: Regulated on Activation, Normal T Expressed and Secreted

RASS: Richmond Agitation-Sedation Scale

RDI: Recognition Discrimination Index

RH: Recombinent Human

SAA: Serum Anticholinergic Activity

SD: Standard Deviation

SE: Standard Error

SIRS: Systemic Inflammatory Response Syndrome

SLE: Systemic Lupus Erythematosus

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

SLI: Somatostatin-Like Immunoreactivity

TGF: Transforming Growth Factor

TIA: Transient Ischaemic Attach

TIVA: Total Intravenous Anaesthesia

TNF: Tumour Necrosis Factor

VEGF: Vascular Endothelial Growth Factor

List of publications

Publications included as chapters in this thesis in an updated format

Hall RJ, Shenkin SD, MacLullich AMJ. A Systematic Literature Review of Cerebrospinal Fluid Biomarkers in Delirium. Dementia and Geriatric Cognitive Disorders 2011, Vol 32 (2), pp79-93

Hall RJ, Ferguson KJ, Green A, Andrews M White TO, Armstrong IR, MacLullich AMJ. Cerebrospinal fluid S100b is high in delirium after hip fracture. American Journal of Geriatric Psychiatry, 2013. 21(12):1239-43

Publications directly related to this thesis but not included as chapters

Tieges Z, McGrath A, Hall RJ, MacLullich AMJ. Abnormal level of arousal as a predictor of delirium and inattention: An exploratory study. American Journal of Geriatric Psychiatry, 2013 doi 10.1016j.jagp.2013.05.003

Watne LO, Hall RJ, Molden E, Ræder J, Frihagen F, MacLullich AMJ, Juliebø V, Nyman A, Meagher DJ, Wyller TB. Anticholinergic activity in CSF and serum in hip fracture patients with and without delirium. Journal of the American Geriatric Society, 2014, Jan 2, doi 10.1111/jgs.12612

Cape E, Hall RJ, van Munster BC, de Vries A, Howie SEM, Pearson A, Middleton SD, Gillies F, Armstrong IR, White TO, Cunningham C, de Rooij SE, MacLullich AMJ. Cerebrospinal Fluid Markers of Neuroinflammation in Delirium: a role for Interleukin-1β in delirium after hip fracture. Journal of Psychosomatic Research, 2014, 77(3) pp219-225

Poljak A, Hill M, Hall RJ, MacLullich AMJ, Raftery MJ, Jian Tai J, Siyu Yan S, Caplan GA. Quantitative Proteomics of Delirium CSF. Translational Psychiatry, 2014, Nov 4, 4e77 doi 10.1038/tp.2012.114

Abstract

Delirium, or “acute confusion” is a common and serious acute neuropsychiatric syndrome mainly affecting older people. It is associated with multiple adverse outcomes, including an increased risk of developing dementia and increased mortality. The underlying mechanisms of delirium are poorly understood, and there are currently no specific treatments. This thesis investigated the roles of the hypothalamic-pituitary adrenal axis and inflammation in the pathophysiology of delirium, persistent delirium and cognitive decline following delirium. It investigated whether levels of cortisol in blood and cerebrospinal fluid (CSF) are elevated in delirium, with elevated pro-inflammatory and reduced anti-inflammatory cytokines. It also investigated whether there is loss of cortisol diurnal rhythm (in saliva) with elevated afternoon cortisol levels. The thesis investigated whether any hypercortisolaemia was sustained during the year after delirium, and whether this was associated with deterioration in cognition during the year after hip fracture. Finally, it also tested whether there are high levels of a marker of central nervous system damage (S100B) and of a dementia marker (tau) in CSF in delirium.

A prospective observational cohort study was conducted in N=108 patients aged over 60 who had sustained a hip fracture, in whom 40% developed delirium. Participants gave informed consent or if they lacked capacity to give informed consent, this was given by their next of kin. Participants were assessed regularly for delirium, according to DSM IV criteria, during the two weeks after hip fracture. A sample of CSF was collected during the spinal anaesthetic performed for the operation to repair their fracture. Samples of blood and saliva were collected during the two weeks after the hip fracture operation. Participants were visited three, six and twelve months after their hip fracture for further delirium assessment, and a cognitive test battery was completed. Further samples of blood and saliva were collected at these visits.

The study found evidence of high levels of cortisol and of S100B in CSF in those with active delirium, but there were no differences in levels of tau or cytokines in CSF. Those with delirium had elevated serum cortisol during the perioperative period, and elevated afternoon salivary cortisol, suggesting flattening of cortisol diurnal rhythm with failure to reach the normal diurnal nadir. After adjusting for confounders in a multivariate logistic regression analysis, serum cortisol was still predictive of delirium, but salivary cortisol AM:PM ratio had a trend towards significance. Those who had persistent delirium features in the months after hip fracture had significantly higher serum cortisol three months after hip fracture. There was a change in serum inflammatory profile in those with delirium, with a shift towards a pro-inflammatory state. Testing the study hypotheses surrounding cognition after delirium was very challenging, due to patient attrition and other factors. Some participants showed a trajectory of cognitive improvement, which was probably due to resolution of delirium during the year after hip fracture. Those with resolved delirium had deficits in verbal and visual memory. This study has improved understanding of the mechanisms of delirium, suggested further avenues for research and identified possible new therapeutic targets.

Lay Summary

Delirium, or “acute confusion” is a common and serious syndrome which mainly affects older people when they become unwell. It is associated with multiple adverse outcomes, including an increased risk of developing dementia and of death. The underlying mechanisms of delirium in the body and the brain are not well understood, and we have no specific treatments. This thesis investigated whether there are increased levels of the stress hormone cortisol and inflammatory markers released in delirium, both into the blood and into cerebrospinal fluid (CSF), which bathes the brain and spinal cord. It also investigated whether there is loss of the natural rhythm of cortisol in the body. The thesis investigated whether any high levels of cortisol were sustained during the year after delirium, and whether this was associated with decline in thinking and memory tested during that year. Finally, it also tested whether there are high levels of a protein marker of brain damage (S100B) and of a dementia marker (tau) in the cerebrospinal fluid in delirium.

A study was conducted in one hundred and eight older people who had sustained a hip fracture, in whom 40% developed delirium. Participants gave informed consent or if they were unable to fully understand the study, this was given by their next of kin. Participants were assessed regularly for delirium, with short tests of thinking and memory, during the two weeks after their hip fracture. A sample of CSF was collected when they had the spinal anaesthetic performed for the operation to repair their fracture. Samples of blood and saliva were collected during the two weeks after the hip fracture operation. Participants were visited three, six and twelve months after their hip fracture and they were tested again for delirium, and completed more detailed thinking and memory tests. Further samples of blood and saliva were collected at these visits.

The study found that there were high levels of cortisol and the brain damage marker S100B in CSF in those with active delirium, but there were no differences in the dementia marker tau or inflammatory proteins. Those with delirium had higher levels

of cortisol in blood, and higher levels in saliva in the afternoons, suggesting some loss of the natural daily rhythm of cortisol. Those who still had symptoms of delirium in the months after hip fracture also had higher levels of cortisol in the blood. There was a change in the pattern of inflammatory markers in the blood in those with delirium, with a shift towards markers that increase the inflammatory response. It was difficult to test the changes in thinking and memory after delirium. Some participants showed improvement, which was probably because they had had delirium but recovered from it during the year after hip fracture. This study have improved our understanding of the mechanisms of delirium, and suggested further avenues for research and new treatments that could be trialled.

1 Chapter 1: General Introduction

1.1 Delirium

In this section, delirium will be defined, and its epidemiology described. Its features will be described, as will methods of assessment and problems often encountered in clinical and research settings with delirium assessment and diagnosis. Outcomes of delirium will also be discussed.

1.1.1 Delirium Definition

Delirium is a serious acute neuropsychiatric condition. It has been recognized for at least two millennia with the term "delirium" deriving from the Latin '*lira'* meaning to wander from one's furrow. Prior to DSM-III (1980) delirium suffered from terminological chaos, with multiple descriptive terms used in different settings (acute organic brain syndrome, acute confusional state, brain failure, toxic encephalopathy, septic encephalopathy, intensive care psychosis). However, these terms do not necessarily describe different pathological processes. Delirium is now the umbrella term in current International Classification of Diseases (ICD) and Diagnostic and Statistical Manual (DSM) classification schemes that subsume these multiple synonyms. This simplification has allowed for a more coherent research effort and a consistent approach to detection and management. Moreover, the use of the label delirium has led to greater appreciation of the considerable impact upon outcomes and independent need for treatment as a brain disorder beyond only treating its underlying aetiological precipitants. The American Psychiatric Association's DSM IV definition will be used here. This describes delirium as a syndrome characterised by an acute and often fluctuating change in mental status with inattention (a difficulty in focussing, shifting or sustaining attention), and the presence of a medical cause (APA 2000). The ICD-10 definition is similar but not used here (WHO 1993). Table 1.1 shows the DSM-IV and ICD-10 criteria for delirium.

Table 1.1 Diagnostic and Statistical Manual version IV and International Classification of Diseases version 10 criteria for diagnosis of delirium

DSM IV criteria for delirium (APA 2000)
(a) A disturbance of consciousness (reduced clarity of awareness of the environment, with reduced ability to focus, sustain, or shift attention)
(b) A change in cognition (eg. memory impairment) or a perceptual disturbance
(c) Onset of hours to days, and tendency to fluctuate
(d) Evidence from the history, physical examination or laboratory findings that the disturbance is caused by the direct physiological consequences of a general medical condition
ICD-10 criteria for delirium (WHO 1993)
A. Clouding of consciousness, i.e. reduced clarity of awareness of the environment, with reduced ability to focus, sustain, or shift attention.
B. Disturbance of cognition, manifest by both:
(1) impairment of immediate recall and recent memory, relatively intact remote memory;
(2) disorientation in time, place or person.
C. At least one of the following psychomotor disturbances:
(1) rapid, unpredictable shifts from hypo-activity to hyper-activity;
(2) increased reaction time;
(3) increased or decreased flow of speech;
(4) enhanced startle reaction.
D. Disturbance of sleep or the sleep-wake cycle, manifest by at least one of the following:
(1) insomnia, which in severe cases may involve total sleep loss, with or without daytime drowsiness, or reversal of the sleep-wake cycle;
(2) nocturnal worsening of symptoms;
(3) disturbing dreams and nightmares, hallucinations or illusions when awake.
E. Rapid onset and fluctuations of the symptoms over the course of the day.
F. Objective evidence from history, physical and neurological examination or laboratory tests of an underlying cerebral or systemic disease (other than psychoactive substance-related) that can be presumed to be responsible for the clinical manifestations in A-D.

1.1.2 Delirium Epidemiology

Delirium is very common, with a prevalence in the Emergency Department (ED) of around 9% (Elie, Rousseau et al. 2000, Kennedy, Enander et al. 2014) and in general medical in-patients of 11-42% (Siddiqi, House et al. 2006). In surgical settings, the

rate is 4-53% in patients with hip fracture, 4-28% in elective orthopaedic patients (Bruce, Ritchie et al. 2007) and 3-72% following cardiac surgery (Sockalingam, Parekh et al. 2005). It is also very common in ICU patients, with rates reported around 30% (Ouimet, Kavanagh et al. 2007) and up to 80% (Ely, Margolin et al. 2001). The difference in incidence rates in different settings is likely due to different underlying patient characteristics, illness severity and co-morbidity.

Despite being common, delirium is often missed, and a missed diagnosis is highly detrimental: non-detection in an ED is associated with higher mortality, with a hazard ratio of 8.22 (95% CI 1.69-39.89) compared to non-delirious patients (Kakuma, Galbaud du Fort et al. 2003). Two studies, one in elderly patients in an ED setting (Elie, Rousseau et al. 2000), and one in acute elderly admissions (Collins, Blanchard et al. 2010) found a broadly similar poor rate of detection, with a sensitivity in the Elie study of 35.3% and a missed diagnosis in the Collins study of 72%. This is due at least in part to delirium's heterogeneous and fluctuating nature, but several issues contribute. Since underlying dementia or cognitive impairment is one of the strongest risk factors for delirium with an odds ratio of 5.2 (Elie, Cole et al. 1998), these two conditions often co-exist in hospital in-patients, leading to difficulties in diagnosis. Patients with hypoactive delirium are more often missed, particularly if they are in a surgical setting (Meagher and Cullen 2012). Clinicians may lack training, experience and confidence in diagnosing delirium, which contributes to poor detection rates (Davis and MacLullich 2009). Busy clinical settings with high patient throughput also make detection, diagnosis and documentation of delirium challenging (Collins, Blanchard et al. 2010).

Multiple risk factors have been identified for delirium; a recent systematic review and meta-analysis found dementia, illness severity, urinary catheterisation, visual impairment, low albumin level and length of hospital stay to be the factors most strongly associated with delirium in older medical in-patients (Ahmed, Leurent et al. 2014).

1.1.3 Delirium Features

The features essential for DSM IV diagnosis are shown in Table 1.1 (APA 2000). Other features commonly seen (in descending order of frequency) are sleep-wake cycle disturbance, short or long-term memory disturbance, poor visuospatial ability, disorientation, psychomotor agitation or retardation, abnormalities in the understanding and use of language, disorganised thinking, lability of affect, and perceptual disturbances with hallucinations or delusions (Meagher, Moran et al. 2007).

1.1.4 Delirium Assessment

Delirium is a complex syndrome that can include a wide range of neuropsychiatric and cognitive features. No single feature is pathognomonic or specific to delirium but the pattern of symptoms that indicates widespread disturbance of brain functions along with the characteristic context (i.e. acute onset, fluctuating course and presence of physical morbidity) are highly suggestive. There are different methods of delirium assessment used in research. Many involve a combination of observation and a semi-structured interview with cognitive testing and tests of conscious level and attention. There are specific tools for delirium diagnosis such as the Confusion Assessment Method (CAM) (Inouye, Vandyck et al. 1990) and its ICU version the CAM-ICU (Ely, Margolin et al. 2001), and tools that can be used to diagnose delirium and assess severity, such as the Delirium Rating Scale Revised ’98 (DRS-R98) (Trzepacz, Mittal et al. 2001) and the Memorial Delirium Assessment Scale (MDAS) (Breitbart, Rosenfeld et al. 1997). The CAM essentially operationalises the DSM III criteria for delirium, and is based on an interview with the patient, often supported by testing of attention (e.g. digit span) and cognitive testing. A period of training is important since the sensitivity of the CAM is poor without training. The CAM-ICU has been designed for use in ventilated, non-verbal patients, with clearly defined tasks assessing attention and disorganised thinking, and the Richmond Agitation Sedation Scale is used to assess conscious level. Some training and resources are required to use the CAM-ICU. Outwith critical care settings, the CAM-ICU has been shown to agree moderately well with the original CAM, but it may not be sensitive

enough for routine use in other settings (Neufeld, Hayat et al. 2011). The DRS-R98 is a detailed assessment of delirium symptomatology and severity, consisting of 13 items (sleep–wake cycle disturbance, perceptual disturbances, delusions, lability of affect, language and thought process abnormalities, motor agitation or retardation, orientation, attention, short and long-term memory and visuospatial ability) with three additional diagnostic items (temporal onset, symptom fluctuation and physical disorder). It involves a semi-structured interview with cognitive testing and assessment of attention, and is usually administered by psychiatrists or trained specialists. The MDAS is a severity instrument that is designed for physician use, and for repeated use within 24h. There are also tools to determine delirium subtype, such as the Delirium Symptom Interview and Delirium Motor Subtype Scale.

Assessment using these tools needs to be combined with information from supporting sources such as casenotes, nursing and medical staff and family members to ascertain baseline level of cognition, the acuity of change, fluctuation and evidence of additional features such as hallucinations or delusions. The frequency of observation is also important to consider, due to the fluctuation of delirium symptoms. Some delirium screening tools are embedded into routine nursing assessments, for example in ICU settings, and are performed once or twice daily. Depending on the patient cohort and setting, there may be “high risk” periods, such as the first few postoperative days following orthopaedic or cardiac surgery, when delirium is most likely to occur, therefore daily assessment during this period is ideal. It is important in research studies of delirium to balance accurate delirium diagnosis and “capture” of delirium episodes by frequent review, without over-burdening research participants.

1.1.5 Delirium Outcomes

It has become increasingly recognised that delirium has multiple adverse consequences. A systematic review included 19 mainly prospective cohort studies examining mortality following delirium and found an overall mortality rate at discharge of 14-37% (Siddiqi, House et al. 2006). The best quality study in the

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review found an increased mortality at discharge and a two-fold independent increase in mortality at one year, with an adjusted hazard ratio of 2.11, using three measures of illness burden and severity (McCusker, Cole et al. 2002). Furthermore, a meta-analysis showed that delirium is associated with an increased risk of mortality which persists post-hospital discharge and is independent of age, sex, co-morbid illness and baseline dementia (Witlox, Eurelings et al. 2010). Some studies have found an association with increased hospital length of stay, and delirium is also associated with an increased risk of institutionalisation, both on discharge and 12 months later (Siddiqi, House et al. 2006, Kennedy, Enander et al. 2014). Delirium is also often distressing, both for the patient themselves, their caregivers and loved ones, and for the nursing staff caring for them (Breitbart, Gibson et al. 2002). For example, the 84 year-old Pulitzer Prize-winning historian Justin Kaplan described his delirious hallucinations which included thousands of weapon-carrying tiny creatures on horseback as “a lot of unpleasantness” (Belluck 2010).

1.1.6 Conclusions

In summary, delirium is a common, serious acute neuropsychiatric syndrome with multiple adverse outcomes. It is underdiagnosed, and its accurate assessment involves a semi-structured interview with testing of attention, level of arousal and cognition, and further information on acuity of onset and fluctuation.

1.2 Delirium and its relationship to cognitive dysfunction

1.2.1 Definitions

Dementia, along with older age, is one of the strongest risk factors for delirium (Young and Inouye 2007, Juliebo, Bjoro et al. 2009), with risk of developing incident delirium increasing proportionately with worse cognitive impairment (Davis, Skelly et al. 2015). There is also a significantly increased risk of developing dementia following delirium (MacLullich, Beaglehole et al. 2009, Witlox, Eurelings et al. 2010). A systematic review in 2004 reviewed nine studies, and despite methodological difficulties, concluded that delirium is associated with an increased risk of long-term cognitive impairment (LTCI) (Jackson, Gordon et al. 2004). A subsequent systematic review in 2009 reviewed a further nine studies from the intervening years and reached the same conclusion, with a relative risk of LTCI of 2-3.5 (MacLullich, Beaglehole et al. 2009). A meta-analysis demonstrated an adjusted odds ratio of developing dementia following delirium of 9.42 (Witlox, Eurelings et al. 2010).

It is unclear at present whether delirium 'unmasks' a pathological process which had already begun in the brain or whether delirium sparks a new pathological process leading to cognitive decline in an otherwise healthily aging brain. There is evidence that an episode of delirium may worsen the trajectory of dementia; Fong *et al* examined the cognitive trajectory of a large cohort of patients with Alzheimer's Dementia, and compared the trajectory of patients with a delirium episode identified by a validated chart review method with patients confirmed not to have had delirium. They found a significant acceleration of the rate of change of cognitive scores in the group with delirium (Fong, Jones et al. 2009). Davis *et al* employed the Vantaa 85+ study, a population-based cohort of the oldest-old, to examine first whether delirium increased the risk of incident dementia, and second whether in those with dementia, a history of delirium increased the degree of standard dementia neuropathology (amyloid plaques, infarcts, lewy body disease). They found that delirium increased the risk of incident dementia with an OR of 8.7, and that it was associated with worsening dementia severity and function. Interestingly, they also found that while

the standard neuropathologies examined were associated with dementia in those with no history of delirium, in those with delirium and dementia, no relationship was found with several markers of classical dementia neuropathology, that is, β-amyloid, α-synucleinopathy, neuronal loss in the substantia nigra and infarcts (Davis, Muniz Terrera et al. 2012). This suggests that there may be alternative pathology involved in dementia that follows delirium.

Delirium in the context of critical illness has been shown to be predictive of LTCI (Girard, Jackson et al. 2010). Imaging in these patients has shown evidence of cerebral atrophy, with ventricular enlargement (Morandi, Jackson et al. 2009), suggesting possible loss of neurons. Longer duration of delirium in critical illness is also associated with smaller brain volumes, and these smaller brain volumes measured on MRI three months after critical illness are associated with LTCI after one year (Gunther, Morandi et al. 2012). A recent large (N=821) study in medical and surgical intensive care patients in which 74% developed delirium and only 6% had cognitive impairment at baseline (according to the Informant Questionnaire on Cognitive Decline in the Elderly) found significant rates of LTCI in the year after critical illness, with 24% of patients having scores consistent with mild Alzheimer's disease at one year. This occurred in both older and younger patients, and longer duration of delirium was independently associated with both worse global cognition and executive dysfunction (Pandharipande, Girard et al. 2013).

In patients undergoing cardiac surgery, Saczynski *et al* performed a prospective cohort study to examine the impact of delirium on cognitive trajectory over one year (Saczynski, Marcantonio et al. 2012). They enrolled N=225 patients, of whom N=103 developed delirium according to the CAM or CAM-ICU. Those who developed delirium had a lower Mini-Mental State Examination (MMSE) score at baseline, and had a greater drop in MMSE score on day 2 postoperatively. A difference in MMSE scores between the groups persisted to one year postoperatively, although after adjusting for the difference at baseline, this was significant at 30 days

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture postoperatively but not thereafter. Patients with postoperative delirium were less likely to have returned to their baseline cognitive level at 6 months postoperatively.

1.2.2 Postoperative cognitive dysfunction

An important construct related to postoperative delirium is postoperative cognitive dysfunction (POCD). There is a lack of consensus as to exactly how POCD should be defined, and it does not form part of formal classification systems such as ICD or DSM. This has caused difficulties for research in this area since differing definitions may give different incident rates and outcomes. However, POCD is generally seen as cognitive dysfunction occurring after elective or emergency surgery. It may occur in the hours or days post-surgery or it may be detected weeks or months later. It tends to be viewed as having longer duration (often weeks to months and perhaps longer) than delirium. Impaired attention may be present, but unlike in delirium it is not a core diagnostic feature. POCD may affect a wide range of cognitive domains, though some authors specify deterioration in memory as the primary complaint (Krenk and Rasmussen 2011). Alteration in conscious level, and the range of neuropsychiatric features that commonly complicate delirium (altered sleep-wake cycle, changes to motor activity, affective changes and psychotic features) are typically not included in the concept of POCD.

There is also an argument that some surgeries, particularly elective operations designed to improve quality of life, alleviate pain or inflammation, may even lead to postoperative cognitive improvement (Nadelson, Sanders et al. 2014). Factors that may affect perioperative cognitive trajectory include underlying age and any dementia process, inflammation, pain, change in medication (e.g. no longer needing pain medication or cardiac drugs), postoperative complications such as pneumonia or acute kidney injury, delirium, cardiopulmonary bypass, general vs. regional anaesthesia, mood and quality of life (Nadelson, Sanders et al. 2014). Postoperative cognitive improvement would be more plausible following certain types of elective surgery, but would be unlikely following acute hip fracture, which is urgent unplanned surgery for a traumatic event in a frail population.

1.2.3 Cognitive decline

The term cognitive decline is usually used to describe deterioration in one or more domains of cognition. It is defined differently in different studies, depending on the methods used to test cognition. It may lead to mild cognitive impairment or dementia, which are now described.

1.2.4 Mild Cognitive Impairment

Mild cognitive impairment is best described in Alzheimer's disease, where there is a growing consensus that a spectrum exists from an asymptomatic phase to a symptomatic predementia phase (MCI or mild cognitive impairment) to dementia onset. The core clinical criteria for MCI due to Alzheimer's disease are (1) concern regarding a change in cognition, (2) impairment in one or more cognitive domains, (3) preservation of independence in functional abilities and (4) absence of dementia (Albert, DeKosky et al. 2011). More than 50% of patients with MCI go on to develop dementia (NICE 2006).

1.2.5 Dementia

Dementia is a clinical syndrome characterised by a chronic impairment in cognition, accompanied by functional decline. DSM IV diagnostic criteria for a dementia syndrome are the presence of an acquired impairment in memory, associated with impairment in one or more cognitive domains, including executive function, language, praxis (learned motor sequences) or gnosis (the ability to recognise faces, objects or other sensory information). These impairments in cognition must be severe enough to interfere with work, usual social activities or relationships with others, and delirium and other major psychiatric disorders must be excluded (APA 2000, McKhann, Knopman et al. 2011). Dementia syndromes include Alzheimer's dementia, Vascular dementia, Frontotemporal dementia, Dementia with Lewy Bodies and Dementia associated with Parkinson's disease, and aetiologically mixed presentations where patients meet the core criteria for Alzheimer's dementia but also

have cerebrovascular disease, features of Dementia with Lewy Bodies or other neurological disease (McKhann, Knopman et al. 2011).

1.2.6 Conclusions

In summary, delirium has a complex relationship with mild cognitive impairment and dementia. Prior dementia is one of the strongest risk factors for delirium. Delirium increases the risk of subsequent dementia, and may worsen the trajectory of existing dementia. Dementia that follows delirium may have an alternative neuropathology to established dementia neuropathology. Delirium is common in surgical settings, and postoperative cognitive dysfunction is an important related construct.

1.3 Delirium pathophysiology

The aim of this section is to introduce and discuss the leading hypotheses in delirium pathogenesis.

1.3.1 Direct brain insults

The pathophysiology of delirium is incompletely understood (MacLullich, Ferguson et al. 2008) and previously little researched, although the number of studies in this area has increased in the past decade. Certain direct insults to the brain cause sufficient global or regional impairment in brain function to induce delirium. These insults primarily cause energy deprivation (hypoxia, ischaemia, hypoglycaemia), metabolic abnormality (hyponatraemia, hypercalcaemia), or in the case of certain drugs, directly disrupt neurotransmission (anticholinergics, L-Dopa) (MacLullich, Ferguson et al. 2008).

1.3.2 Neurotransmitter disruption

Disruption in the functioning of several neurotransmitter systems has been hypothesised to be involved in delirium pathophysiology (Trzepacz 2000). Acetylcholinergic neurotransmission is involved in many of the brain functions affected in delirium including arousal, attention, sleep, perception and orientation. Anti-cholinergic medication may precipitate delirium, and acetylcholine deficiency is an established mechanism in dementia (Trzepacz 2000). Small early studies measuring serum anticholinergic activity (SAA) levels in delirium have had mixed results (Trzepacz 2000); a more recent larger longitudinal serum study in N=142 patients with hip fracture found higher SAA in the delirium group, however this was confounded by cognitive impairment and IL-6 levels, and the authors concluded that any independent relationship of SAA and delirium was unlikely (van Munster, Thomas et al. 2012). Serotoninergic neurotransmission has been implicated in both deficiency and excess (Flacker and Lipsitz 1999, Trzepacz 2000). Dopaminergic neurotransmission is also involved in many of the features of delirium including

attention, thought, perception, motivation and motor activity. Delirium in patients with Parkinson's disease may be precipitated by their L-dopa medication, and often involves hallucination, and current standard treatment of delirium symptoms is with neuroleptic medication such as the dopamine D2 receptor blocker Haloperidol (Trzepacz 2000). Elevated levels of CSF homovanillic acid, a breakdown product of dopamine, have been associated with psychotic features of delirium (Ramirez-Bermudez, Ruiz-Chow et al. 2008).

1.3.3 Aberrant stress responses

One of the major hypotheses in delirium pathophysiology has been that delirium represents an aberrant response to stress, both of the Hypothalamic-Pituitary-Adrenal (HPA) axis, and the immune system (Olsson 1999, MacLullich, Ferguson et al. 2008). Multiple different stressors such as trauma, surgery, infection, acute illness and even psychological stress may precipitate delirium (Fong, Tulebaev et al. 2009). Ageing and dementia may both be associated with an increased and prolonged cortisol response to stressors due to impaired negative feedback, and consequently "shut-off" of the HPA axis (Lupien and McEwen 1997, Olsson 1999, Ferrari and Magri 2008). This is tied in with the idea of sickness behaviour, and it has been argued that delirium may be a maladaptive form of sickness behaviour. This thesis will discuss evidence that delirium involves exaggerated stress system and inflammatory activity. The two systems are of course intertwined, and given recent evidence that glucocorticoids may be pro-inflammatory in the central nervous system (CNS) (Sorrells and Sapolsky 2007), it seems plausible that any abnormal CNS glucocorticoid and inflammatory response in delirium could potentially become damaging if sustained.

1.4 The glucocorticoid hypothesis of delirium

1.4.1 The Hypothalamic-Pituitary-Adrenal (HPA) axis

Primary control of cortisol level in the blood is by the HPA axis. Cortisol, the primary endogenous glucocorticoid in humans, is secreted into the blood from the adrenal cortex in response to Adrenocorticotropic Hormone (ACTH) from the pituitary (Chapman and Seckl 2008). The pituitary gland is in turn stimulated to release ACTH by Corticotropin-Releasing Hormone from the paraventricular nucleus of the hypothalamus, and negative feedback is exerted on the anterior pituitary gland and hypothalamus by circulating cortisol levels. Higher cortical feedback is exerted by the hippocampus, limiting stress-induced HPA axis activation (Ferrari and Magri 2008). In health, cortisol has roles in multiple immune, metabolic and neuronal pathways. Myriad stressors including trauma, infection and psychological stress lead to a rapid activation of the HPA axis and rise in blood levels of cortisol. Cortisol circulates in the blood bound to the high-affinity, saturable transporter corticosteroid-binding globulin (CBG), with albumin acting as an additional low-affinity transporter. Free, unbound cortisol may also be measured in saliva, although at much lower levels. Levels of salivary cortisol have been shown to correlate with serum cortisol, including in older adults (Reid, Intrieri et al. 1992).

1.4.2 Cortisol in the central nervous system

Corticosteroids are highly lipid-soluble, and the major source of CNS cortisol is diffusion from the blood across the blood-brain barrier (BBB) (Herbert, Goodyer et al. 2006, Mason, Pariante et al. 2010). There may also be a small amount of local synthesis (Herbert, Goodyer et al. 2006). Glucocorticoid transporters such as the multi-drug resistance P-glycoprotein transporter and the organic anion transporter polypeptide family are present in different brain regions and export cortisol from the brain, but are not thought to play a major role in access of cortisol to the brain (Herbert, Goodyer et al. 2006, Mason, Pariante et al. 2010). Levels in CSF are

usually around 5% of those in the periphery (95% of blood cortisol is bound to CBG), and sudden peripheral rises in cortisol in response to stress may lead to disproportionate rises in CSF cortisol due to the limited binding capacity of CBG (Herbert, Goodyer et al. 2006). CSF cortisol rises with age in some people (Herbert, Goodyer et al. 2006). *In vitro*, hydrocortisone has been shown to strengthen mechanical properties of the BBB (Schrot, Weidenfeller et al. 2005, Weidenfeller, Schrot et al. 2005), but *in vivo*, acute immobilization stress has also been shown in rats to increase BBB permeability by activating brain mast cells (Esposito, Gheorghe et al. 2001). In neurological patients with evidence of BBB disruption on CT scanning, elevated serum cortisol was associated with increased BBB disruption, along with increased blood pressure and temperature (Tomkins, Kaufer et al. 2001).

There are two types of intracellular receptors for glucocorticoids within the brain, high affinity mineralocorticoid receptors (MR) and low affinity glucocorticoid receptors (GR) (Ferrari and Magri 2008). MR are activated at low basal levels of cortisol, having a 10-fold higher affinity for cortisol (Gil-Bea, Aisa et al. 2010) whereas GR are activated once MR are saturated, in the presence of peak cortisol levels, during stress or the peak of the circadian rhythm (Ferrari and Magri 2008). MR are expressed in high numbers in the hippocampal-limbic area. GR are more widespread, being found in the pituitary, hypothalamus and thalamus, prefrontal cortex and cortex, with a dense population in the hippocampus (Ferrari and Magri 2008). Within the brain there is a further level of regulation of glucocorticoid levels, namely the activity of 11ß-hydroxysteroid dehydrogenase (11ß-HSD). The two isoforms of this enzyme work in opposing directions, 11ß-HSD-1 generating active cortisol or corticosterone from inert 11-keto derivatives and 11ß-HSD-2 inactivating them. 11ß-HSD-1 is widespread in the brain as well as in liver and adipose tissue. By contrast there is little brain activity of 11ß-HSD-2; it is predominantly found in epithelial cells of the kidney, colon and salivary glands (Seckl and Walker 2004). The relative activity of these enzymes is thought to have a significant impact on the exposure of the brain to glucocorticoids, as shown by studies in knock-out rodent models. 11ß-HSD-1 knockout mice with a 129/MF1 background have increased basal corticosterone levels and exaggerated stress responses, but corticosterone levels

are lower within the hippocampus, and they are resistant to the decline in the ability to learn a hippocampal-dependent water maze task seen in some normal older mice (Yau, Noble et al. 2001). However, there is heterogeneity, with 11βHSD-1 knockout mice with other genetic backgrounds such as C57Bl/6J showing unaltered basal corticosterone levels, suggesting inter-individual variation in the role of 11βHSD-1 in HPA axis regulation (Carter, Paterson et al. 2009). In humans, two small randomized controlled trials have shown that 11ß-HSD inhibition using carbenoxolone improved verbal fluency in healthy older men and verbal memory in older men with type II diabetes, without alteration in plasma cortisol (Sandeep, Yau et al. 2004).

1.4.3 Cortisol diurnal rhythm

There is a diurnal rhythm of cortisol levels, which track the body's daily cycle of activity and rest during the hours of light and darkness. This is linked to a central clock in the hypothalamus, the suprachiasmatic nucleus. Peripheral levels of cortisol may vary up to eight-fold in this circadian rhythm, and it is present in all humans except in certain pathological states (Herbert, Goodyer et al. 2006). In addition to the diurnal rhythm of cortisol, there is also seasonal variation, with basal cortisol levels higher in May than in November (Arsenault-Lapierre, Chertkow et al. 2010). A large (N=2802) population-based study of older people, the Whitehall II study, identified two common patterns of cortisol diurnal rhythm. These were the "normative curve" and the "raised curve", with the raised curve showing higher waking and daytime cortisol and a flatter slope. Those with a raised curve were more likely to be male, a smoker, sleep for a shorter period and report more stress (Kumari, Badrick et al. 2010). Disruption of the circadian rhythm is found in several disorders including depression, Cushing's disease and Alzheimer's dementia, where the sleep-wake cycle and cortisol rhythm may no longer be in harmony (Herbert, Goodyer et al. 2006). Sleep-wake cycle disturbance is an almost universal feature of delirium (Meagher, Leonard et al. 2010).

1.4.4 Stress and the HPA axis

The natural circadian rhythm of cortisol allows room for increased activity during daylight hours, with declining levels in the evening. Multiple external and internal stressors exert effects through the HPA axis, stimulating an increase in cortisol release from the adrenal gland in the short term to aid the response of the organism to the insult. Stressors relevant to the current study, that all patients were exposed to greater or lesser degrees, include trauma (both the initial fall and hip fracture and the ensuing surgery), fasting, dehydration, pain, anaesthesia, and psychological stress. The Trier Social Stress Test, commonly used to induce psychological stress in humans, causes an HPA axis response in all age groups. Kudeilka *et al* compared the responses of children, young adults and healthy older adults to the test; younger adults had a greater ACTH response to stress, although there was no difference between plasma cortisol levels. Older women had higher cortisol levels overall, although older men had higher salivary cortisol levels (Kudielka, Buske-Kirschbaum et al. 2004). In addition to the clear effects of acute stressors, chronic stress (resulting in increased exposure of the brain to glucocorticoids), is thought to have detrimental effects on brain structure and cognitive function (Lee, Glass et al. 2007).

1.4.5 The effect of trauma on the HPA axis

Elderly women with hip fracture have been shown to have an abnormally persistent trauma-induced elevation in cortisol levels. This has been shown at two to three weeks to be a greater increase in cortisol than young subjects with a comparable injury (Frayn, Stoner et al. 1983), shown to persist for at least eight weeks (Roberts, Barton et al. 1990, Barton, Horan et al. 2001) compared to elderly but probably healthier women, and increased compared with frail bedridden elderly patients without fracture (Roberts, Barton et al. 1990). This rise is not suppressed by dexamethasone (Roberts, Barton et al. 1990). Further examination of the cortisol kinetics in a group of thirteen elderly women with hip fracture showed a lower cortisol metabolic clearance rate after hip fracture, leading to a threefold increase in plasma cortisol bioavailability three weeks post fracture (Barton, Horan et al. 2001). Patients were not assessed for delirium in these early studies; given the high

prevalence of delirium after hip fracture it may have been present or patients may have been recovering from it in these studies, enough to give informed consent to participate.

Cortisol and Dehydroepiandrosterone sulphate (DHEAS) work in interplay. The role of DHEAS is not fully understood, but it generally acts to enhance immunity. Cortisol was previously thought of as anti-inflammatory, and steroids are used to treat many immune and inflammatory diseases, however endogenous cortisol and stress itself have more varied and subtle effects on the immune system and inflammation depending on the context and concentration of glucocorticoids, acting both to activate or prepare the immune response and to shut it down (Sorrells and Sapolsky 2007, Cruz-Topete and Cidlowski 2014). DHEAS levels fall variably with age, while cortisol levels mainly stay static or rise. Butcher *et al* tested the hypothesis that an increase in the cortisol:DHEAS ratio in elderly patients following trauma (hip fracture) would be involved in the immunosuppression and increased susceptibility to infection frequently seen in this frail population (Butcher, Killampalli et al. 2005). They monitored a small cohort of elderly patients with hip fracture (N=35) for infection in a six-week postoperative period, and compared them with a group of young trauma patients. Thirteen of the elderly cohort developed predominantly bacterial infections. The young trauma patients had higher serum DHEAS levels, while the elderly patients had higher cortisol levels, and on calculating the ratios of these hormones, they confirmed that, strikingly, the elderly trauma patients had an almost *seven*-fold higher cortisol:DHEAS ratio than the young trauma patients (Butcher, Killampalli et al. 2005). Comparing these ratios with healthy young and elderly controls, they found that there was no significant difference between the healthy young and injured young ratios (the young trauma patients having proportionate rises in both hormones), however, the healthy elderly ratio was higher than both young groups but lower than the injured elderly group. Furthermore, those in the elderly trauma group who developed a postoperative infection had an even greater imbalance in the cortisol:DHEAS ratio (Butcher, Killampalli et al. 2005). They found that although the elderly patients had a comparable neutrophilia response to injury, neutrophil function (superoxide release) was impaired. Cortisol was shown

in vitro to be capable of causing this impairment in neutrophil function, and co-incubation with DHEAS was shown to overcome it (Butcher, Killampalli et al. 2005). This suggests therefore that the cortisol and DHEAS response to trauma is profoundly different in young and elderly patients. Persistent elevation of cortisol with a consequent "catabolic" state is likely to hinder recovery (Frayn, Stoner et al. 1983). There are also important consequences for neutrophil function, and susceptibility to infection. However, there is still a lack of clarity as to the mechanisms of action of DHEAS, and further work is needed in this area.

1.4.6 The relationship between age and the HPA axis

Cortisol levels, particularly in the evening, generally rise with age, although there is some heterogeneity (Deuschle, Gotthardt et al. 1997, Otte, Hart et al. 2005, Varadhan, Walston et al. 2008, Vreeburg, Kruijtzer et al. 2009). In the healthy elderly, cortisol response to challenge is greater than in young controls. A meta-analysis of studies comparing the cortisol response to either a pharmacological or psychological challenge in young vs. old healthy volunteers found that overall, age increases the HPA axis response to challenge, defined as either a stronger response to stimulation or less inhibition in a test of HPA axis suppression (Otte, Hart et al. 2005). Lupien *et al* suggested the existence of three subgroups in a longitudinal study over 3-6 years in N=51 healthy elderly people. The authors measured cortisol circadian rhythm yearly, and then measured the slope of cortisol changes over time. One subgroup showed a rise in cortisol, a further subgroup showed a fall and the final subgroup had no change (Lupien, Roch Lecours et al. 1996). Some of the heterogeneity in HPA axis responses with age may be related to gender and frailty. The effect of aging on the response of cortisol to challenge is 3-fold greater in women than men (Otte, Hart et al. 2005). Frailty also impacts on cortisol levels and circadian rhythm. Varadhan *et al* made detailed measurements of cortisol circadian rhythm in a cohort of N=214 community-dwelling women aged 80-90. They found higher evening cortisol levels and blunted diurnal variation in women with a greater frailty burden than women with lesser frailty scores, after adjusting for important

confounders such as age, education, cognition, depression, diabetes and cardiovascular disease (Varadhan, Walston et al. 2008).

It has been debated whether the rise in cortisol with age seen in some people is adaptive or pathological (Herbert, Goodyer et al. 2006). Physiological aging has been associated with hippocampal atrophy, which is more exaggerated in the presence of chronic glucocorticoid excess (Ferrari and Magri 2008). The hippocampus is particularly vulnerable to the effects of excess glucocorticoids due to the density of glucocorticoid receptors. More recent studies have demonstrated that hippocampal volume loss in this context is primarily due to loss of dendrites and synapses (Tata and Anderson 2010). This can lead to persistent stress-induced activation of the HPA-axis and hypercortisolaemia as we age, due to loss of negative feedback (Ferrari and Magri 2008).

1.4.7 The relationship between cognitive impairment or dementia and the HPA axis

The majority of evidence suggests that there is hyperactivity of the HPA axis in dementia, but again there is heterogeneity among studies. In the CNS, an increase in CSF cortisol has been demonstrated in post mortem samples from patients with dementia (Swaab, Raadsheer et al. 1994), and in living subjects (Gil-Bea, Aisa et al. 2010), where CSF cortisol correlated negatively with MMSE score (worse cognition with higher cortisol) (Gil-Bea, Aisa et al. 2010). A relationship between CSF cortisol levels and ApoE genotype has also been demonstrated (Peskind, Wilkinson et al. 2001), with a stepwise increase in levels in Alzheimer's dementia patients from E3/E3 to E3/E4 to E4/E4 (Peskind, Wilkinson et al. 2001). CSF cortisol has not been shown to be elevated in patients with MCI, however (Popp, Schaper et al. 2009, Gil-Bea, Aisa et al. 2010). A post mortem brain study has shown an increase in MR expression in the frontal cortex in Alzheimer's dementia, along with a reduction in GR expression (Gil-Bea, Aisa et al. 2010). Johansson *et al* did not find elevated CSF cortisol in a group of older patients with newly diagnosed cognitive impairment, but did find this in the female subgroup (Johansson, Johansson et al. 2011).

Dementia has been associated with HPA axis dysregulation by means of positive dexamethasone suppression tests, where administration of the steroid dexamethasone fails to suppress endogenous secretion of cortisol (Giubilei, Patacchioli et al. 2001, Robertsson, Blennow et al. 2001). An inverted-U shape has been demonstrated in serum cortisol levels in dementia, with highest levels in those with moderate dementia, and lower levels in those with mild or severe dementia (Robertsson, Blennow et al. 2001). Where serum studies are used for assessment of cortisol circadian rhythm, this requires repeated sampling which may be burdensome (Giubilei, Patacchioli et al. 2001). The first study to measure salivary cortisol in Alzheimer's dementia found it to be elevated both in the morning and evening in those with dementia compared to age- and sex-matched controls. This study also showed that morning salivary cortisol correlated with both the severity of cognitive impairment and the degree of cerebral atrophy on CT (Giubilei, Patacchioli et al. 2001). In Nursing Home residents with advanced dementia, a study using salivary measurements of cortisol circadian rhythm has demonstrated heterogeneity in cortisol patterns (Woods, Kovach et al. 2008). Whilst some residents had a loss of circadian rhythm with a flattened profile and elevated evening values, others had both a morning and evening rise, and some had a relatively normal diurnal rhythm despite significant dementia pathology. Where serum cortisol has been examined in relationship to ApoE genotype, although higher morning serum cortisol levels were found in groups with cognitive-impairment-no-dementia and dementia compared to healthy elderly controls, there was no relationship found with ApoE genotype (Lara, Caramelli et al. 2013), in contrast to the relationship found between CSF cortisol and ApoE status (Peskind, Wilkinson et al. 2001). Greater baseline HPA axis activity (morning plasma cortisol) has also been shown to be associated with more rapid deterioration in scores on a neuropsychological test battery over 4 years in patients with Alzheimer's dementia (Csernansky, Dong et al. 2006).

Results in mild cognitive impairment (MCI) have been more mixed. One study found no difference in salivary cortisol levels in MCI compared to healthy elderly and

young adult controls (Wolf, Convit et al. 2002). A further study found no difference in basal salivary cortisol levels but a did find greater cortisol awakening reponse in MCI patients compared with healthy elderly controls, including after adjusting for age, since the control group was significantly older (Lind, Edman et al. 2007). After controlling for seasonal variation, a further study has shown higher salivary cortisol in MCI and dementia patients compared to healthy elderly controls, with levels in MCI patients between healthy controls and dementia (Arsenault-Lapierre, Chertkow et al. 2010). Souza-Talarico *et al* compared salivary cortisol levels between groups of healthy elderly, MCI and mild probable Alzheimer's dementia. They found similar cortisol levels between groups, however higher cortisol levels were associated with better memory performance in healthy elderly, but with worse performance in the group with MCI, and there was no relationship in the group with probable dementia. The authors suggested that the inverse association between memory performance and cortisol between those with and without a diagnosis of cognitive impairment may have been related to GR and MR receptor occupancy, with saturated receptor occupancy in MCI patients with a degree of hippocampal atrophy (Souza-Talarico, Chaves et al. 2010).

In population-based studies, the KORA-Age study of older adults in Germany, which classified participants as normal (N=599), or having MCI (N=101) or dementia (N=33) based on a telephone-administered cognitive test, examined salivary cortisol measures in relation to cognition. The study found lower morning cortisol levels and a trend towards elevated evening levels (which was significant in men) in groups with MCI and dementia. After adjusting for covariates with a multiple linear regression analysis, they found that those with lower morning cortisol and higher evening cortisol were more likely to be cognitively impaired. When stratified for gender, this relationship was significant for men but not for women (Johar, Emeny et al. 2015). In another population based study where participants were free from dementia at baseline, there was no association found between baseline serum cortisol and subsequent incident dementia (Schrijvers, Direk et al. 2011).

In summary, CSF cortisol is elevated in dementia but this is not universal, and may be related to the severity or stage of the dementia process. There appears to be hyperactivity of the HPA axis in dementia, and this is related to severity of disease and cerebral atrophy, and predictive of disease progression. Findings are less clear in MCI, which may be related to the diagnostic criteria used. Finally, it is unclear if this increased activation of the HPA axis is a cause or a consequence of the dementia process, that is whether hippocampal damage from other pathological processes leads to loss of higher cortical feedback of the HPA axis and increased activation, or whether it is a consequence of the disease process itself (Gil-Bea, Aisa et al. 2010).

1.4.8 Cortisol and delirium

1.4.8.1 Behavioural effects of corticosteroid use in clinical practice and "steroid psychosis"

Short-term corticosteroid use in clinical practice has been shown to be associated with psychological effects in around 5% of patients, including altered mood, hyperactivity, insomnia and psychosis (Buchman 2001), all component symptoms of delirium. "Steroid psychosis" usually occurs in those taking at least 40mg of prednisolone, within the first five days of treatment (Buchman 2001). "Dementia in the absence of psychosis" has also been described, along with decreased attention, concentration and verbal memory (Buchman 2001). Delirium may also occur after corticosteroid withdrawal (Campbell and Schubert 1991), along with sleep and mood disturbance (Buchman 2001). Higher doses of steroids are more likely to be associated with behavioural symptoms, but there is much individual variability in the type and severity of reactions that may occur (Wolkowitz, Reus et al. 1997).

Wolkowitz *et al* (Wolkowitz 1994) conducted a series of animal and human experiments in the 1980s designed to elucidate the mechanisms of steroid psychosis and the behavioural effects of steroids. They first administered healthy male Sprague-Dawley rats either corticosterone or placebo for 7 days, and measured levels of neurotransmitter metabolites in the caudate. They found increases in the dopamine

metabolite homovanillic acid (HVA), and a trend to increased levels of the serotonin metabolite 5-hydroxyindole-acetic acid. These changes were accompanied by behavioural changes in keeping with activation of those neurotransmitter systems (Wolkowitz 1994). They later showed that pre-treatment with lithium blocked all these effects (Wolkowitz 1994). Of note, later human studies of CSF in delirium have shown increased HVA associated with psychotic features of delirium (Ramirez-Bermudez, Ruiz-Chow et al. 2008) and increased 5-hydroxyindole-acetic acid has been found in subgroups of delirium (Hall, Shenkin et al. 2011).

Wolkowitz *et al* also performed two studies in healthy human volunteers. They first examined the effects of a single dose of corticosteroid, namely 1mg of dexamethasone administered at 2300 hours, on neurotransmitter metabolites and behaviour. They found that HVA levels rose following dexamethasone administration, but not MHPG (3-methoxy-4-hydroxypheylglycol) (Wolkowitz 1994). The second study involved administration of placebo for five days, then prednisolone at 80mg od for five days followed by placebo for seven days. Serial blood tests, lumbar punctures (in a subgroup of the participants) and quantitative electroencephalograms were also performed, and mood and behavioural changes were documented (Wolkowitz 1994). Seventy-five percent of the study group reported various mild behavioural and mood changes, such as tearfulness, irritability, insomnia, confusion, mood elevation or depression and increased energy (Wolkowitz 1994). They found that some changes in neurotransmitters or their metabolites in CSF were associated with changes in mood and behaviour (Wolkowitz 1994). Quantitative electroencephalogram showed an increase in central theta activity, meaning a slowing of brain wave activity, during steroid administration, and decreases in alpha wave activity (also representing slowing) associated with the increase in theta activity (Wolkowitz 1994). The authors also conducted a test of verbal vigilance and attention in both groups of participants, at baseline and following steroid administration. There was no change in overall attention, but both steroid-treated groups demonstrated increases in “errors of commission”, whereby they were less able to filter out irrelevant information (Wolkowitz 1994).

1.4.8.2 Glucocorticoids and thinking and memory

Glucocorticoid actions within neurons, via their nuclear receptors, alter the transcription of various enzymes involved in the activity of neurotransmitters and catecholamines (Wolkowitz 1994), with additional effects on neuronal metabolism, receptor expression and glial functions (Bellavance and Rivest 2014). When a learning task is being performed, a transient increase in circulating corticosteroids facilitates consolidation of the memory (Buchanan and Lovallo 2001, Abercrombie, Kalin et al. 2003). Learning and memory retrieval may be disrupted, however, by the administration of systemic glucocorticoids (Roozendaal, Williams et al. 1999). In adults with Cushing's syndrome, a correlation between volume of the hippocampal formation and scores on tests of verbal learning and memory has also been demonstrated, but this was not correlated with IQ score (Starkman, Gebarski et al. 1992).

1.4.9 Clinical studies of serum cortisol in delirium

1.4.9.1 Postoperative delirium: cardiac surgery

A meta-analysis of studies examining predisposing and precipitating risk factors for delirium following cardiac surgery found elevated serum cortisol to be a precipitating risk factor (Lin, Chen et al. 2012), based on the results of studies by Mu *et al* (Mu, Wang et al. 2010) and Plaschke *et al* (Plaschke, Fichtenkamm et al. 2010).

Mu *et al* (Mu, Wang et al. 2010) conducted a prospective cohort study examining the role of serum cortisol, measured on the first postoperative day, in delirium in patients undergoing elective coronary-artery bypass grafting. In their cohort of N=243, 50.6% developed postoperative delirium according to CAM-ICU, at a median time of postoperative day 1, and 97% developed symptoms within 3 days of surgery. The delirium group had higher serum cortisol levels, and also had higher APACHE II scores, longer surgery and spent longer on mechanical ventilation, and developed

more postoperative complications. Following logistic regression analysis, serum cortisol level remained an independent predictor of postoperative delirium with an odds ratio of 3.09.

Plaschke *et al* (Plaschke, Fichtenkamm et al. 2010) examined serum cortisol as a measure of the stress response to surgery and the bispectral index (BIS, an electro-encephalography based measure of cortical brain activity) in a cohort of N=114 patients on the first postoperative day following cardiac surgery, of whom 28% developed postoperative delirium. Blood samples were collected once, immediately after BIS measurement. Cortisol levels were higher in the delirium group. There was no difference in timing or length of surgery between groups, nor medication administered. However, the authors only tested for delirium once on the morning of the first postoperative day, and may therefore have missed some cases. They also excluded those with a prior diagnosis of dementia, depression, schizophrenia, stroke or alcohol abuse. Since there was no baseline or informant assessment of cognitive impairment, undiagnosed cognitive impairment may have been a confounding factor.

Kazmierski *et al* have shown that higher preoperative serum cortisol, along with major depression, predicted development of postoperative delirium after coronary-artery bypass surgery, after controlling for other factors in a multivariate analysis (Kazmierski, Banys et al. 2013). In the same cohort, the authors examined the influence of a diagnosis of mild cognitive impairment (MCI) (preoperative MoCA score of 25 or less, and fulfils National Institute on Aging and Alzheimer's Association criteria) on risk factors for development of postoperative delirium (Kazmierski, Banys et al. 2014). They found that those with MCI were more likely to develop delirium and had higher pre- and postoperative (day 1) serum cortisol levels. It is therefore difficult to assess causal relationships between MCI, higher cortisol levels and delirium.

1.4.9.2 Postoperative delirium: non-cardiac surgery

In a small (N=7) cohort of middle-aged men undergoing elective surgery, McIntosh *et al* studied cortisol circadian rhythm in relation to delirium (McIntosh, Bush et al. 1985). They sampled blood four-hourly over a 24-hour period two days prior to surgery and again for a 24-hour period on the second postoperative day. The authors demonstrated a rise in postoperative serum cortisol and β-endorphin levels in the three patients who developed delirium diagnosed according to DSM III criteria. This study was the first to suggest that those who developed postoperative delirium had loss of the normal circadian rhythm of both cortisol and β-endorphin during delirium.

In a cohort of N=80 elderly patients undergoing elective abdominal surgery for resection of stage I or II malignant tumours, Kudoh *et al* found that those who developed postoperative delirium according to DSM IIIR criteria had higher postoperative serum cortisol levels, in samples collected at the end of surgery and 24 and 48 hours later (Kudoh, Takese et al. 2005). There was no difference in preoperative levels. Cortisol levels were higher in the group who developed delirium in samples collected at the end of surgery, 24h and 48h later. There was also no difference in postoperative pain levels or preoperative cognition as assessed by the MMSE score. This suggests that there might be a difference in the HPA axis response to surgery in those who develop delirium, although causation cannot be inferred from this observational study.

The same investigators also examined the effects of local (epidural) anaesthesia for perioperative pain relief in postoperative confusion in patients with chronic schizophrenia, and the involvement of cortisol in these processes (Kudoh, Takese et al. 2003). Patients with chronic schizophrenia are at high risk of postoperative delirium. They randomised N=70 patients with chronic schizophrenia who were undergoing abdominal surgery to receive either epidural plus general anaesthesia, or general anaesthesia alone. A further control group without schizophrenia (N=35) received general anaesthesia for their procedures. Postoperative emergence psychosis was diagnosed by psychiatrists, and ‘postoperative confusion’ was assessed daily

with the CAM until postoperative day 7. Blood samples for cortisol were collected before anaesthesia, 15 minutes after incision, at the end of surgery, 24 hours and 48 hours later. Postoperative confusion occurred in 21% of the schizophrenia group who were receiving epidural anaesthesia, 30% in those with schizophrenia without an epidural and 3% of the control group. Cortisol levels were lower 15 minutes post-incision and at the end of surgery in the epidural group compared to the other schizophrenia group, and lower at the end of surgery in the epidural group than the control group. Within-group comparisons found postoperative confusion was associated with higher cortisol levels at 15 minutes post-incision and at the end of surgery in patients with schizophrenia who had solely general anaesthesia. There were no differences in plasma cortisol levels in the epidural group between those who did and did not develop confusion (Kudoh, Takese et al. 2003). These results suggest there may be a lesser HPA axis response with epidural anaesthesia. However, it is difficult to generalise these findings as the pathogenesis of postoperative confusion or delirium may be different in patients with chronic schizophrenia, either due to their underlying psychotic disorder or their antipsychotic medication, which all patients were receiving. Also, subgroup numbers within groups comparing those with and without confusion were quite small.

In surgical ICU patients who had undergone non-cardiac surgery, Shi *et al* conducted a study examining risk factors for and incidence of postoperative delirium, and the relationship between delirium and serum cortisol levels (Shi, Wang et al. 2010). In their fairly large cohort of N=164 patients, 30.5% developed delirium while still in surgical ICU, and 44.5% during their hospital stay. High serum cortisol on the first postoperative day was identified as a risk factor for postoperative delirium in a logistic regression analysis.

Cerejeira *et al* (Cerejeira, Batista et al. 2013) studied cortisol and inflammatory profile in a cohort of N=101 patients without dementia undergoing elective hip arthroplasty. Morning blood samples were collected on the day before surgery and the first postoperative day. Delirium assessments were on the evening of the day of

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture surgery and on the second and third postoperative days. They excluded patients who were delirious preoperatively; 36.6% met DSM-IV-TR criteria for delirium postoperatively. They found that those who developed postoperative delirium had a greater rise between pre- and postoperative cortisol levels compared with those who did not develop delirium (Cerejeira, Batista et al. 2013). Due to the timing of samples and assessments and the observational nature of the study, it is difficult to know whether a greater rise in cortisol predisposes to delirium or is a consequence. The greater rise in serum cortisol in those who developed delirium may be due to predisposition to disruption of the HPA axis with a consequential greater rise in cortisol due to stress in those who were more prone to delirium, or may be because those who had a more complicated or physically stressful surgical episode with greater rise in cortisol were also more likely to develop delirium. This study is discussed further in section 1.6.6.2.

Deiner *et al* performed a prospective cohort study investigating the relationship between anaesthetic choice, stress response and incidence of postoperative delirium (Deiner, Lin et al. 2014). They hypothesised that total intravenous anaesthesia (TIVA) with propofol compared to inhalational anaesthesia would lead to a lesser stress response and lower incidence of delirium. N=76 elderly patients underwent elective non-cardiac surgery under either TIVA or gas anaesthetic, determined by the clinical anaesthetic team, according to the requirements of the procedure. On the day of surgery, 4h after surgery started and 2h after surgery, blood was collected for measurement of cortisol, epinephrine and norepinephrine. Delirium was assessed using CAM in the recovery room and daily postoperatively. Cortisol levels were higher at all timepoints in the gas group, and levels of epinephrine were higher intraoperatively and postoperatively, and norepinephrine was higher intraoperatively. There was no difference in delirium incidence between groups. The gas group was older, more of the group were ASA 3 or 4, more had malignancy, MMSE score was lower (median 27 vs. 29), and they were undergoing a greater range of procedures; all but four of the N=40 TIVA group underwent spinal surgery. A multivariable logistic regression modelling postoperative delirium as the outcome found that predictors were diabetes, surgical duration and postoperative norepinephrine level.

1.4.9.3 Acute illness and medical ICU delirium

Higher early morning serum cortisol on the first morning after admission has been shown in a relatively small study (N=52) to predict incidence of delirium in patients with acute coronary syndrome admitted to a coronary care unit (Colkesen, Giray et al. 2013). There was also a significant correlation between cortisol levels and delirium severity. The authors found 48% of their cohort, with a mean age in their 60s, developed delirium according to DSM IV.

Pfister *et al* examined several biomarkers including serum cortisol, and measures of cerebral blood flow and oxygenation, in a small cohort of ICU patients with sepsis-associated delirium (Pfister, Siegemund et al. 2008). Biomarkers were measured at the same time as cerebral blood flow measurement, within 48 hours of admission and stabilisation. In their cohort of critically ill patients with extracranial sepsis, 12/16 developed delirium according to the CAM-ICU, and serum cortisol levels were significantly higher in the delirium group. However, the authors did not control for other factors such as illness severity, so those who were more unwell or who had a greater systemic stress or inflammatory response may have had higher cortisol levels and also developed delirium. Also, several patients received hydrocortisone as part of their therapy.

Van den Boogaard *et al* examined multiple biomarkers including cortisol in a cohort of ICU patients who they split into “inflamed” and “non inflamed” (van den Boogaard, Kox et al. 2011). They monitored patients for delirium using the CAM-ICU three times daily, and 50% of the 100 patients included in the study developed delirium. They divided those with delirium into “inflamed” and “non-inflamed”; “inflamed” was defined as a positive culture for which the patient was given antibiotics, and “non-inflamed” as the absence of proven or suspected infection, and having no more than one systemic inflammatory response syndrome criterion. The control group were likewise split into “inflamed” and “non-inflamed”. Cortisol level,

collected once within 24 hours of development of delirium, was associated with delirium in both subgroups of "inflamed" and "non inflamed" patients in a univariate logistic regression analysis, however not in a multivariate analysis.

Nguyen *et al* performed a prospective observational cohort study in N=140 ICU patients to study the effect of cortisol on "brain dysfunction", encompassing coma and delirium, in severe sepsis and septic shock (Nguyen, Huyghens et al. 2014). They looked at the predictive value of cortisol and of the CNS damage marker S100B. Dementia was amongst the exclusion criteria. They assessed brain dysfunction using the Glasgow Coma Scale, the CAM-ICU and the RASS, and it was considered present if GCS was ≤13, or if CAM-ICU was positive. Serum cortisol and S100B were measured, first within 6-12 hours of admission and stabilisation, and then each morning for four consecutive days. Twelve patients who died while still sedated were excluded, N=22 patients remained comatose, 107/128 patients developed "brain dysfunction", and delirium developed in 85/107 (80%) of patients. Cortisol levels throughout the measurement period were higher in those with brain dysfunction than those without, after adjusting for age and gender, but over time the cortisol levels in the brain dysfunction group fell while those in the non-brain dysfunction group rose. Using logistic regression analysis, they found two risk factors for delirium in severe sepsis, which were cortisol level and cortisol combined with age.

1.4.9.4 Hip fracture

Bisschop, van Munster *et al* studied the role of cortisol, insulin and glucose in a cohort of N=143 elderly patients with acute hip fracture, using serial blood sampling, before during and after delirium (Bisschop, de Rooij et al. 2011). They showed using a linear mixed models approach that the group who developed perioperative delirium (N=70) had higher plasma cortisol levels. However, this relationship was no longer significant after adjusting for prior cognitive impairment (as defined by the

Informant Questionnaire on Cognitive Decline in the Elderly or a prior history) in a multivariate analysis.

1.4.9.5 Dexamethasone suppression tests

Robertsson *et al* examined the HPA axis response in a cohort of patients admitted for assessment of dementia, with no acute medical illness. In those with DSM-III-R delirium superimposed on dementia, the authors found the highest basal serum cortisol levels in those with mild delirium, with lower levels in those with no or moderate-to-severe delirium. There was no relationship between cortisol levels and age or differences between different aetiologies of dementia, but those with moderate severity dementia had the highest basal cortisol levels, and the authors also found increasing HPA axis dysfunction with increasing severity of dementia. They also found that the strong relationship between delirium and positive dexamethasone suppression test results was independent of age and dementia severity (Robertsson, Blennow et al. 2001).

Impaired negative feedback of the HPA axis, as demonstrated by positive dexamethasone suppression tests in patients with delirium, has also been demonstrated in patients with lower-respiratory tract infection (O'Keeffe and Devlin 1994). In sixteen consecutive patients admitted to an acute geriatric ward with a lower respiratory tract infection, the authors found that in 7/9 patients who had delirium (in the absence of dementia or depression), dexamethasone failed to suppress cortisol levels, compared to 1/7 patients without delirium, despite comparable illness severity. Furthermore, in six patients who had had delirium and a positive dexamethasone suppression test who they were able to follow-up eight weeks later, although their delirium and acute illness had resolved, 5/6 still had a positive test. Since this was an observational study, causation cannot be established. This may indicate that these patients had underlying HPA axis pathology and were very prone to developing high cortisol levels in response to acute illness and to

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture developing delirium. It may be that the delirium process led to abnormal HPA axis activity which persisted after resolution of the delirium.

1.4.9.6 Genetic predisposition; glucocorticoid receptor signalling

Glucocorticoid receptor (GR) signalling may be of importance in the risk of delirium development; homozygous carriage of GR haplotype *Bcl*I and *TthIII*I was found to be associated with a 92% decreased risk of delirium in a cohort of elderly patients with acute hip fracture (Manenschijn, van Rossum et al. 2011).

1.4.9.7 CSF cortisol

Pearson *et al* undertook a prospective cohort study in patients over the age of 60 undergoing spinal anaesthetic for repair of a hip fracture, with the aim of determining if CSF cortisol levels are elevated in delirium (Pearson, de Vries et al. 2010). N=27 patients were assessed for delirium preoperatively with an assessment battery which informed completion of the Confusion Assessment Method, but only N=20 could be included due to change to general anaesthetic or blood contamination of the sample. A blood sample was collected at the same time. CSF specimens were taken at the onset of spinal anaesthetic, before administration of the anaesthetic agent. The investigators found CSF cortisol levels to be higher in those with delirium than those without. Plasma cortisol was also significantly higher in cases than controls. This suggests an association between elevated CSF cortisol and delirium, although causation could not be inferred in this cross-sectional, observational study. As the authors comment, dementia, severity of co-morbid disease and physiological stress are potential confounding factors which should be assessed in future studies.

1.4.10 Glucocorticoids and sleep

In clinical practice, steroids are not generally administered late in the day due to the potential for sleep disruption. Both Cushing's syndrome and major depressive disorder which display hypercortisolism are associated with sleep disorders (Shipley, Schteingart et al. 1992), which are also a very common feature of delirium (Meagher, Leonard et al. 2010). Shipley *et al* have shown using polysomnography that both disorders are associated with similar sleep problems, including taking longer to reach stage 2 sleep, and shorter total sleep time and poorer efficiency (Shipley, Schteingart et al. 1992). Those with Cushing's had even lighter sleep than those with major depression. It has also been demonstrated that sleep continuity and the level of blood cortisol are well correlated, with lighter sleep when cortisol levels are higher in the morning and deeper sleep when levels are lower in the evening in normal subjects (Shipley, Schteingart et al. 1992). Older age is commonly associated with a variety of sleep problems, with as many as 50% of older adults complaining of difficulty falling asleep or maintaining sleep (Crowley 2011). This poor night-time sleep has been shown in a small cohort of healthy older adults to be associated with higher serum cortisol and interleukin-6 than healthy younger adults who had normal sleep patterns, and cortisol level was shown to be negatively associated with the amount of REM sleep in both young and older adults (Vgontzas, Zoumakis et al. 2003). Salivary cortisol level on wakening is significantly lower in middle-aged patients with primary insomnia, and lower waking cortisol correlates with poorer sleep quality (Backhaus, Junghanns et al. 2004).

1.4.11 Cortisol in cognitive decline and dementia

Chronic elevation of glucocorticoid levels has been shown to be associated with cognitive impairment, depression, structural CNS abnormalities including hippocampal atrophy and neuroendocrine abnormalities (Chapman and Seckl 2008).

The Baltimore Memory Study is an epidemiological study (N=967) looking at risk factors for cognitive decline in urban residents. At a single timepoint in the study,

researchers collected four salivary cortisol samples during study visits which included cognitive testing (deemed as a mild psychosocial stress), before, during and after the testing and finally at the end of the visit. They found in multivariable analyses that higher cortisol was significantly associated with poorer performance in cognitive testing, independent of age, gender, education and other covariates. The authors concluded that there was a dose-response relationship between cortisol exposure, potentially due to chronic stress, and worse cognitive performance in domains encompassing the hippocampus and neocortex (Lee, Glass et al. 2007). A smaller (N=79) longitudinal study assessed the relationship between cognition and cortisol diurnal rhythm annually for three years in healthy older adults. They found that higher average and 23.00h cortisol level were both associated with poorer performance on tests of declarative memory and executive function. They also found that higher salivary cortisol levels predicted decline in verbal memory over three years (Li, Cherrier et al. 2006).

Elevation in glucocorticoids, precipitated by the significant stressor of a surgical procedure, has been hypothesised to be involved in postoperative cognitive dysfunction (POCD). Rasmussen *et al* performed a study investigating the hypothesis that changes in perioperative cortisol secretion are involved in POCD (Rasmussen, O'Brien et al. 2005). They included N=187 patients from ten centres, and performed a neuropsychological test battery preoperatively, on day 7 postoperatively and at 3 months, along with diurnal salivary cortisol measurements at the same timepoints plus the first postoperative day. There was no specific testing for postoperative delirium. POCD was defined according to their criteria, that 2/7 Z scores from individual tests or the combined Z score was greater than 1.96. 18.8% of tested patients had POCD at one week, and 15.2% at three months. Morning and afternoon salivary cortisol increased after surgery, with a statistically significant increase in both values on the first postoperative day and in the afternoon value at one week, with flattening of the normal diurnal variation. There was a trend towards a lower am/pm salivary cortisol ratio in those who developed POCD. Log transformation of the non-normally distributed am/pm ratio, examined in a linear interaction model, found a significant change over time in the ratio, and that this was related to POCD

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture at one week but not at three months (Rasmussen, O'Brien et al. 2005). It is interesting that the association found between loss of normal diurnal rhythm of cortisol and POCD was demonstrated acutely but not at longer-term follow-up. It is impossible to know how many of those patients had also developed postoperative delirium, and whether this might have impacted on cognitive testing, or whether the same mechanisms are in play for the development of postoperative delirium and POCD.

1.4.12 Cortisol and neuronal toxicity

In clinical practice, glucocorticoids are used for their anti-inflammatory and immune-suppressing qualities. However, there is some evidence that they may worsen the excitotoxic insult in some inflammatory brain injury states, and clinical trials of steroids in cerebral oedema after stroke and traumatic brain injury have found them to worsen rather than improve outcomes (Sorrells and Sapolsky 2007). Exposure of neurons to excess cortisol increases their vulnerability to damage and cell death from various insults such as glutamate excess, hypoxia, hypoglycaemia and oxidative stress (Kimonides, Spillantini et al. 1998, Herbert, Goodyer et al. 2006), with hippocampal neurons seemingly particularly vulnerable (Dinkel, MacPherson et al. 2003). Recent studies have also challenged the traditional view that steroids are anti-inflammatory in the central nervous system (Sorrells and Sapolsky 2007). In a rat model comparing the effects of low (adrenalectomy), intact and high (corticosteroid treatment) glucocorticoid levels on a kainic acid-induced brain injury, Dinkel *et al* showed that endogenous glucocorticoids had an overall pro-inflammatory effect on cellular infiltrate and anti-inflammatory effect on synthesis of mRNA for cytokine production. They also found that chronically high glucocorticoids had pro-inflammatory effects in the CNS (Dinkel, MacPherson et al. 2003). Stress, via glucocorticoids, has also been shown to be capable of priming pro-inflammatory responses of microglia in an animal model; rats were given a stressor (an inescapable tail shock), and then microglia isolated from their hippocampi were exposed to LPS, and this potentiated the pro-inflammatory response, e.g. expression of IL-1β gene. Pre-treating the rats in vivo with a glucocorticoid antagonist (RU486) or removing

the adrenal glands, prevented the potentiation of the response (Frank, Thompson et al. 2012).

1.4.13 Conclusions

In summary, cortisol is a steroid hormone with roles in multiple homeostatic mechanisms and is central to the body's response to stress. Cortisol and its circadian rhythm may be influenced by age and dementia, although this is heterogeneous. Exposure to high levels of cortisol and exogenous steroids acutely may lead to psychosis, and chronic exposure may be associated with depression, dementia and hippocampal atrophy. Several studies have found evidence of elevated cortisol levels in delirium in medical, surgical and critical care settings.

1.5 The inflammatory hypothesis of delirium

1.5.1 Definitions

The immune response has two main components, comprising the innate and humoral immune responses. Innate immunity involves cells which respond to insults using receptors that do not need gene rearrangement for their activity. These cells include neutrophils, which circulate in the blood and macrophages which are resident in tissues (microglia in the brain), and they initiate an inflammatory response including phagocytosis, and recruit dendritic cells involved in adaptive immunity. Dendritic cells present antigens to prime specific T-lymphocytes. Neutrophils and macrophages interact; macrophages activate neutrophils, and neutrophils produce the chemokines interleukin-8, MIP-1α and MIP-1β which recruit and activate macrophages (Solana, Tarazona et al. 2012).

There has been interest in the potential role of an abnormal inflammatory response in delirium (Perry, Cunningham et al. 2007, MacLullich, Ferguson et al. 2008). Peripheral infection is a common precipitant of delirium, although the pathway from a seemingly minor infection without significant systemic upset to the neuropsychiatric symptoms of delirium is unclear. In clinical practice, evidence for possible urinary tract infection is often sought in delirium, and a recent systematic review found that there is an association between delirium and urinary tract infection, but the studies looking specifically at this are methodologically flawed (Balogun and Philbrick 2014). Sepsis is also a common precipitant of ICU delirium in young, fit patients; this is sometimes referred to as “septic encephalopathy”.

Cytokines form an integral part of the metabolic response to stress, as well as to infection. After administration of endotoxin, Tumour Necrosis Factor (TNF)-α is the first cytokine to appear (Stouthard 1996) and is involved in inducing a catabolic or septic state with changes to glucose, protein and fat metabolism. Interleukin(IL)-6 is released following TNF-α administration, and is therefore thought to be another

primary mediator of this process. Stouthard *et al* studied the metabolic effects of recombinant human (rh)IL-6 administration in N=8 patients with metastatic renal cell carcinoma, who were receiving it as part of a phase II clinical trial; each patient also received a saline infusion at a different time, and acted as their own control (Stouthard, Romijn et al. 1995). Infusion of rhIL-6 induced a febrile response and release of norepinephrine, cortisol, glucagon and plasma free fatty acids. Resting energy expenditure increased.

The effects of the administration of the bacterial endotoxin lipopolysaccharide (LPS) in order to induce a transient state of low-grade inflammation, on emotional and cognitive aspects of sickness behaviour, have been studied in healthy adult men. In a study by Reichenberg *et al*, the administration of bacterial endotoxin or saline IV to N=20 healthy adult men, increased serum levels of cortisol, TNF-α, soluble TNF receptors, IL-6 and IL-1-receptor antagonist (Reichenberg, Yirmiya et al. 2001). Following endotoxin administration, the subjects had heightened anxiety, depressed mood and decreased verbal and non-verbal memory on neuropsychological testing (Reichenberg, Yirmiya et al. 2001).

Grigoleit *et al* found that intravenous injection of LPS caused a transient rise in body temperature, inflammatory cytokines including TNF-α and IL-6, neutrophils, salivary and plasma cortisol, and ACTH, in a cohort of N=24 healthy young men (Grigoleit, Oberbeck et al. 2010). They observed that LPS did not induce any change in performance on the revised Wechsler Memory Scale-R nor in its subscales (including attention), nor in the Stroop colour test which tests executive function. There was a correlation between attention subscale scores in the LPS treated group and plasma prolactin (Grigoleit, Oberbeck et al. 2010). It is possible that the inflammatory insult was too mild or too short lived to see any significant effect on the memory tasks, particularly in a cohort of cognitively resilient young adults.

1.5.2 Sickness behaviour syndrome

The mental status changes often referred to as 'sickness behaviour syndrome', are thought to represent a coordinated set of behavioural changes which, in health, promote survival. The features of sickness behaviour; malaise, somnolence, decreased locomotion and cognition, and anorexia (Perry, Cunningham et al. 2007), are similar to hypoactive delirium, and delirium in older patients may represent an exaggerated and pathological response to an infective or immune stressor.

1.5.3 Animal modeling

This section will discuss animal (mouse and rat) experiments that explicitly aim to model delirium.

Cunningham and Perry were among the first to use an animal model to examine the role of inflammation in delirium pathophysiology. They have predominantly employed the ME7 prion disease mouse model to study the role of inflammation in chronic neurodegeneration. The ME7 model is produced by injecting female C57BL/6 mice in the dorsal hippocampus with either normal mouse brain homogenate (control) or infected ME7 mouse brain homogenate. The ME7 mice then have a predictable course of deterioration of neurological function over weeks, and experiments are usually performed just before clinical signs develop at 19 weeks. This research group first demonstrated in 2005 that microglia (the resident macrophages in the brain) are "primed", probably by the ongoing neurodegenerative pathology, to produce a greater response to subsequent inflammatory challenges. The microglia have morphological changes suggesting that they are active, but they do not produce inflammatory cytokines such as IL-1β. However, even a low-moderate dose peripheral challenge of LPS (bacterial endotoxin lipopolysaccharide) in the form of an intraperitoneal injection leads to the exaggerated production of IL-1β, IL-6 and TNF-α in the brain, along with exaggerated sickness behaviour responses. Importantly, there was also associated neuronal cell death (Combrinck, Perry et al. 2002, Cunningham, Wilcockson et al. 2005).

Further investigation of the effects of these peripheral inflammatory challenges (100μg/kg LPS injected intraperitoneally) showed that in the ME7 mice, they lead to transient and reversible deteriorations in working memory as assessed by a T-maze task where the mice are walking in shallow water and need to remember to turn in the opposite direction to the previous trial in order to exit the maze (Murray, Sanderson et al. 2012). The group have also shown that after these inflammatory challenges, the rate of deterioration in the prion-induced neurodegenerative disease accelerates in the ME7 mice (Cunningham, Campion et al. 2009). The authors postulated that this might be a similar process to when patients with dementia develop delirium, since delirium is commonly triggered by infection and infections have been shown to accelerate the progression of dementia.

This group have recently adopted a model of cholinergic deficiency, in which mice are lesioned with murine-p75-saporin immunotoxin in the basal forebrain to induce chronic cholinergic depletion similar to early dementia. Interestingly, this lesion did not induce microglial priming. When these mice are given a systemic inflammatory challenge with LPS injection (100μg/kg), they display transient deficits in working memory compared to unlesioned mice and lesioned mice that had a saline challenge. Interestingly, the central inflammatory response was similar in lesioned and unlesioned controls. Peripherally administered donepezil, given shortly after the infective challenge, was partially protective against the working memory deficits. These experiments support the hypothesis that chronic cholinergic deficiency may predispose to delirium as well as acutely-induced cholinergic deficiency, but that there might be some protection from acetylcholinesterase inhibitors (Field, Gossen et al. 2012).

In aged animals, similar processes have been demonstrated. Godbout *et al* have shown in aged mice (20-24 months old, where natural lifespan is 26 months) that peripheral LPS injection at a dose which usually causes mild transient sickness behaviour in young adult mice, led to greater anorectic responses and greater

reduction in social behaviour, which lasted longer than young adult mice. They also found a protracted and exaggerated neuroinflammatory response, with increased production of IL-1β and IL-6 in the brains of the aged mice. This response was not found to the same extent in the periphery (Godbout, Chen et al. 2005).

Culley *et al* also set out to study inflammatory mechanisms in an animal model of delirium (Culley, Snayd et al. 2014). They used a task that tested attention and executive function in rats, the attentional set-shifting task (AST); this task involves the prefrontal and parietal cortex, and uses cholinergic neurotransmission, giving it relevance for attention in human delirium. They used older (24 month old) Fischer-344 rats, and gave them a peripheral inflammatory stimulus with an intraperitoneal injection of lipopolysaccharide (50μg/kg) or saline. The rats were trained in the AST, given either LPS or saline, and then tested on different aspects of the attentional task for three consecutive days. TNF-α and CCL-2 were measured in plasma and prefrontal cortex of a further group of age-matched rats given either LPS or saline, at 2, 24 and 48 hours. Analysis of variance testing demonstrated a main effect of LPS on behaviour, selectively impairing performance on tasks involving shifting attention. Plasma levels of TNF-α and CCL-2 rose 2h after LPS, and levels of TNF-α had returned to control levels by 24h, whereas CCL-2 returned to control levels by 48h. TNF-α levels did not rise significantly above control levels in prefrontal cortex, whereas CCL-2 rose in LPS treated animals at 2h only. These results suggest that in aged rats, peripheral inflammatory stimuli are capable of selectively impairing attention and executive function. Associative learning was not affected, and LPS only impaired more complex attentional tasks requiring cognitive flexibility. The deficits were also present after the measured inflammatory responses had resolved; this is often seen in human delirium when an infection triggers delirium that persists after the infective insult has been treated.

1.5.4 Effects of directly administered cytokines

Direct administration of cytokines used for cancer chemotherapy, such as Interferon (IFN)-α (Capuron, Ravaud et al. 2004) and Interleukin (IL)-2 (Rosenberg, Lotze et

al. 1989, Walker, Wesnes et al. 1996, Walker, Walker et al. 1997), can lead to acute mental status change, such as disorientation, somnolence, reduced reaction times (Rosenberg, Lotze et al. 1989, Walker, Wesnes et al. 1996, Walker, Walker et al. 1997) and depressive symptoms (Capuron, Ravaud et al. 2004). The neurotoxicity associated with interferons has been observed to include delirium, depression and "sickness behaviour" type symptoms, as well as manic and psychotic syndromes, but not always falling within usual psychiatric diagnostic criteria (Patten 2006). Disordered serotonin metabolism is thought to be involved in the pathogenesis of IFN-α-induced depression. Serotonin reuptake inhibitors are helpful in its management, and IFN-α can activate pathways involved in serotonin processing (Miller 2009). IFN-α may also alter the metabolism of dopamine, with potential consequences of fatigue, psychomotor slowing and sleep-wake cycle disturbance. It can also cause hypomanic symptoms, irritability and hyperactivity (Miller 2009).

1.5.5 Human studies of inflammation in delirium

1.5.5.1 Postoperative delirium: cardiac surgery

Rudolph *et al* examined the hypothesis that delirium is a CNS response to a systemic inflammatory insult in a state of blood-brain barrier compromise in a cohort of patients undergoing cardiac surgery (Rudolph, Ramlawi et al. 2008). A prospective cohort of N=42 patients without preoperative delirium underwent surgery under cardiopulmonary bypass. Blood samples were collected preoperatively, and 6 hours and 4 days postoperatively. Patients were assessed for delirium preoperatively and daily from the second postoperative day. Twelve patients developed postoperative delirium, and were matched according to age, duration of surgery and baseline MMSE score. They found no differences in baseline inflammatory profile between groups. At 6 hours postoperatively, levels of chemokines (EOTAXIN, RANTES, CCL-2 and IL-8) were increased in those who went on to develop delirium, but there was no difference by day 4. At day 4 there was a trend towards lower levels of cytokines promoting T-helper 1 and 2 activity.

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Kazmierski *et al* performed a prospective cohort study of the role of mild cognitive impairment in the development of postoperative delirium after coronary artery bypass grafting, and the relationship with cortisol, cytokines, cobalamin and homocysteine (Kazmierski, Banys et al. 2014). This study is also described in section 1.4.9.1. A blood sample for IL-2 and TNF-α was collected on the morning of the first postoperative day. Both cytokines were elevated in the delirium group compared to those who did not develop delirium. However, these associations were lost after the results were entered into a multivariate analysis. The MCI group had higher IL-2 levels after surgery. Further analysis of this cohort, examining whether postoperative delirium is associated with raised pro-inflammatory cytokines (IL-2 and TNF-α), and examining whether any relationship between inflammation and delirium is mediated by surgical, anaesthetic, or premorbid disease factors has also been described (Kazmierski, Banys et al. 2014). Those with raised pro-inflammatory cytokines were at increased risk of postoperative delirium, including after controlling for other variables that were positive in the univariate analysis (trend for TNF-α; other variables included AF, depression, dose of midazolam, reperfusion time, trail-making B time). Several of the medical and surgical factors under investigation correlated with inflammatory cytokine levels, including correlations with advancing age, poorer MoCA score, and poorer trail-making scores, and longer perfusion and reperfusion time (with IL-2 only).

Plaschke *et al* (Plaschke, Fichtenkamm et al. 2010) examined serum IL-6 and CRP as measures of the inflammatory response to surgery and the bispectral index (BIS, a measure of cortical brain activity) in a cohort of N=114 patients on the first postoperative day following cardiac surgery, of whom 28% developed postoperative delirium. This study also measured cortisol, the results of which were discussed in section 1.4.9.3. Blood samples were collected once, immediately after BIS measurement. They found that both cortisol and IL-6 levels were higher in the delirium group, but the markers themselves were not correlated. CRP was measured at the same timepoint, and there was no difference between groups.

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Bokesch *et al* studied a large cohort (total N=557) of high-risk patients undergoing cardiac surgery with cardiopulmonary bypass, looking at risk factors for neurological complications including delirium or stroke/TIA (Bokesch, Izykenova et al. 2006). Little information is provided on how delirium was assessed, however. The majority (49/55) of those encountering neurological complications developed "moderate-severe confusion or delirium". The authors found no association between preoperative CRP level and postoperative neurological complications. A higher preoperative S100B level was associated with an increased risk of neurological complications, but those with a higher NMDA receptor antibody concentration preoperatively were at much higher risk.

Delirium has also been studied as a predictor for other postoperative complications including sepsis. Martin *et al* used a retrospective data review of patients in two cardiac surgery data registries to identify patients who had had both delirium (defined as per the Society of Thoracic Surgeons as "mental disturbance marked by illness, confusion, cerebral excitement, and having a comparatively short course") and sepsis (Martin, Buth et al. 2010). Sepsis was the primary outcome of interest. Patients were considered to be delirious first if they had those symptoms for 48 hours prior to the signs of sepsis. Of just over 14,000 patients on the registry, 981 developed delirium, 227 became septic and 70 were also delirious. Patients who developed delirium were more likely to develop pneumonia, urinary tract infections, deep sternal wound infections and sepsis. Thirty-four patients were delirious 2-10 days prior to developing sepsis. Logistic regression analysis found that delirium was associated with the development of postoperative sepsis, with an adjusted odds ratio of 2.32. This finding suggests that delirium may increase the risk of developing sepsis. The authors discuss that this may be because of an increase in the need for instrumentation, e.g. of the urinary tract due to self-removal of catheters, the use of sedating agents which reduce the ability to self care and clear secretions, and poor oral intake and malnutrition increasing patients' vulnerability to infection (Martin, Buth et al. 2010). Delirium in this context may also be an early marker of sepsis, and should prompt clinicians to search for infection. Delirium here was defined differently to DSM-IV or ICD 10. The Society of Thoracic Surgeons definition is

still likely to be describing delirium but with a bias towards hyperactive subtypes, so some patients with hypoactive delirium may not have been included.

1.5.5.2 Postoperative delirium: non-cardiac surgery

Liu *et al* conducted a large prospective cohort study examining the role of IL-6 in delirium following major non-cardiac surgery (Liu, Ya-wei et al. 2013). They enrolled N=338 patients over the age of 60 who were due to undergo elective non-cardiac surgery. They excluded those with prior dementia, Parkinson's disease and schizophrenia, severe liver or renal dysfunction and those who appeared delirious preoperatively. Patients were assessed twice daily for delirium (CAM-ICU) on the first three postoperative days, and blood samples were collected to measure IL-6 before anaesthesia and on the first postoperative morning. Fifty patients (14.8%) developed postoperative delirium. High pre- and postoperative IL-6 levels were associated with delirium. Multivariate Cox regression analysis identified high postoperative IL-6 as an independent predictor of delirium, along with age, poor preoperative NYHA class, MMSE <24 and high postoperative pain scores.

In a cohort of N=101 cognitively healthy patients aged over 60 undergoing elective arthroplasty, Cerejeira *et al* screened for postoperative delirium and measured markers of both inflammation and the cholinergic system (Cerejeira, Nogueira et al. 2012). Blood samples were collected on the day before surgery and the first postoperative day, and delirium assessments were on the evening of the operation day and on days 2 and 3. The surgical trauma itself induced an increase in CRP, IL-6, IL-8 and IL-10, and an increase in the ratio of pro/anti-inflammatory cytokines in both groups. Thirty-seven patients developed delirium, and they found that those who developed delirium had lower preoperative plasma esterase levels. There were no differences in the inflammatory markers measured (IL-1β, IL-6, IL-8, IL-10, CRP and TNF-α) between those who did and did not develop delirium, but the delirium group had a higher pro/anti-inflammatory ratio postoperatively. They also found correlations between preoperative plasma esterase activity and change in levels of

CRP, IL-6 and pro/anti-inflammatory ratio in the delirium group only (Cerejeira, Nogueira et al. 2012). Plasma Insulin-like Growth Factor-1 (IGF-1) was also measured in this cohort, and they did not find a significant difference between groups. The elevation in cortisol described in section 1.4.9.2 correlated with IL-6 and IL-10 in those with postoperative delirium, but not in those without delirium (Cerejeira, Batista et al. 2013).

In their study of postoperative delirium in patients undergoing abdominal surgery, Kudoh *et al* examined cortisol, IL-6, CRP and norepinephrine levels before anaesthesia, at the end of surgery, on the next day and on the day following that (Kudoh, Takese et al. 2005). This study has been described in more detail in section 1.4.9.2. The authors found no difference in baseline IL-6 levels, but found that levels were higher in those with delirium at all 3 postoperative timepoints. They observed the same pattern in cortisol levels, and found that the two were positively correlated at all postoperative timepoints in the delirium group, but only in the sample at the end of surgery in the group without delirium. No differences were detected in norepinephrine and CRP levels between groups.

The same investigators also examined the effects of local (epidural) anaesthesia for perioperative pain relief in postoperative confusion in patients with chronic schizophrenia, and the involvement of IL-6 and cortisol in these processes (Kudoh, Takese et al. 2003). This study has been discussed in more detail in section 1.4.9.2. Blood samples for IL-6 and cortisol were collected before anaesthesia, 15 minutes after incision, at the end of surgery, 24 hours and 48 hours later. Postoperative confusion occurred in 21% of the schizophrenia group who were receiving epidural anaesthesia, 30% in those with schizophrenia without an epidural and 3% of the control group. No differences were found in levels of IL-6 between treatment groups. The epidural group had lower IL-6 levels at the end of surgery and 24 hours later than the control group without schizophrenia. Patients with schizophrenia who developed postoperative confusion had higher IL-6 levels at the end of surgery and 24 hours later than those who did not. These results suggest there may be an

association between higher IL-6 and cortisol levels and postoperative confusion. However, it is difficult to generalise these findings as the pathogenesis of postoperative confusion or delirium may be different in patients with chronic schizophrenia, either due to their underlying psychotic disorder or their antipsychotic medication, which all patients were receiving. Indeed, high serum IL-6 levels have been documented in schizophrenia (Naudin, Mege et al. 1996). Also, subgroup numbers within groups comparing those with and without confusion were quite small.

Pol *et al* performed a prospective cohort study in N=277 consecutive patients undergoing vascular surgery, with the aim of examining whether there is a inflammatory basis to postoperative delirium in vascular surgery (using CRP levels), and what the main risk factors were for delirium in that setting (Pol, van Leeuwen et al. 2014). The rate of postoperative delirium in the study was relatively low at 6%. In univariate analyses, they found that the presence of multiple co-morbidities, elevated CRP postoperatively, intensive care admission, and open aortic or amputation procedures were related to postoperative delirium. CRP level was shown to be related to the development of postoperative delirium in a multivariate logistic regression analysis, but the odds ratio was only 1.01 (95% confidence interval 1.00-1.03, p=0.04). Compared with a normal CRP level of 5, a CRP level of 50 was associated with a 35% increased risk of postoperative delirium, and a level of 100 with a 90% increased risk.

Capri *et al* performed a secondary analysis of a subcohort of patients from a case-control study into postoperative delirium to examine the predictive role of preoperative "inflammageing" cytokines in delirium (Capri, Yani et al. 2014). In their original cohort of N=351 patients aged over 65 years and without known dementia, who underwent any emergency or elective general or transplant surgery, N=47 developed postoperative delirium. N=37 of these patients were age- and sex-matched with controls who did not develop delirium, and a panel of cytokines (TNF-α, IL-1β, IL-2, IL-6, IL-8, and IL-10) was measured with ELISA, in a serum sample

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture taken on the day of hospital admission. The authors found that IL-1β and TNF-α were largely undetected, there was no difference in IL-8 or IL-10 levels, but IL-2 was lower and IL-6 was higher in the group that went on to develop delirium. After adjusting for potential confounders (age, co-morbidity, functional level, anxiety and depression, benzodiazepine use) with logistic regression analysis, IL-2 was no longer significantly associated with delirium, but IL-6 was still predictive of delirium. There was no mention of assessment of preoperative delirium, although given that 28/37 patients in the delirium group had emergency surgery, some may have developed delirium preoperatively.

1.5.5.3 Hip fracture

Van Munster *et al* examined the time-course of cytokine changes in relation to delirium in a cohort of patients with acute hip fracture, and related this to delirium subtype (van Munster, Korevaar et al. 2008). They prospectively recruited a cohort of N=221 patients, and assessed them daily for delirium on weekdays, using the Delirium Symptom Interview to ascertain subtype and the DRS-R98 to assess severity. Blood samples were collected a maximum of four times, on weekdays, at 11am. Levels of TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12 were measured with a cytometric bead array immunoassay. In order to examine the time-course, samples were classed as either "non-delirious", or "before", "during" or "after" delirium. N=98 patients could be included in the analysis. Unfortunately, TNF-α, IL-1β and IL-10 were undetected for the majority of samples. The authors found that IL-6 and IL-8 were significantly higher in the delirium group. Comparing the kinetics of the cytokines over time in those who were continuously delirious, non-delirious or delirious for a discrete period postoperatively, it can be seen that levels of these cytokines start high, presumably a response to the hip fracture trauma and subsequent operation, with an exaggerated rise in those with delirium, with a degree of dose-dependency. Levels fall over the coming days but remain slightly higher for longer in the delirium group (van Munster, Korevaar et al. 2008). The authors also found that IL-6 levels were higher in those with hyperactive or mixed subtypes of delirium.

Van Munster *et al* also examined IL-6 and IL-8 levels (along with cortisol as described in section 1.4.9.4) in a cohort of patients with acute hip fracture (van Munster, Bisschop et al. 2010). They included N=120 patients, of whom 52% developed delirium. Patients had up to four blood samples taken during the perioperative period, at 11am on weekdays, with blood samples classed as "non-delirious", "before" "during" or "after" delirium. Using a linear mixed models approach, IL-6 and IL-8 were both associated with delirium, and were both influenced by surgery, and IL-8 was affected by age. In a multivariate analysis, log IL-6 was associated with delirium but log IL-8 was not. After adjusting for premorbid cognitive impairment, log IL-6 was no longer associated with delirium.

Beloosesky *et al* conducted a prospective cohort study in elderly patients with acute hip fracture, aiming to examine the relationships between cytokines and the inflammatory marker C-reactive protein (CRP) over the month after hip fracture, postoperative complications including delirium, and mortality at 6 months (Beloosesky, Hendel et al. 2007). They stratified the cohort by MMSE score, due to the hypothesis that there is a state of chronic inflammation in dementia. In N=41 patients, 27% developed delirium according to the CAM. Comparing kinetics curves for the different cytokines and CRP (timepoints: within 10 hours of fracture and pre-surgery, 48-60 hours, day 7, day 30), the authors found different kinetics curves for CRP and IL-8 between those with delirium and those without complications. In the subgroup of patients with "impaired mental status", defined as an MMSE score (measured at baseline, at stabilisation and during rehabilitation, with the higher score used) of 23 or less, those who developed postoperative complications had higher CRP than those who did not develop complications. Patients with impaired mental status also had higher IL-6, IL-8 and IL-10, related to the impaired cognition and independent of complications. This is a relatively small study, although the use of longitudinal measures increases its power.

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Lemstra *et al* examined the association between cytokines and incident delirium in a cohort of patients with hip fracture, to see whether elevated cytokines are part of the disease state of delirium or whether they form a trait which puts patients at increased risk of delirium (Lemstra, Kalisvaart et al. 2008). This cohort was originally part of a prospective nested case control trial of haloperidol prophylaxis for delirium. Blood samples were collected preoperatively within 12 hours of admission and IL-6, IGF-1 and CRP were measured. Patients who developed incident delirium within 5 days of admission were matched with a control group who did not. Two hundred and fifty-two participants had a blood sample stored. Twenty-eight developed postoperative delirium, and were matched with fifty patients who did not. No differences were found in levels of any of the inflammatory markers between those who did or did not go on to develop delirium. IL-6 was negatively correlated with MMSE score.

1.5.5.4 Elderly medical in-patients

Adamis *et al* examined the role of physical illness severity in delirium in elderly medical in-patients, studying whether ApoE4 as a predisposing factor and levels of CRP and cytokines were involved in the perpetuation of delirium (Adamis, Treloar et al. 2007). Patients were assessed for delirium every three days on four occasions and then 28 days after admission. Blood tests were taken in the morning at the same time as the clinical assessments, and levels of IL-1α, IL-1β, IL-1ra, IL-6, TNF-α, Insulin Growth Factor (IGF)-1, IFN-γ and LIF (Leukaemia inhibitor factor) measured with ELISA. For financial reasons only 60 of 116 patients who consented to blood withdrawal could be included, from a total cohort of 164. Lower serum IGF-1 was associated with prevalent delirium. Those who recovered from prevalent delirium had higher initial IFN-γ levels. No associations were found between levels of the other cytokines and development of or recovery from delirium.

In the same cohort, Adamis *et al* examined the null hypotheses that delirium would have no effect on in-hospital and six-month mortality (Adamis, Treloar et al. 2007). In the patients where blood samples were available, associations between biological

factors involved in inflammation and frailty, and mortality were also tested. Those surviving initial hospitalisation who died within 6 months had higher initial IL-6 and CRP, and lower albumin levels. The authors' final predictive model of mortality using logistic regression found significant predictors of mortality included lower albumin and higher IL-6 and IFN-γ, as well as cognitive impairment and the interaction of MMSE with male gender. In this study, delirium was not associated with increased mortality.

In the same cohort of N=164 acutely admitted elderly patients, Adamis et al examined the relationship between delirium and functional impairment, and the modifying influences on any relationship of inflammatory cytokines and ApoE genotype (Adamis, Treloar et al. 2011). The cytokines measured were IL-1α, IL-1β, IL-1ra, IL-6, IFN-γ, TNF-α, LIF and IGF-1 as in the preceding study. Delirium was assessed using the CAM and the original Delirium Rating Scale. Using multivariate analysis, the authors found that function, as measured by the Barthel Index, was affected by MMSE, ApoE, IL-1α, IL-6, LIF and TNF-α, but there was no significant effect of delirium. There was evidence of functional decline in those with delirium only where the delirium did not resolve.

In a prospective cohort of elderly medical in-patients, Adamis *et al* investigated the associations between serum cytokines, IGF-1, ApoE4, illness severity, cognition and gender with both delirium presence and severity (Adamis, Lunn et al. 2009). They enrolled N=70 elderly medical patients, and assessed them in the morning twice per week for three weeks, with blood sampling at the first four assessments. The cytokines measured were IL-1α, IL-1β, IL-1ra, IL-6, IFN-γ, TNF-α, LIF and IGF-1. They used two types of statistical modelling to examine the relationships between the variables under study, Generalised Estimating Equations and Penalised Quasi Likelihood methods. They diagnosed prevalent delirium in 37.3% of patients and incident delirium in 4.5%. When they examined the effects of their explanatory variables on the presence or absence of delirium, they found that MMSE score, IGF-1 and IL-1ra had significant effects, while the other cytokines tested did not. When

they examined the relationships with delirium severity, they found that a high MMSE and high IGF-1 were associated with lower DRS-R98 score, while high IFN-γ was associated with high DRS-R98 score. These results suggest that IGF-1 and IL-1ra may have neuroprotective properties, and that high IFN-γ may be associated with delirium.

Wilson *et al* studied the relationship between premorbid IGF-1 levels and development of delirium in a cohort of N=100 elderly acute medical in-patients (Wilson, Broadhurst et al. 2005). They recruited those with significant physical illness, defined as an APACHE II score of >8, and excluded those with prevalent delirium or insulin dependent diabetes. Patients were assessed daily for delirium using the CAM with confirmation by a psychiatrist according to DSM III. Twelve patients developed incident delirium. Multivariate logistic regression found an association between incident delirium and IGF-1 (OR 0.82), along with depression and IQCODE score (OR 3.26).

De Rooij *et al* conducted a study among consecutively admitted elderly medical patients to examine the expression of pro (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines and CRP in relation to delirium (de Rooij, van Munster et al. 2007). Patients were reviewed within 48 hours of admission and blood samples collected in the morning within 3 days of admission. Sixty-four of the 185 patients in whom they could measure blood markers developed delirium. They found no difference in CRP levels between those with and without delirium. They found levels of most cytokines to be below the detection limit of the assays used, but the delirium group were more likely to have detectable levels of IL-6 and IL-8, and after adjusting for age, cognitive impairment and infection, an IL-6 level of >10pg/ml was significantly associated with delirium.

MacDonald *et al* studied the role of CRP in predicting both incident delirium and recovery of delirium (MacDonald, Adamis et al. 2007). They enrolled a cohort of N=94 acutely ill elderly medical patients. They were assessed within 3 days of

admission with MMSE for cognitive function and CAM for delirium, and then every 3 days until day 18, and further on day 28. Blood was collected for CRP measurement at the first assessment, with a final N=86. Twenty-six patients had prevalent delirium and a further six developed incident delirium, with 37.2% of patients developing delirium at any stage. Those with prevalent and incident delirium had higher CRP levels. In a logistic regression analysis, CRP independently predicted the onset of incident delirium. Higher initial MMSE score and low CRP predicted recovery from delirium.

White *et al* tried to replicate these findings in a larger (N=283) cohort of emergency elderly medical admissions (White, Eeles et al. 2008). They also found higher baseline CRP levels in those with prevalent and incident delirium, but no difference between those two delirium groups. In a small number who developed incident delirium and where they had CRP levels at baseline and the time of development of delirium, there was no significant difference in levels. CRP did not correlate with delirium severity nor duration.

Speciale *et al* found in a cohort of N=110 geriatric patients in a rehabilitation facility with delirium, 98 prevalent and 11 incident delirium, that CRP levels were high on admission in those with prevalent delirium and low at delirium resolution (Speciale, Bellelli et al. 2008). In those with incident delirium, they found it to be low on admission, higher at delirium onset and low at resolution. These patients had heterogeneous precipitating causes of delirium, and the CRP levels were higher in those with “infective” or “surgical” causes of delirium than in those with “non-infective” causes.

Morandi *et al* studied the association of CRP with prevalent and incident delirium further in a large cohort of elderly sub-intensive care patients (Morandi, Sleiman et al. 2007). These patients were critically ill and required higher care levels than on a general ward, but did not require “global intensive care”. Of 1,369 patients, 301

developed delirium, 132 prevalent and 162 incident delirium. They found that prevalent delirium was associated with higher CRP levels, as well as a worse prognosis, health status and higher disability scores than incident delirium.

Ritchie *et al* retrospectively examined the relationship between CRP, delirium, delirium severity and medical diagnosis in a database of patients over the age of 70 admitted to an acute medical unit (Ritchie, Newman et al. 2014). The database was constructed for a study examining the prevalence and mortality of patients with dementia admitted acutely, and all patients were assessed for delirium using the CAM for both diagnosis and severity within 48 hours. Routine blood tests including CRP were recorded. Of the N=710 included participants, 12.3% had delirium within 72 hours of admission. Raised CRP on admission was associated with delirium, and this relationship remained after the authors adjusted for illness severity, co-morbidity, gender, age and the atropine risk score for medication risk. The relationship between CRP and delirium differed depending on the broad category of medical diagnosis; there was no relationship between the two variables in those with an infective diagnosis, but the relationship was stronger in those with a musculoskeletal or metabolic diagnosis. There was no relationship between CRP and delirium severity. Although the diagnosis of delirium was made prospectively, the retrospective use of the database to perform post hoc analyses limits the study somewhat, the use of routine blood tests may introduce bias, and not all (but most) patients had CRP measured.

1.5.5.5 Acute stroke

McManus *et al* found in a cohort of patients with acute stroke (N=82), of whom 28% developed delirium, that high admission CRP (>5mg/L) was independently associated with development of delirium, with an odds ratio of 10.2 (McManus, Pathansali et al. 2009). Most cases of delirium were present at stroke onset. The authors speculate that this may be related to those with a larger stroke being more at

risk of delirium and infective complications, although these may not have occurred by the first assessment, which was within four days of admission.

1.5.5.6 ICU delirium

McGrane *et al* performed a prospective cohort study in critically ill patients (N=83) to test the hypothesis that systemic inflammation would lead to a longer duration of delirium and acute brain dysfunction, using two inflammatory biomarkers, CRP and procalcitonin which is an early marker of sepsis (McGrane, Girard et al. 2011). The study was nested within a randomised controlled trial of dexmedetomidine vs. lorazepam for sedation in ICU. Blood samples were collected within 24 hours of enrolment, and patients were assessed daily for delirium for up to 12 days (CAM-ICU and RASS). Delirium and coma-free days were the outcomes of interest. Proportional odds logistic regression was used to examine the relationship between the two inflammatory biomarkers and delirium and coma-free days. Higher baseline procalcitonin was associated with more brain dysfunction (fewer delirium and coma-free days), and higher CRP trended towards the same outcome. This relationship was independent of sepsis. Procalcitonin was not associated with other types of organ dysfunction, but higher CRP levels were associated with kidney dysfunction in septic patients. Inflammation, as identified by higher procalcitonin and CRP levels, may therefore contribute to delirium and acute brain dysfunction, independent of sepsis and other organ dysfunction.

Zhang *et al* performed a study in ICU patients with the aim of better defining the relationship between CRP and delirium (Zhang, Pan et al. 2014), having found in a meta-analysis of critically ill patients that prolonged elevation of CRP is associated with higher mortality (Zhang and Ni 2011). They performed a prospective observational cohort study in a mixed medical and surgical ICU. They excluded those with delirium on admission, and monitored for delirium with the CAM-ICU eight-hourly. Plasma CRP was measured routinely on admission to ICU and 24 hours later, and the change in the two values was also calculated. Of the N=223 patients

eligible, delirium developed in 24%. The mean age was 57 years. The delirium group had higher CRP levels, and CRP remained independently associated with delirium after adjusting for confounding variables including age, gender, APACHE score, intubation status, medical condition, smoking, alcohol, physical restraint, hospital length of stay before ICU admission and living alone. Change in CRP was also associated with an increased risk of developing delirium. Delirium subtype also had a role; patients with mixed or hypoactive subtypes had higher CRP than those without delirium.

Pfister *et al* examined several biomarkers including serum IL-6, CRP, cortisol and S100B, and measures of cerebral blood flow and oxygenation, in a small cohort of ICU patients with sepsis associated delirium (Pfister, Siegemund et al. 2008). Patients with pre-existing central neurological disorders such as dementia were excluded, as were patients with delirium felt to be secondary to a cause other than sepsis. Biomarkers were measured at the same time as cerebral blood flow measurement, within 48 hours of admission and stabilisation. In their cohort of critically ill patients with extracranial sepsis, 12/16 developed delirium according to the CAM-ICU. They found higher levels of CRP but not IL-6 in the delirium group, and CRP correlated positively with disturbance in auto-regulation. S100B and cortisol were also elevated, and S100B discriminated between those who survived and those who did not. Numbers in the study were small, however, and they did not control for other factors such as illness severity.

Girard *et al* tested the hypothesis that inflammation and deranged coagulation are risk factors for delirium in critical illness (Girard, Ware et al. 2012). Mechanically ventilated medical ICU patients were recruited to a randomised controlled trial of a paired sedation and ventilator weaning strategy, and this study was performed as a nested case control study. Blood samples were collected on the morning after enrolment and on day 5, and nine intermediates of inflammation or coagulation measured; CRP, matrix metalloproteinase-9 (MMP-9), myeloperoxidase, neutrophil gelatinase-associated lipocalin, soluble TNF-receptor 1, d-dimer, protein C,

plasminogen activator inhibitor type 1 and von Willebrand factor antigen. Patients were assessed daily for delirium using the CAM-ICU until death or ICU discharge; patients with RASS -4 or -5 were considered to be comatose. N=138 patients were included, the majority were aged over 65, and 78% were delirious during their ICU stay. After adjusting for age, illness severity and severe sepsis, the group of biomarkers studied was found to be associated with delirium. Specifically, higher MMP-9 was associated with a reduced probability of delirium, whereas high soluble TNF-receptor and low protein C were associated with an increased probability of delirium.

Ritter *et al* tested the hypothesis that the association between systemic inflammation and delirium would be different in patients with and without sepsis (Ritter, Tomasi et al. 2014). They prospectively enrolled ICU patients and assessed them for delirium twice daily for the first 72h after admission with the CAM-ICU. Blood was collected within 12h of ICU admission, and TNF-α, soluble TNF receptor-1 and -2, adiponectin, IL-1β, IL-6 and IL-10 were measured. Patients were classed as septic or not according to international criteria. Seventy-eight patients were included, of whom 31 developed delirium. The delirium group had higher levels of IL-1β, soluble TNF receptors and adiponectin, and these markers were associated with delirium in a multivariate logistic regression analysis. Sepsis did not modify the relationship between the markers and delirium.

Van den Boogaard *et al* examined multiple biomarkers in a cohort of ICU patients who they split into “inflamed” and “non inflamed” (van den Boogaard, Kox et al. 2011). This study has also been discussed in section 1.4.9.3. They monitored patients for delirium using the CAM-ICU three times daily, and 50% of the 100 patients included in the study developed delirium. They divided those with delirium into “inflamed” and “non-inflamed”; inflamed was defined as a positive culture for which the patient was given antibiotics, and absence of inflammation as the absence of proven or suspected infection, and having no more than one SIRS criterion. The control group were likewise split into “inflamed” and “non-inflamed”. In addition to

cortisol, the pro-inflammatory intermediates they measured were TNF-α, IL-1β, IL-6, IL-8, IL-17, IL-18 and macrophage migration inhibitory factor (MIF), the anti-inflammatory intermediates were IL-1ra, IL-10, and also the chemotactic marker MCP-1. They also measured CRP and pro-calcitonin as indicators of the acute phase response, and plasma defensin (human neutrophil peptide-1). Finally, they measured amyloid$\beta_{1\text{-}40}$ and amyloid$\beta_{1\text{-}42}$ as well as their truncated forms, and S100B and tau. The delirium group had higher levels of TNF-α, IL-6, IL-8, MIF, IL-1ra, IL-10, MCP-1 and procalcitonin. No difference was found between groups in levels of IL-1β, IL-17, IL-18, plasma defensin or CRP. Of the brain specific proteins, the ratios of amyloid$\beta_{1\text{-}42/40}$ and amyloid$\beta_{N\text{-}42/40}$ were lower in the delirium group, and truncated amyloid$\beta_{N\text{-}40}$ levels were higher. No differences were found in S100B, tau and the other amyloid isoforms. In inflamed patients, several biomarkers were associated with delirium, but in a multivariate logistic regression analysis, only IL-8 (OR 9.0) was associated with delirium in inflamed patients. In the non-inflamed group, multivariate logistic regression analysis found associations between the ratio of amyloid$\beta_{1\text{-}42/40}$ (OR 0.03) and IL-10 (OR 2.6) with delirium in non-inflamed patients. Given the differences between the biomarker profiles in "inflamed" and "non-inflamed" delirious patients, the authors suggest that there are different pathogenetic mechanisms for delirium in these types of patients. However, this was an exploratory study with relatively small numbers for the number of biomarkers measured.

1.5.5.7 CSF cytokines

Katsumata *et al* conducted a prospective cohort study recruiting 59 patients with SLE, acutely admitted for assessment or treatment to the Rheumatology Unit of a General Hospital in Tokyo, Japan (Katsumata, Harigai et al. 2007). Patients were grouped according to whether or not they had CNS manifestations of SLE, and in the CNS group, the presence or absence of delirium according to DSM criteria. They examined pro-inflammatory cytokines in the CSF of these patients with the aim of developing a diagnostic tool for delirium in this context. They measured IL-6, IL-8 and IFN-α, plus IgG index, an indicator of intrathecal immunoglobulin synthesis and Q-albumin, a measure of blood-brain-barrier function. Katsumata *et al* found IL-6 to

be higher in the delirious group compared to those with no CNS features of SLE. IL-6 also positively correlated with the SLE Disease Activity Index (SLEDAI) when this score included CNS syndrome but not when the score did not. IL-8 and IFN-α were not significantly different in delirium. The rate of positive IgG index was higher in delirium suggesting that there may be intrathecal production of immunoglobulins, perhaps signaling damage or providing protection against attack, or augmenting the inflammatory response (that might then worsen any CNS damage). There was also a trend towards higher Q-albumin in delirium, suggesting subtle blood-brain-barrier dysfunction.

MacLullich *et al* undertook a prospective cohort study in patients over the age of 60 undergoing hip fracture repair under spinal anaesthesia, with the aim of investigating whether CNS pro-inflammatory cytokines are elevated in delirium (MacLullich, Edelshain et al. 2011). Thirty-six patients in two centres (Edinburgh and Amsterdam) were assessed for delirium preoperatively and 3-4 days postoperatively using the CAM. CSF specimens were taken at the onset of spinal anaesthesia prior to the administration of anaesthetic agent. They measured TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12p70 by cytometric bead array. The investigators found IL-8 to be detected in 33/36 CSF samples, and IL-6 in 3/36 samples. The other cytokines were not detected. CSF IL-8 was significantly higher in cases than controls. Cases also had higher serum IL-6 levels. Dementia was not associated with raised IL-8, and when the investigators excluded patients with known dementia from the analysis, the pattern of results did not change. These results suggest an association between delirium and elevated CSF IL-8, although causation cannot be implied in this cross-sectional, observational study.

Westhoff *et al* performed an exploratory study in patients with acute hip fracture, with the hypothesis that those with a dysfunctional immune response would be more vulnerable to postoperative delirium (Westhoff, Witlox et al. 2013). The investigators monitored for delirium using the CAM, with severity scoring using the DRS-R98 until day 5 postoperatively, or longer if delirium was still present at that

time. They collected blood preoperatively to measure CRP, IL-6 and ESR (erythrocyte sedimentation rate). CSF was collected at the onset of spinal anaesthetic, and a panel of 42 cytokines and chemokines measured using a Luminex assay. Some of the cytokines and chemokines were not detectable in the majority of samples, and they compared levels of each marker where there was sufficient detected (16 markers) between those who did and did not develop delirium postoperatively using Mann Whitney U test. Of N=122 patients enrolled, N=61 patients were included in the analysis due to a change to general anaesthesia, the exclusion of those with preoperative delirium and logistical reasons. The authors found that those who developed postoperative delirium had lower levels of CSF IL-6, IL-1ra and Flt-3L (FMS-like tyrosine kinase 3 ligand) preoperatively. However, the delirium group had higher levels of serum IL-6 preoperatively. They found no difference in CRP and ESR levels. There is no comment on whether there was any correlation between serum and CSF levels of IL-6, but there is also no information on how IL-6 was measured in serum, since if this was by a different assay technique they would be harder to compare.

1.5.5.8 Genetic polymorphisms

In a cohort of N=881 elderly patients, some with acute hip fracture and some acutely unwell medical patients, van Munster *et al* examined whether genetic polymorphisms in the genes for IL-6, IL-8 and the IL-6 receptor were associated with delirium (van Munster, Zwinderman et al. 2011). Delirium was diagnosed with the CAM, and the incidence was 50% in the patients with hip fracture and 34% in the medical patients. The single nucleotide polymorphisms the authors examined were not associated with delirium.

1.5.5.9 Autopsy studies

The presence of neuroinflammation in delirium has been assessed in an autopsy study by van Munster *et al*, looking for the presence of microglial, astrocyte and cytokine activation in those who died with delirium compared to those who did not.

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N=9 cases with delirium were compared with N=6 age-matched controls (van Munster, Aronica et al. 2011). Those with CNS infection were excluded. The diagnosis of delirium had been made pre-morbidly by a member of a team of geriatricians following patients with delirium in their centre, however in the controls the absence of delirium could only be confirmed by retrospective case-note review. Using immunocytochemistry, they found more active microglia in the brains of those who died with delirium, and more astrocyte activity in the dentate gyrus. There was also more IL-6 immunoreactivity but IL-1β was not detectable.

Lemstra *et al* performed a case control autopsy study to test the hypothesis that peripheral infection induces microglial activation in the brain (Lemstra, Groen in't Woud et al. 2007). This has previously been demonstrated in a rat model by Semmler *et al* (Semmler, Okulla et al. 2005), and it is thought that activated microglia are involved in inducing sickness behaviour changes associated with sepsis. Thirteen cases who died with sepsis and had an absence of CNS infection or other supratentorial pathology, were compared with 17 controls who had no signs of infection 0-3 days prior to death or at autopsy. Using immunohistochemistry, the authors demonstrated activated microglia in the brains of the patients who died with sepsis from a peripheral source. These patients were sourced from the autopsies performed at their centre where permission was given for use in research, and no information is given on delirium, although it is speculated that microglial activation in this context may be involved in delirium (Lemstra, Groen in't Woud et al. 2007).

1.5.6 Summary of human studies of inflammation in delirium

In summary, delirium has been shown to be associated with several different derangements in inflammatory cytokines and chemokines, but results for individual cytokines and cytokine families are mixed, and this may depend on the factors precipitating the delirium, the timing of the samples and covariates taken into account. There seems to be a generally pro-inflammatory and pro-chemotactic milieu in delirium. Some studies find a lack of anti-inflammatory cytokines such as IL-1ra

and IL-10, and some find higher levels. Findings for each group of markers are now summarised.

1.5.6.1 Interleukin-1 family

Serum IL-1α, IL-1β and IL-1ra were not different in elderly medical patients with delirium (Adamis, Treloar et al. 2007), but IL-1ra level was shown to be a protective modifier of delirium risk (Adamis, Lunn et al. 2009). IL-1β has been shown to be associated with ICU delirium after controlling for covariates (Ritter, Tomasi et al. 2014). A further study in those with critical illness found higher serum IL-1ra but not IL-1β (van den Boogaard, Kox et al. 2011). In CSF, lower IL-1ra has been demonstrated in delirium after hip fracture (Westhoff, Witlox et al. 2013).

1.5.6.2 Interleukin-2

After cardiac surgery, elevation of IL-2 is seen in the immediate postoperative period in those with postoperative delirium (Kazmierski, Banys et al. 2014). In a cohort undergoing general surgery, those with lower IL-2 went on to develop delirium, but this was no longer associated after adjusting for covariates (Capri, Yani et al. 2014).

1.5.6.3 Interleukin-6

Serum interleukin-6 has been shown to be elevated in the immediate postoperative period following cardiac surgery (Plaschke, Fichtenkamm et al. 2010), and it has also been demonstrated to be an independent predictor of delirium following non-cardiac surgery (Liu, Ya-wei et al. 2013, Capri, Yani et al. 2014). It was not different in those with delirium following elective arthroplasty (Cerejeira, Nogueira et al. 2012), and in one study of delirium after hip fracture (Lemstra, Kalisvaart et al. 2008), but three further studies in those with hip fracture found it to be elevated (van Munster, Korevaar et al. 2008, MacLullich, Edelshain et al. 2011, Westhoff, Witlox et al. 2013), but not after adjusting for covariates including prior dementia (van Munster,

Bisschop et al. 2010). IL-6 level was a predictor of mortality in elderly medical patients (Adamis, Treloar et al. 2007). One study in elderly medical patients found higher IL-6 in delirium after adjusting for covariates including prior dementia (de Rooij, van Munster et al. 2007), but a further study did not find a difference (Adamis, Treloar et al. 2007). In ICU delirium, two studies have not shown a difference in IL-6 (Pfister, Siegemund et al. 2008, Ritter, Tomasi et al. 2014), but one found it to be elevated (van den Boogaard, Kox et al. 2011). Where IL-6 has been measured in CSF, it was shown to be higher in delirium associated with SLE (Katsumata, Harigai et al. 2007), and lower in those with delirium after hip fracture (Westhoff, Witlox et al. 2013). Polymorphisms in the genes for IL-6 and IL-6 receptor were not found to be associated with increased risk of delirium (van Munster, Zwinderman et al. 2011). An autopsy study also found more IL-6 immunoreactivity in the brains of those whe died with delirium than in controls (van Munster, Aronica et al. 2011).

1.5.6.4 Tumour Necrosis Factor

After cardiac surgery, elevation of TNF-α is seen in the immediate postoperative period in those with postoperative delirium (Kazmierski, Banys et al. 2014), but not after adjusting for covariates. TNF-α was not shown to be different in elderly medical patients with delirium (Adamis, Treloar et al. 2007). Soluble TNF-receptors have been shown to be associated with ICU delirium in two studies (Girard, Ware et al. 2012, Ritter, Tomasi et al. 2014). Two studies have found elevated TNF-α in ICU delirium, but not after controlling for covariates (van den Boogaard, Kox et al. 2011, Ritter, Tomasi et al. 2014).

1.5.6.5 Chemokines including IL-8

After cardiac surgery, chemokines have been found to be raised in those who go on to develop delirium (Rudolph, Ramlawi et al. 2008). In patients with hip fracture, IL-8 has been shown to be elevated in those with delirium (Beloosesky, Hendel et al. 2007, van Munster, Korevaar et al. 2008), and IL-8 was still associated with delirium after adjusting for covariates including prior dementia (van Munster, Korevaar et al.

2008, Bisschop, de Rooij et al. 2011). It was not shown to be different preoperatively in those who developed delirium after general surgery (Capri, Yani et al. 2014). IL-8 has also been shown to be elevated in ICU delirium, including after adjusting for covariates in those with "inflamed delirium", and MCP-1 was found to be higher in ICU delirium (van den Boogaard, Kox et al. 2011). In CSF, IL-8 has been shown to be elevated in delirium after hip fracture (MacLullich, Edelshain et al. 2011). A polymorphism in the gene for IL-8 was not shown to be associated with delirium risk (van Munster, Zwinderman et al. 2011).

1.5.6.6 Interleukin-10

IL-10 has been found to be elevated in ICU delirium in two studies (van den Boogaard, Kox et al. 2011, Ritter, Tomasi et al. 2014). In one it was no longer associated after adjusting for covariates (Ritter, Tomasi et al. 2014), but in a further study, those with "non-inflamed" delirium had higher IL-10 following a multivariate analysis (van den Boogaard, Kox et al. 2011). It was not found to be different preoperatively in those who developed delirium after general surgery (Capri, Yani et al. 2014).

1.5.6.7 Balance of pro- and anti-inflammatory cytokines

In elective arthroplasty, an imbalance of pro and anti-inflammatory cytokines in favour of a pro-inflammatory state has been demonstrated (Cerejeira, Nogueira et al. 2012).

1.5.6.8 IGF-1

In elderly acute medical patients, IGF-1 has been shown to be lower in prevalent delirium, and higher levels of IGF-1 were associated with less severe delirium (Adamis, Lunn et al. 2009), and lower risk of incident delirium (Wilson, Broadhurst

et al. 2005). After hip fracture, IGF-1 was not associated with incident delirium (Lemstra, Kalisvaart et al. 2008).

1.5.6.9 IFN-γ

Elderly medical patients with higher initial IFN-γ were more likely to recover from prevalent delirium (Adamis, Treloar et al. 2007), but IFN-γ also correlated with delirium severity (Adamis, Lunn et al. 2009). Higher IFN-γ was a predictor of mortality (Adamis, Treloar et al. 2007).

1.5.6.10 CRP

Multiple studies in different populations have measured C-reactive protein, with very mixed results. In ICU delirium, one study found an association between higher CRP and delirium in a multivariate analysis (Zhang, Pan et al. 2014), a small study found higher levels (Pfister, Siegemund et al. 2008), one study found a trend towards a relationship between CRP and brain dysfunction (McGrane, Girard et al. 2011) and one study found no difference (Girard, Ware et al. 2012). There was no association between CRP and postoperative delirium following cardiac surgery (Bokesch, Izykenova et al. 2006, Plaschke, Fichtenkamm et al. 2010), elective arthroplasty (Cerejeira, Nogueira et al. 2012) and abdominal surgery (Kudoh, Takese et al. 2005). One study found an association with delirium following vascular surgery but with an OR of only 1.01 (Pol, van Leeuwen et al. 2014). In delirium after hip fracture, two studies found no association between CRP measured at a single timepoint (Lemstra, Kalisvaart et al. 2008, Westhoff, Witlox et al. 2013), and a further study did find different “CRP kinetics”, over a wide timeframe from 10h to 30 days post-fracture in those with delirium (Beloosesky, Hendel et al. 2007). Results in incident and prevalent delirium in elderly medical in-patients are more mixed; it has been found to be higher in delirium (MacDonald, Adamis et al. 2007, Morandi, Sleiman et al. 2007, White, Eeles et al. 2008, Ritchie, Newman et al. 2014), but not always (de Rooij, van Munster et al. 2007, Adamis, Lunn et al. 2009), and it has been shown to be an independent predictor of incident delirium in elderly patients (MacDonald, Adamis et

al. 2007) and after stroke (McManus, Pathansali et al. 2009). It does differ depending on the precipitating illness (Ritchie, Newman et al. 2014), and while some studies have suggested CRP may track the onset and resolution of delirium (Speciale, Bellelli et al. 2008), others have not (White, Eeles et al. 2008).

1.5.7 Immunosenescence

There has been an increasing understanding in recent years of the effect of ageing primarily on the humoral but also on the innate immune response. This has been termed immunosenescence, and more recently the term inflammageing has been coined to reflect that the aged immune system is remodelled rather than simply being depressed, with some functions static or even increased (Ginaldi, Mengoli et al. 2007). Drivers of immunosenescence include oxidative stress and a lifetime's accumulation of exposure to antigens. Thymic involution also leads to a reduction in numbers of lymphoid precursor T cells and B cells, which are important to the adaptive immune response (Malaguarnera, Ferlito et al. 2001, Ginaldi, Mengoli et al. 2007). There are fewer lymphocyte subgroups, and T cells have diminished proliferative capacity. Aged B lymphocytes are qualitatively different, with reduced antibody responsiveness, which is of particular importance for immunisation (Malaguarnera, Ferlito et al. 2001). Changes to the innate immune response affect different cell types. Aged neutrophils and macrophages both exhibit reduced chemotaxis, phagocytosis, superoxide production and signal transduction. Macrophages also show reduced MHC class II production and cytokine production, and increased prostaglandin E2 production (Solana, Tarazona et al. 2012).

1.5.8 Human studies of inflammation and ageing

Serum levels of chemokine-cytokine factor (sIL-4R, IL-6 and IL-8) have been found to be associated with atrophy on cerebral MRI in the Memory and Morbidity in Augsberg Elderly study, after adjusting for several co-variates including age, gender, education, cardiovascular disease and smoking. No association was found between single cytokines and MRI changes such as white matter lesions, atrophy or lacunar

infarcts, and it was thought that a pattern of cytokine and chemokine changes may be responsible for changes in the ageing brain (Baune, Ponath et al. 2009).

Higher baseline high-sensitivity (hs)CRP has been shown to predict poorer memory after 12 years in an epidemiological study in older women. Komulainen *et al* studied N=97 women with an age at baseline of 60-70 years, without dementia, measured global cognitive function with the MMSE and collected serum for hsCRP (Komulainen, Lakka et al. 2007). Participants were followed-up 12 years later, with repeat MMSE and a fuller cognitive test battery with measures of memory and cognitive speed. The authors found that higher baseline hsCRP was associated with poorer memory at follow-up, including after adjustment for age, education, depression, hormone replacement therapy, smoking, serum LDL cholesterol and BMI. This population-based study suggests inflammation may be associated with the development of cognitive impairment, and the authors suggest hsCRP may be useful as a biomarker for risk of cognitive decline.

1.5.9 Human studies of inflammation and POCD

Visvanathan *et al* investigated the role of TNF-α in postoperative cognitive dysfunction (Visvanathan, Sundararajan et al. 2003). They enrolled patients (N=44) scheduled to undergo elective cardiac surgery, and collected blood preoperatively. Mononuclear cells were stimulated to produce TNF-α, and levels measured. They tested cognition preoperatively and 6 months postoperatively, with a battery covering linguistic function, theory and psychomotor function. Those with a "clinically important" decline in their scores on the cognitive tests had a higher preoperative stimulated TNF-α level. This indicates that those with a greater pro-inflammatory state may be more prone to POCD.

1.5.10 Human studies of inflammation in dementia

In recent years a chronic inflammatory process has been implicated in dementia, with a focus on the role of glial cells. They are thought to instigate a cytokine-mediated

inflammatory response, following an insult to neurons (Lee, Chung et al. 2009). This may lead to the production of acute phase proteins including amyloid precursor protein.

Holmes, Cunningham *et al* performed a study in a cohort of elderly patients with Alzheimer's disease, with the hypothesis that these patients would show evidence of permanent cognitive decline following an infection, even in the absence of delirium (Holmes, El-Okl et al. 2003). This phenomenon is well known to clinicians, families and carers of patients with dementia. Animal modelling in mice with neurodegenerative disease has shown that a peripheral inflammatory stimulus invokes an exaggerated cytokine response in the CNS from microglia that are already "primed" by the ongoing pathology of the neurodegenerative disease. This is accompanied by a dip in cognitive functioning mimicking delirium, and with an acceleration of the neurodegenerative disease process. The authors suspect that a parallel process occurs in the brains of patients with Alzheimer's dementia. Eighty-five patients with Alzheimer's dementia and no evidence of systemic infection at baseline were studied, assessed for delirium with the CAM, cognitive function with the MMSE and baseline serum IL-1β was measured. The patients and their carers were questioned about symptoms or signs of infections in the previous two months, and they were assessed again in the same manner two months later. No patients fulfilled CAM criteria for delirium at either assessment. They found that the fifteen patients with evidence of infection in the two months prior to baseline assessment had a higher rate of cognitive decline in the two months between assessments than the group without infection. Only six patients had detectable serum IL-1β, but these patients also had a greater degree of cognitive decline than those with undetectable IL-1β. Those developing an infection between the two assessments had a trend towards greater cognitive decline. Although this is a relatively small study with retrospective diagnosis of infection, and low rates of detection of IL-1β, it does suggest that systemic infection may drive cognitive decline in dementia, even in the absence of delirium.

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Holmes *et al* (Holmes, Cunningham et al. 2009) performed a prospective cohort study in patients with Alzheimer's disease to test the hypothesis that acute systemic inflammatory events, accompanied by a rise in peripheral TNF-α, would be associated with long-term cognitive decline. They recruited N=300 patients with Alzheimer's disease, and performed a cognitive assessment at baseline designed to detect change in cognitive function, and collected a blood sample to measure CRP and TNF-α. The patient's caregiver was interviewed about systemic inflammatory events in the preceding two months. This procedure was repeated at two-monthly intervals for six months, including assessment for delirium using the CAM. N=275 patients were included, following exclusion of patients with end-stage dementia at baseline. N=18 had delirium at baseline, and N=39 patients had had a total of fifty systemic inflammatory events in the two months preceding baseline assessment. N=222 patients completed follow-up, with N=110 having a total of one-hundred and fifty systemic inflammatory events. The authors found no difference in the change in cognitive function over six months in those with high vs. low CRP levels at baseline, however those with high TNF-α levels at baseline suffered a four-fold greater rate of cognitive decline. Those who had a systemic inflammatory event during follow-up also had a faster rate of cognitive decline than those with no inflammatory events. N=25 subjects had delirium diagnosed during the follow-up period, and this was more frequent in those with higher baseline TNF-α. Systemic inflammatory events were associated with cognitive decline independent of delirium. The same group also hypothesised that in this cohort of patients with Alzheimer's disease, serum IL-6 and TNF-α, which are involved in initiating sickness behaviour, would be associated with the development of neuropsychiatric symptoms similar to sickness behaviour (Holmes, Cunningham et al. 2011). Those who developed delirium over the six months had higher scores on the Neuropsychiatric Inventory. Those with low TNF-α or IL-6 also had a lower Neuropsychiatric Inventory score over the six months, and the three core symptoms of sickness behaviour (depression, anxiety and apathy) were more frequent in those with higher proinflammatory cytokines (Holmes, Cunningham et al. 2011).

Non-steroidal anti-inflammatory drugs have been suggested as a possible treatment for Alzheimer's disease, following epidemiological data showing lower than expected prevalence or delayed onset of the disease in patients on long-term NSAIDs. MacKenzie *et al* (MacKenzie and Munoz 1998) conducted a post-mortem study in elderly patients without dementia, comparing those with arthritis who have used NSAIDs long-term with those with no diagnosis which would have led to them using NSAIDs regularly. The authors did not find any difference in the number of amyloid plaques or neurofibrillary tangles, but they found fewer activated microglia in the patients with long-term NSAID use, suggesting that prevention of microglial activation may be a mechanism by which NSAIDs confer their protective effects.

1.5.11 The immune system and sleep

There is an important relationship between the immune system and sleep. This is pertinent because sleep is often disrupted in hospital settings (Pilkington 2013), and sleep-wake cycle disturbance is a very prevalent feature of delirium (Meagher, Moran et al. 2007). Levels of IL-6 and TNF-α have been shown to be elevated with sleep restriction. Vgontzas *et al* (Vgontzas, Zoumakis et al. 2003) showed by comparing two cohorts of older and younger "normal sleepers" that IL-6 levels were associated with total waking time, particularly in the older adults, and in the young adults that IL-6 was negatively associated with REM sleep. They found no difference in TNF-α levels between groups.

1.5.12 Conclusions

In summary, the immune system is integral in the body's response to trauma and infection, and there are important changes with ageing. Some studies have found associations between delirium and a pro-inflammatory cytokine milieu or lack of anti-inflammatory cytokines. However, the results have been heterogeneous; this may reflect the wide range of patient cohorts, underlying delirium aetiology and the types of measurement used.

1.6 Central nervous system damage in delirium

1.6.1 Definitions

As discussed in section 1.1.5, delirium is associated with multiple adverse outcomes including an increased risk of institutionalisation and of mortality (Siddiqi, House et al. 2006, Witlox, Eurelings et al. 2010), which is independent of age, sex, co-morbid illness and baseline dementia (Witlox, Eurelings et al. 2010). There is also a complex relationship with long-term cognitive decline (MacLullich, Beaglehole et al. 2009). Dementia, along with older age, is one of the strongest risk factors for delirium (Young and Inouye 2007, Juliebo, Bjoro et al. 2009, Davis, Muniz Terrera et al. 2012), and there is a significantly increased risk of developing dementia following delirium (MacLullich, Beaglehole et al. 2009, Witlox, Eurelings et al. 2010, Davis, Muniz Terrera et al. 2012). It is currently unclear whether delirium is 'unmasking' a dementia process that had already begun but not been recognised, or whether a new pathological process has been sparked in a brain that had otherwise been ageing healthily. However, there is evidence that an episode of delirium worsens the trajectory of cognitive decline in Alzheimer's dementia (Fong, Jones et al. 2009). In middle-aged patients (median age 58) with ICU delirium, a longer duration of delirium has been shown to be associated with smaller brain volumes three months after ICU admission (Gunther, Morandi et al. 2012). Although this was an observational study and pre-existing ventricular enlargement cannot be excluded, it raises the possibility of neuronal loss during delirium. There is also evidence that incident dementia that follows an episode of delirium may not be associated with standard dementia neuropathologies (Davis, Muniz Terrera et al. 2012). Due to these associated deleterious outcomes, an obvious question is whether there is evidence of acute damage to the CNS during a delirium episode.

S100B is a Ca^{2+} binding protein, found in the CNS primarily in astrocytes, oligodendrocytes, some neurons and populations of lymphocytes, ependymal cells and epithelial cells of the choroid plexus (Steiner, Bernstein et al. 2007). It is also found peripherally. It has multiple functions intracellularly, being involved in

cytoskeleton assembly and calcium homeostasis, protein phosphorylation and the glycolytic pathway (Donato, Sorci et al. 2009, Donato and Heizmann 2010, Leclerc, Sturchler et al. 2010). It is also secreted by astrocytes under conditions of metabolic stress, and has cytokine-like properties, transmitting messages among glia and between glia and neurons (Steiner, Bernstein et al. 2007).

Due to its cellular location and properties, raised levels of S100B in cerebrospinal fluid (CSF) and serum have been seen as a marker of astrocyte damage or dysfunction, and of CNS damage. It has been used as a biomarker of traumatic brain injury (Bloomfield, McKinney et al. 2007, Petzold 2007), and in stroke it correlates with stroke severity score, infarct volume and functional outcome (Brouns, De Vil et al. 2010). S100B has also been found to be elevated in CSF in early Alzheimer's disease (Peskind, Griffin et al. 2001), and in CSF and serum in schizophrenia (Rothermundt, Ahn et al. 2009). It has been found to be elevated following cardiopulmonary bypass, and its level in serum was associated with confusion and neurocognitive dysfunction after cardiac surgery (Shaaban Ali, Harmer et al. 2000).

1.6.2 Human studies of markers of CNS damage in delirium and POCD

Herrmann *et al* (Herrmann, Ebert et al. 2000) examined the predictive value of serum S100B and Neuron-Specific Enolase (NSE) for neurocognitive outcomes following cardiac surgery. N=113 patients were enrolled, and they had blood samples from 78. Patients had a neuropsychological and neuropsychiatric evaluation preoperatively, three and eight days postoperatively and at six months. This included assessment for delirium based on DSM-III-R criteria. Blood samples were collected before surgery, and 1h, 6h, 20h and 30h after skin closure. Seven patients were delirious at three days postoperatively, and two were delirious at eight days. S100B levels peaked one hour postoperatively and fell back towards preoperative levels thereafter. S100B rose higher and the rise lasted longer in those with delirium. This pattern was also the case for those with adverse neuropsychological outcomes.

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture

Rasmussen *et al* conducted a prospective cohort surgery in N=35 elderly patients undergoing cardiac bypass surgery, with the hypothesis that serum NSE and S100B would rise after surgery and correlate with change in cognitive function (Rasmussen, Christiansen et al. 1999). Patients had a neuropsychological test battery performed preoperatively, at discharge from hospital and three months later. They did not test specifically for delirium. Blood samples were collected preoperatively, and 24h and 48h postoperatively. As expected, levels of these markers of CNS damage rose postoperatively, and the level of NSE 24h after surgery correlated with the degree of POCD at the time of hospital discharge. S100B level only correlated with the duration of cardiopulmonary bypass.

In their study in ICU patients with sepsis associated delirium described in section 1.4.9.3 and 1.5.5.6, Pfister *et al* examined S100B in addition to other biomarkers and measures of cerebral blood flow and oxygenation (Pfister, Siegemund et al. 2008). Biomarkers were measured within 48 hours of admission and stabilisation. In those patients with extracranial sepsis, 12/16 developed delirium. S100B levels were elevated in the whole sample, and they found that S100B discriminated between those who did or did not survive. As previously noted, numbers in the study were small, however, and they did not control for other confounders.

Rasmussen *et al* conducted a prospective cohort study with the hypothesis that serum S100B and NSE would be increased in elderly patients after abdominal surgery, and that that increase would correlate with POCD (Rasmussen, Christiansen et al. 2000). N=65 patients were assessed for delirium according to DSM III criteria for the first three mornings postoperatively, and a neuropsychological test battery was performed preoperatively, and at one week and three months postoperatively. Patients were only included if their preoperative MMSE was >23/30. Blood samples were collected for S100B and NSE preoperatively, and at 24, 48 and 72 hours postoperatively. The authors found that 8/65 (12.3%) patients developed delirium postoperatively, 17/52 (32.7%) had POCD one week postoperatively, and 5/53 (9.4%) at three months. There was a postoperative rise in S100B, and this rise was greater at 48 hours in the

delirium group, but this was no longer significant after applying Bonferroni's correction. They found no correlation between the degree of POCD and either biomarker.

Van Munster *et al* examined S100B levels in a cohort of patients with acute hip fracture (van Munster, Bisschop et al. 2010). They included N=120 patients, of whom 52% developed delirium. Patients had up to four blood samples taken during the perioperative period, at 11am on weekdays, with blood samples classed as "non-delirious", "before" "during" or "after" delirium. Using a linear mixed models approach, they showed that S100B was associated with delirium, and influenced by surgery. In a multivariate analysis, S100B was associated with delirium even after adjusting for premorbid cognitive impairment. A further paper from this group reports more detailed associations with S100B levels, and they found that delirious state, day of blood sampling, pre- or postoperative condition and type of fracture were associated with S100B levels (van Munster, Korse et al. 2009). There was no association with delirium subtype. They also measured NSE, but no difference was found in NSE in those with and without delirium.

van Munster *et al* also examined S100B levels in elderly general medical in-patients (van Munster, Korevaar et al. 2010). They approached all general medical admissions aged over 65 within 48 hours, and assessed daily for delirium using the CAM, subtype with the delirium symptom interview and severity using DRS-R98. Blood samples were collected in the morning, within one week of admission. Four hundred and twelve patients were included, for whom they had blood samples and the CAM assessment was known for the day of blood sampling. One hundred and twenty-six patients developed delirium. Blood samples were classed as either "during", "after" or "without" delirium. In the cognitively intact subgroup, levels of S100B were higher in those during and after delirium. The highest levels were found in the oldest patients with infection and pre-existing cognitive impairment, after delirium. However, S100B was only independently associated with delirium in the group without prior cognitive impairment, and not in those with prior disease.

Infection led to higher S100B levels, after adjustment for age, prior cognition and delirium. There was no difference in S100B levels between subtypes, nor was there any correlation with severity.

Grandi *et al* aimed to determine the relationship between serum levels of Brain Derived Neurotrophic Factor (BDNF), NSE and S100B and delirium in critically ill patients in ICU (Grandi, Tomasi et al. 2011). A prospective cohort of N=130 patients were recruited in ICU and monitored for delirium with CAM-ICU. N=30 developed delirium, and they were retrospectively matched for gender, age, disease severity, sedation use, mechanical ventilation, hypotension and vasopressor therapy with N=30 controls. Due to the relatively small cohort, there was some imbalance in the case-control matching. Daily blood samples were collected, and levels of the three biomarkers were measured on the day of ICU admission and the day before delirium diagnosis (or the corresponding day post-admission for control patients). In univariate analyses, BDNF and NSE were higher on admission in the group who went on to develop delirium, but there was no difference in S100B levels. Levels of the markers fell during ICU stay. This is a relatively small cohort and levels of delirium are relatively low for an ICU cohort, as the authors state. Given that they had collected daily blood samples, a longitudinal analysis would have been very interesting and potentially more informative.

Khan *et al* examined the hypothesis that S100B could act as a biomarker in delirium, with higher levels predicting longer duration of delirium. They performed a prospective observational cohort study in ICU patients with CAM-ICU delirium, collecting two blood samples for S100B one week apart, on days 1 and 8 after enrolment. They used a cut-off of an S100B level of greater than 0.1ng/ml as abnormal, based on a previous population-based study. They found a trend towards longer delirium duration in those with abnormal S100B levels after adjusting for age, cognitive impairment and illness severity. Although a small sample, this provides further evidence towards elevation of S100B in delirium and suggests it may be

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture useful as a prediction marker, if confirmed in a larger study (Khan, Farber et al. 2013).

1.6.3 Biomarkers of CNS damage in CSF

Caplan *et al* (Caplan, Kvelde et al. 2010) undertook a prospective cohort study with the aim of comparing CSF, blood and clinical markers of delirium and neuronal cell death between patients with persistent delirium and an external control group of outpatients with known Alzheimer's Dementia but no delirium. There were N=20 patients in each group. The two groups were relatively well-matched, however the average IQCODE in the delirium group was the same as in the dementia group (3.7 in both groups) suggests a high prevalence of dementia in the delirium group. Delirium was diagnosed using the CAM and Delirium Index. The Acute Physiology, Age, Chronic Health Evaluation (APACHE III) score was significantly higher in the delirium group. Lumbar puncture was performed during the delirium episode or on an outpatient basis for the dementia group. Lactate, protein, glucose, S100B and NSE were measured in CSF. The investigators found higher CSF lactate in the inpatient group with delirium compared to the outpatient group with Alzheimer's dementia, lower NSE, higher protein, and no difference in S100B and glucose. In all participants, CSF lactate correlated positively with APACHE III score, CAM and Delirium Index. NSE correlated negatively with CAM and Delirium Index. Those within the delirium group who died in hospital had higher CSF lactate than those discharged home, and those who required institutional care had an intermediate level. The finding that CSF lactate was higher in persistent delirium, and was also associated with poorer outcomes, suggested that delirium may be associated with neuronal injury, or failure of normal aerobic energy metabolism such as ischaemia. However, here the group with delirium was acutely unwell, compared to the group with dementia who were stable outpatients, therefore differences may be due to acute illness rather than delirium. A third group with acute illness but without delirium would have strengthened the study, as the group comment. The somewhat surprising finding of lower NSE in delirium in the context of high lactate was explained by the investigators as possibly due to lactate suppressing NSE. This could occur via

glutamate stimulation suppressing glycolysis in neurons, which instead gain energy from astrocytic lactate, as per the shuttle hypothesis (Caplan, Kvelde et al. 2010), but these findings require replication.

1.6.4 S100B in severe sepsis and "septic encephalopathy"

Nguyen *et al* performed a cohort study aiming to examine serum levels of S100B and NSE in early sepsis, relate these to any septic encephalopathy and brain injury observed, and see whether the two markers were relevant in predicting outcome compared with Glasgow Coma Scale (GCS) (Nguyen, Spapen et al. 2006). Patients with severe sepsis and severe septic shock according to standard definitions were recruited. They diagnosed two different types of encephalopathy; type A characterised by "agitation, disorientation, confusion, irritability or seizures", and type B, characterised by "somnolence stupor and coma", when two or more of the symptoms were persistently observed during the 72 hours after weaning from sedation. They do not use the term delirium and no delirium specific monitoring or diagnostic tools were used, although the two types of encephalopathy described are likely to be analogous to delirium. Blood samples for S100B and NSE were collected within 24 hours of admission and stabilisation, and repeated daily for four days. Of N=170, almost half had GCS scores >13 on admission. S100B was elevated in 42% and NSE in 53% of the cohort. There was a non-significant trend to an inverse correlation between GCS score and S100B level. The highest levels of both biomarkers were found in participants who died early. Multivariate analysis found that S100B and age were the most powerful predictors of ICU survival. N=27 (16%) patients were diagnosed with encephalopathy after withdrawal of sedation, and elevation of S100B was associated with type B encephalopathy.

Nguyen *et al* conducted a further study to examine the predictive value of both S100B and cortisol in "brain dysfunction" , encompassing coma and CAM-ICU delirium, in severe sepsis and septic shock (Nguyen, Huyghens et al. 2014). This study has also been discussed in section 1.4.9.3. They found S100B (measured serially over four consecutive days) to be elevated in brain dysfunction, with the

highest levels in those with coma. A logistic regression analysis did not find S100B level to be predictive of brain dysfunction.

S100B has been found to be elevated in CSF and serum in children with sepsis and evidence of septic encephalopathy, compared to children with sepsis but without encephalopathy and to healthy children (Hamed, Hamed et al. 2009). Piazza *et al* did not find elevated CSF S100B in a small number of adult patients (N=4) with septic encephalopathy, but did find it to be elevated in serum in three of the four (Piazza, Cotena et al. 2009).

1.7 Dementia neuropathology markers in delirium

1.7.1 CSF studies of dementia neuropathology markers in delirium

Witlox *et al* examined levels of β-amyloid1-42, tau and phosphorylated tau in CSF as pathological indicators of cognitive impairment, with the hypothesis that β-amyloid1-42 would be low and tau and phosphorylated tau would be elevated in patients who developed delirium, due to the high risk of delirium in those with cognitive impairment (Witlox, Kalisvaart et al. 2011). They recruited N=122 patients with acute hip fracture to a trial of taurine vs. placebo in reducing 1-year morbidity and mortality of patients with hip fracture. They collected CSF from N=76 patients, N=30 of whom developed delirium postoperatively. None had syndromal delirium preoperatively. The investigators found no significant difference in levels of any of the dementia markers between those who did or did not develop delirium postoperatively, although the levels of all three markers were different between groups in the directions hypothesised. They also examined the ratios between β-amyloid1-42 to tau and to phosphorylated tau, and there was no difference in either ratio between groups.

Xie *et al* performed a prospective cohort study in older patients undergoing elective orthopaedic surgery, to investigate whether there is an association between the ratio of β-amyloid-40 or β-amyloid-42 to tau protein in CSF at baseline and both the incidence and severity of postoperative delirium (Xie, Swain et al. 2014). Given that tau is usually elevated in dementia and β-amyloid-40 and -42 proteins are usually lowered, they hypothesised that lower β-amyloid-40/tau and β-amyloid-42/tau ratios preoperatively would be associated with greater delirium incidence and severity. They enrolled N=244 participants into the study and were able to analyse samples from N=153. CSF was collected at the onset of spinal anaesthesia, and participants had standardised perioperative care. They were assessed for delirium on the first and second postoperative days only, using the CAM (Inouye, Vandyck et al. 1990) and

MDAS (Breitbart, Rosenfeld et al. 1997); delirium developing later in the postoperative course may therefore have been missed. Although the investigators excluded patients with known dementia or other neurological or psychiatric disease, they did not include an informant interview such as IQCODE (Jorm and Jacomb 1989), or baseline cognitive testing to assess for undiagnosed dementia; the authors qualify this by describing the study as a proof of concept study. Thirty-one participants (20%) were diagnosed with postoperative delirium. There was no significant difference in CSF β-amyloid-42/tau ratio or β-amyloid-40/tau ratio between groups with and without delirium. When they examined quartiles of β-amyloid/tau ratios, they found a higher incidence of delirium in those in the lowest quartile for both ratios. They also found that the median MDAS score in the group within the lowest quartile β-amyloid-40/tau ratio was higher than the median score in the other three quartiles. Using linear regression, they also found an inverse association between MDAS score and both β-amyloid/tau ratios, which remained significant after adjusting for age and gender. This study suggests that lower β-amyloid/tau ratio is associated with increased risk of developing delirium postoperatively; it seems likely that this would be related to the presence of incipient or active dementia, and future studies should take this into account.

1.8 Hip fracture

Peripheral trauma is a major cause of morbidity and mortality in the elderly; hip fracture is the clearest example of this. There are estimated to be 1.26 million hip fractures per year worldwide, projected to increase to 2.6 million in 2025 (Gullberg, Johnell et al. 1997). In the UK, there are around 65,000 hip fractures per year according to the National Hip Fracture Database (NHFD) (Boulton, Burgon et al. 2014), with around 6,000 in Scotland (SIGN 2009). It is a serious and costly injury for older patients. The average hospital cost for each patient over the age of 60 is £12,163 (SIGN 2009). It often heralds deterioration in mobility and functional independence (Keene, Parker et al. 1993). Mean length of stay in orthopaedic wards is 15.3 days, and in acute hospitals 19.8 days. Hip fracture mortality has changed little in the past four decades. Thirty day mortality following hip fracture is around 8% (Boulton, Burgon et al. 2014), 11-23% at 6 months and 22-29% at 12 months (Haleem, Lutchman et al. 2008).

Risk factors for mortality after hip fracture include the use of diuretics, a history of coronary artery disease, male gender, functional dependence identified by a Barthel index of ≤18/20, heart rate >100 and body mass index <20, whereas the use of statins may be protective (Juliebo, Krogseth et al. 2010). A large prospective study (N=1000 consecutive proximal femoral fractures in N=972 patients) has also identified that extracapsular fractures are associated with higher morbidity and mortality at one year than intracapsular (38% vs. 29%). However, those with extracapsular fractures were older, less mobile and less independent at the time of their fracture, and these confounding factors were not accounted for, nor was there any assessment of co-morbidity or cognitive status (Keene, Parker et al. 1993).

1.8.1 Types of hip fracture

Hip fractures can be either intracapsular or extracapsular. Intracapsular fractures occur within the fibrous capsule which encloses the hip joint, and contains much of the joint's blood supply; these fractures cause more disruption in blood supply and may lead to necrosis of the femoral head if not treated. Extracapsular fractures may be either intertrochanteric or subtrochanteric (Boulton, Burgon et al. 2014).

1.8.2 Surgical and anaesthetic options

The UK NICE guidelines recommend that patients are offered a choice between general or regional anaesthetic techniques, after discussing risks and benefits (NICE 2011). The NHFD found no difference in 5 or 30 day mortality among those who received general or spinal anaesthesia, after adjusting for age and physical status. NICE also recommends that patients have surgery on the day of, or the day after fracture. Extracapsular fractures are repaired with a screw and plate (e.g. dynamic hip screw), or an intramedullary nail if the fracture is more complex. Intracapsular fractures are usually repaired with a hemiarthroplasty or total hip arthroplasty (Boulton, Burgon et al. 2014).

1.8.3 Delirium, cognitive impairment and hip fracture

Delirium is common after hip fracture, with rates ranging from 4 to 61% (Schuurmans, Duursma et al. 2003, Juliebo, Bjoro et al. 2009). Cognitive impairment is also very common in patients with hip fracture. Gruber-Baldini *et al* found in a large (N=674) cohort of community-dwelling patients with hip fracture that 50% had some form of cognitive impairment, with 28% having prefracture dementia or reduced MMSE score, 8% had cognitive impairment detected preoperatively and a further 14% had impairment detected postoperatively (Gruber-Baldini, Zimmerman et al. 2003).

Delirium present on admission or developing preoperatively may have a different profile of causative factors to delirium developing postoperatively. Those with

preoperative delirium may have fallen due to an intercurrent illness. Postoperative delirium may be related to the surgical procedure, anaesthetic and postoperative complications. Risk factors for preoperative delirium include male gender, cognitive impairment, previous delirium, indoor injury, anticholinergic drugs, fever and preoperative waiting time (Edlund, Lundstrom et al. 2001, Juliebo, Bjoro et al. 2009). Risk factors for postoperative delirium include age, male gender, cognitive impairment or psychiatric disorders, indoor injury, premorbid ADL dependency, abnormal serum sodium, having several co-morbidities, perioperative fall in blood pressure, infective postoperative complications and body mass index below 20 (Edlund, Lundstrom et al. 2001, Galanakis, Bickel et al. 2001, Schuurmans, Duursma et al. 2003, Juliebo, Bjoro et al. 2009). Analysis of the probable causes of delirium in patients with hip fracture according to a classification instrument (drug-induced, infection-induced, fluid-electrolyte disturbance, metabolic/endocrine disturbance, intracranial process, cardiopulmonary compromise and/or hypoxia, alcohol and drug withdrawal, sensory/environmental) has shown that many episodes have several causes. However, the rate of delirium was low in this study at 9.3%, and the information used to guide classification is dependent on assessments and investigations performed by clinical staff, which may be heterogeneous or incomplete (Brauer, Morrison et al. 2000).

The presence of dementia has been identified as an independent risk factor for mortality following hip fracture, but results for delirium are mixed (Juliebo, Krogseth et al. 2010). A study by Juliebø *et al* which aimed to recruit a natural population of patients with hip fracture, including patients with dementia and severe dementia, aimed to address this question. In their cohort of N=331, the estimated mortality at one year was 20.5%, which was higher in those with delirium at any stage (28.0%), and particularly in those with delirium preoperatively (42.0%). Delirium was associated with mortality in univariate analyses, however, when they adjusted for the presence of prior dementia (IQCODE), the relationship disappeared. Severity of prior dementia, assessed using the IQCODE, was associated with mortality post-hip fracture. Kaplan-Meier plots showed a cumulative effect of delirium and dementia on mortality (Juliebo, Krogseth et al. 2010). Dolan *et al* also found that delirium on

admission was not a significant predictor of mortality after adjusting for age, gender, race, co-morbidity and functional status. They did find that delirium, in the absence of prior cognitive impairment, was associated with poorer physical, cognitive and affective functioning at 6 months, and slower recovery post-fracture, after adjusting for confounding factors (Dolan, Hawkes et al. 2000). There may be a difference in outcomes following prevalent (preoperative) and incident (postoperative) delirium; Edlund *et al* found that patients with preoperative delirium were more likely to be discharged to an institution and had poorer mobility than those with only postoperative delirium, which was often shorter in duration (Edlund, Lundstrom et al. 2001). Givens *et al* examined the individual and cumulative effects of delirium, dementia and depression on outcomes post-hip fracture (Givens, Sanft et al. 2008). In N=126 patients, they found that depression increased the odds of new nursing home admission or death at one month, prior dementia increased these odds and the likelihood of decline in mobility, and delirium increased the likelihood of all of these poor outcomes and of decline in function. They also found a cumulative effect of the three psychiatric disorders, where the stepwise addition of each increased the likelihood of poor functional outcomes (Givens, Sanft et al. 2008). Nightingale *et al* also examined the effects of delirium, dementia and depression on mortality after hip fracture. In N=731 patients, only 28% were psychiatrically "well", 40% had dementia, 15% had delirium and 13% had depression, and 4% had other psychiatric diagnoses. At 2 years, 53% were alive with no loss to follow-up; a multivariate survival analysis demonstrated hazard ratios for death of 2.62 for dementia, 2.40 for delirium and 1.57 for depression (Nightingale, Holmes et al. 2001).

Delirium has also been shown to be a risk factor for the development of dementia after hip fracture. A prospective study by Krogseth *et al* aimed to assess the impact of delirium in hip fracture patients on the risk of dementia at 6 months, adjusted for prior cognitive impairment (Krogseth, Wyller et al. 2011). They assessed patients for perioperative delirium using the CAM, and used informant history via the IQCODE as a proxy for prefracture cognitive impairment. Patients were followed-up at 6 months with a series of cognitive tests, and two expert clinicians then decided whether each patient fulfilled the DSM-IV criteria for dementia both at baseline and

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture at 6 months. Of their cohort of N=266, N=92 were lost to follow-up, around half due to death, N=174 were seen at 6 months and N=106 were deemed free from prefracture dementia. In a multivariate logistic regression analysis in the group free from prefracture dementia, delirium perioperatively was shown to be the strongest risk factor for dementia at six months.

1.8.4 Physiological responses to trauma

The body's response to trauma, leading to protein loss and fatigue which can be persistent, is likely to contribute to poor outcomes in the elderly following hip fracture. Injury or trauma is associated with neuroendocrine, inflammatory and metabolic responses (Hill 2000, Chuang, Power et al. 2005). The first stages of injury lead to hypoperfusion and then reperfusion of the injured tissues, with activation of afferent peripheral nerves and the involvement of factors from vascular endothelium (Hill 2000). Events are then controlled on two levels, in the CNS and at a local tissue level. Early hormonal release of glucocorticoids, catecholamines and glucagon leads to lipolysis and nitrogen loss. It is now thought that this neuroendocrine response is an initiating event, with the subsequent involvement of a complex cascade of pro- and anti-inflammatory cytokines, oxygen free radicals and arachidonic acid metabolites. The results of these CNS and local processes are anorexia, pyrexia and immobility in the initial stages, and in the later stages protein loss, organ dysfunction and persistent fatigue which may last several months (Hill 2000).

There is also evidence that peripheral injury such as hip fracture may invoke a CNS inflammatory response. Chuang *et al* (Chuang, Power et al. 2005) measured inflammatory cytokines in CSF and serum of elderly women undergoing either hip fracture repair or elective joint replacement. They found the pro-inflammatory cytokines IL-8 in CSF and IL-6 in serum to be higher in trauma patients compared to elective patients. Of note, however the trauma group were significantly older than the elective group (81 years vs. 69 years), and they make no comment on the presence or absence of delirium. This neuroinflammation, apparently induced by trauma, with

what the authors conclude to be intrathecal cytokine synthesis, is very intriguing given the high rate of delirium after hip fracture and the overall poor outcomes.

1.9 Clinical trials in the prevention and management of delirium

This section will briefly discuss the current evidence for interventions aimed at preventing or managing delirium, with a particular focus on interventions which target the HPA axis or inflammation.

1.9.1 Multicomponent interventions

Much of the early evidence for delirium prevention comes from multicomponent interventions such as proactive geriatric consultation. Inouye *et al* developed a multicomponent intervention termed the Hospital Elder Life Program (HELP), which was aimed at reducing delirium incidence in elderly medical in-patients. The intervention involved protocols to manage six delirium risk factors; cognitive impairment, sleep deprivation, visual impairment, hearing impairment and dehydration. In N=852 patients, the intervention reduced delirium incidence from 15.0% in the usual care group to 9.9% in the intervention group, with an odds ratio for delirium of 0.60 (Inouye, Bogardus et al. 1999). The elements of the HELP program have been further adapted and shown to reduce delirium incidence and improve quality of care where they are integrated into clinical practice (Vidan, Sanchez et al. 2009).

Marcantonio *et al* have shown in a prospective randomized blinded single-centre trial that proactive geriatric consultation compared with usual care can reduce delirium incidence (odds ratio 0.64) and cases of severe delirium in patients with acute hip fracture (Marcantonio, Flacker et al. 2001). The intervention included targeted recommendations such as oxygen delivery, fluid balance, medication review, pain management and regulation of bladder and bowel function. Lundstrom *et al* have shown that a multicomponent intervention involving postoperative care of patients with hip fracture in a specialized geriatric ward can reduce delirium incidence,

duration and length of stay (Lundstrom, Olofsson et al. 2007). However, a recent single-centre trial in Oslo, Norway, examined whether orthogeriatric care in a dedicated unit staffed by geriatric nurses and geriatricians, with comprehensive geriatric assessment, might prevent perioperative delirium and longer-term cognitive decline, compared to standard orthopaedic care. Three-hundred and twenty-nine patients with acute hip fracture were randomised to care in an acute geriatric ward or standard orthopaedic care. Primary outcome was cognitive function four months after hip fracture, and secondary outcomes were pre- and postoperative delirium, delirium severity and duration, mortality and mobility. No significant differences were found between treatment groups in delirium rates, severity or duration, in cognitive outcomes at four months, nor in mortality (Watne, Torbergsen et al. 2014).

Although multi-component interventions have been shown to be effective in preventing delirium, they are not always effective at managing cases of delirium or shortening its duration once delirium has developed. Cole *et al* performed two randomized controlled trials to evaluate whether the systematic detection of delirium in elderly medical in-patients, followed by a consultation with a geriatrician and liaison nurse follow-up would lead to cognitive improvement. In the first trial of N=88, the intervention led to small improvements in the Short Portable Mental Status Questionnaire and Crichton Behavioural Rating Scale (Cole, Primeau et al. 1994). However, the second trial of N=227 found no significant difference in time to or rate of improvement in the Delirium Index or other outcome measures such as length of stay, function or survival (Cole, McCusker et al. 2002). A randomized controlled trial of a multicomponent intervention with tailored geriatrician input in N=174 elderly medical patients in Finland has shown a significantly faster reduction in delirium severity, as measured by the MDAS, in the intervention group, but did not impact on rates of mortality or institutionalization (Pitkala, Laurila et al. 2006). Nurse-led detection of delirium in post-acute care facilities has been shown to increase delirium detection, but a subsequent multicomponent intervention in cases to address delirium causes and complications and restore function was not effective (Marcantonio, Bergmann et al. 2010).

1.9.2 Anti-psychotic medication

Typical and atypical anti-psychotic medications are commonly used in clinical practice for the management of delirium symptoms, particularly agitation and psychotic features. The current UK NICE guidance is to consider short-term use of an anti-psychotic such as haloperidol or olanzapine where patients are at risk of harm to themselves or others, and where other de-escalation techniques have failed (NICE 2010). Evidence for the efficacy of anti-psychotics in delirium is modest. A Cochrane review comparing haloperidol with atypical antipsychotics in the management of delirium and the incidence of adverse reactions identified three trials comparing haloperidol to risperidone, olanzapine and placebo. The studies were small, and the conclusions limited. Low dose haloperidol decreased delirium severity and duration in postoperative patients, although not incidence, when compared to placebo. There were no significant differences when low dose haloperidol was compared to the atypical antipsychotics, nor were there more adverse effects with its use (Lonergan, Britton et al. 2007). Prophylactic use of anti-psychotic medication may be more promising. A recent meta-analysis of trials examined prophylactic use of anti-psychotic medication in high-risk surgical populations, and identified five randomized prospective clinical trials with a total of N=1491 elderly participants. Three trials compared haloperidol to placebo, one olanzapine to placebo and one risperidone to placebo, across a range of surgical populations. The pooled analysis suggested antipsychotic prophylaxis reduced delirium incidence, with an odds ratio of 0.42 (Gilmore and Wolfe 2013). The Hope-ICU trial however did not find any benefit in early use of haloperidol versus placebo in decreasing duration of delirium or coma in N=141 critically ill patients (Page, Ely et al. 2013).

1.9.3 Cholinesterase inhibitors

Since one of the hypotheses of delirium pathogenesis is that there is a deficiency in acetylcholine neurotransmission, cholinesterase inhibitors have been trialled in its therapy. A single-centre double-blind randomized controlled trial of prophylactic rivastigmine versus placebo in N=120 elderly patients undergoing elective cardiac

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture surgery found no difference in delirium incidence between groups (Gamberini, Bolliger et al. 2009).Pilot studies of cholinesterase inhibitors have been performed in several settings. When donepezil was piloted versus placebo in delirium after hip fracture, there were no differences in delirium duration or severity, and those receiving donepezil suffered more side effects and adverse events (Marcantonio, Palihnich et al. 2011). A pilot study of oral rivastigmine in severe and persistent delirium after stroke concluded that in the N=17 patients included, rivastigmine was safe and well-tolerated (Oldenbeuving, de Kort et al. 2008). A double-blind randomized pilot study of rivastigmine (N=8) versus placebo (N=7) in established delirium in elderly medical patients found no difference in delirium duration but it was suggested that rivastigmine was safe in this setting (Overshott, Vernon et al. 2010). A pilot double-blind randomized placebo-controlled trial of donepezil in delirium prevention in N=33 patients undergoing elective hip arthroplasty found that donepezil was well tolerated with no adverse events. There was no difference in delirium incidence between groups (Sampson, Raven et al. 2007). A multicentre, randomized double-blind placebo controlled trial of rivastigmine as an adjunct to usual care with haloperidol in patients with delirium associated with critical illness had to be stopped early due to a higher mortality rate in the rivastigmine arm. N=104 patients were included compared to N=440 planned. Median duration of delirium was longer in the rivastigmine group (van Eijk, Roes et al. 2010).

1.9.4 Melatonin

Melatonin is a hormone released from the pineal gland which in health promotes sleep (Uchida, Aoki et al. 1999). Due to the characteristic sleep-wake cycle disturbance in delirium, Melatonin has been trialled as prophylaxis against delirium in the hope of preserving sleep-wake cycle. Al Aama *et al* performed a randomized placebo-controlled trial of melatonin in elderly patients admitted to an acute medical unit. N=145 patients were randomized but due to loss to follow-up they were only able to analyse date for N=121. Delirium occurrence was lower in the melatonin group (number needed to treat 6.4), including after excluding a small proportion who were delirious at enrolment (Al-Aama, Brymer et al. 2011). De Jonghe *et al*

performed a multicentre randomized placebo-controlled trial of melatonin as prophylaxis for delirium in N=378 patients with acute hip fracture; the study was possibly underpowered due to post-randomization exclusions. No difference was observed in delirium incidence between groups. Median delirium duration was the same at two days, but a smaller proportion of patients in the melatonin group had long-lasting delirium (> 2 days) (de Jonghe, van Munster et al. 2014). In ICU and general medical wards, Hatta *et al* trialled Ramelteon, a melatonin agonist, in N=67 elderly patients as prophylaxis against delirium. Delirium incidence was significantly lower in the treatment group, at 3% compared with 32% in the placebo group. Although N=1059 screened patients did not meet inclusion criteria, primarily due to expected length of stay of <48h or intubation, the numbers in each arm of the study met those in their power calculation (Hatta, Kishi et al. 2014). A prospective clinical observational study examined the role of melatonin prophylaxis in delirium prevention in elective or urgent cardiac surgery. The first group of N=250 served as controls, and the second group of N=250 received melatonin. The melatonin group experienced less delirium but also had significantly lower levels of alcoholism and spent less time on cardiopulmonary bypass. The study design may also have meant that delirium rates were lower in the second group, recruited later, due to increased staff awareness and knowledge of delirium in their unit. Melatonin was not associated with less delirium in their multivariate analysis (Artemiou, Bily et al. 2015). Results for melatonin have therefore been mixed; a recent meta-analysis of four randomized controlled trials of its use for delirium prevention found a trend towards effectiveness, with a subgroup analysis of use in elderly medical patients showing benefit (Chen, Shi et al. 2015).

1.9.5 Dexmedetomidine

Dexmedetomidine is an α2-adrenoreceptor agonist which has also been shown to have anti-inflammatory properties, enhancing macrophage phagocytosis and bacterial clearance (Pandharipande, Sanders et al. 2010, Ji, Li et al. 2013). In post-operative delirium and delirium in critical illness, there has been recent evidence that sedation or anaesthesia using dexmedetomidine reduces the duration or incidence of

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture delirium. A large retrospective observational study of N=1134 patients has shown in a multivariate analysis that dexmedetomidine reduced delirium incidence after cardiac surgery, with an adjusted odds ratio of 0.53 (Ji, Li et al. 2013). A recent meta-analysis examined twenty studies with N=2612 patients where dexmedetomidine was tested in anaesthesia or sedation with regard to beneficial effects on neurocognitive dysfunction, which included delirium. The authors concluded that dexmedetomidine use was associated with less neurocognitive dysfunction, but not in subgroup analyses when delirium according to the CAM-ICU was the outcome (Li, Wang et al. 2015). A meta-analysis of trials comparing dexmedetomidine to propofol sedation for mechanical ventilation in ICU examined ten trials with N=1202 patients and concluded that dexmedetomidine reduced ICU length of stay and incidence of delirium, but did not influence ICU mortality, and was associated with increased hypertension (Xia, Chen et al. 2013). Pandharipande *et al* specifically hypothesised that sedation for mechanical ventilation with dexmedetomidine compared to lorazepam would be beneficial, particularly in patients with sepsis, due to its anti-inflammatory effects. They performed an *a priori* subgroup analysis of septic and non-septic patients from a randomized double-blind placebo controlled trial of sedation with dexmedetomidine or lorazepam. They found that in the N=63 patients with sepsis, those receiving dexmedetomidine (N=31) had on average 3.2 more delirium or coma-free days, and had a lower risk of developing delirium or of death. The beneficial effects of dexmedetomidine were more pronounced in the group with sepsis (Pandharipande, Sanders et al. 2010).

1.9.6 Drugs which disrupt the HPA axis

A search of the literature found no studies testing pharmacological agents such as the 11-β-HSD inhibitor carbenoxolone or experimental 11β-HSD-1 inhibitors which would limit the HPA axis response as prophylaxis or treatment in delirium. Case reports only reported the use of metyrapone in patients with delirium or other neuropsychiatric complications of Cushing's disease.

1.9.7 Anti-inflammatory agents

1.9.7.1 Corticosteroids

Since there are also multiple threads of evidence of an inflammatory nature to delirium, as discussed in detail in section 1.5, some investigators have tried to tackle this inflammatory response by administering dexamethasone. Mardani *et al* studied the effects of intravenous dexamethasone administration in N=93 patients undergoing coronary-artery bypass grafting and recovering in ICU (Mardani and Bigdelian 2012, Mardani and Bigdelian 2013). Patients were given dexamethasone preoperatively and for two days postoperatively. They demonstrated that those who did not receive dexathethasone were 1.2 fold more likely to develop postoperative delirium. However, their methods for capturing delirium were flawed, since they screened using the MMSE, which is an insensitive tool for delirium detection, and only conducted further psychiatric interviews to make a diagnosis of delirium according to DSM IV criteria if the MMSE score was 23 or below. Also, it is unclear who and how well matched the control group were. Sauer *et al* conducted a single centre substudy nested within a larger placebo controlled randomised controlled trial, hypothesising that the administration of high dose dexamethasone intraoperatively would reduce the rate of postoperative delirium by reducing the inflammatory response associated with CABG surgery and cardiopulmonary bypass (Sauer, Slooter et al. 2014). Patients were randomised to receive either 1mg/kg dexamethasone or placebo at the onset of anaesthesia, and they were monitored for delirium daily postoperatively for four days using the CAM-ICU or CAM after ICU stepdown. Of N=768 patients, N=737 had complete data. The incidence of delirium was similar in both the treatment and placebo group (around 14% in both groups), as was the median duration of delirium (2 days in both groups). Surrogate markers including the use of restraint and the administration of haloperidol, benzodiazepines and opioids were also similar in both groups. Dexamethasone administration therefore did not alter the incidence or duration of postoperative delirium in the four days following CABG. The steroids in cardiac surgery trial examined whether administration of methylprednisolone as an anti-inflammatory versus placebo would improve outcomes in patients undergoing cardiopulmonary bypass. Their primary outcome

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was thirty-day mortality and one of the secondary safety outcomes was delirium on postoperative day three. The investigators found no difference in incidence of delirium between groups, nor in mortality nor major morbidity (Whitlock, Devereaux et al. 2015).

There is, therefore, very little evidence of a beneficial effect of dexamethasone prophylaxis or treatment in delirium, where it is used as an anti-inflammatory agent. As already discussed, steroid use in clinical practice is historically associated with delirium (Wolkowitz, Reus et al. 1997). Steroid administration in a critical care setting has been shown in a multivariate analysis to be independently associated with increased likelihood of transitioning into delirium in patients with acute lung injury (Schreiber, Colantuoni et al. 2014). A systematic literature review of N=14 prospective studies investigating the association between medication use and delirium risk found that there was uncertainty with steroid use, however, with only one included low quality study examining steroids, with an odds ratio of 0.50 (Clegg and Young 2011).

1.9.7.2 Non-steroidal anti-inflammatory drugs (NSAIDs) including COX II inhibitors

A review of the literature did not find any studies testing whether non-steroidal anti-inflammatory drugs, including newer COX II inhibitors or biological agents are useful in the prophylaxis or treatment of delirium. Use of NSAIDs in the elderly is with great caution, due to the risk of side effects including acute kidney injury and gastrointestinal haemorrhage. The systematic literature review cited above which investigated the association between medication use and delirium risk found that there was also uncertainty with NSAID use, with one low quality study reporting an odds ratio of 0.40 (Clegg and Young 2011). COX II inhibitors were found in one case report to induce delirium (MacKnight and Rojas-Fernandez 2001).

1.9.7.3 N-3 fatty acids

N-3 fatty acids are proposed to alter the balance between pro- and anti-inflammatory mediators in favour of anti-inflammatory pathways, in the hope that this modulation of the inflammatory response will be beneficial in severe sepsis and sepsis-associated delirium. A small randomized study of IV therapy with n3 fatty acids versus placebo in N=50 ICU patients with sepsis found no effect on incidence of delirium, nor in plasma levels of the CNS damage markers S100B and NSE nor in CRP or inflammatory mediators. The study population was small and heterogeneous, around half of the intervention group could not receive the full dose of n3 fatty acids due to high triglyceride levels, and some delirium assessments were missing, limiting the conclusions that can be drawn (Burkhart, Dell-Kuster et al. 2014).

1.9.7.4 Ketamine

The anaesthetic agent ketamine has been proposed to have neuroprotective effects, potentially by reducing excitotoxic injury and limiting CNS inflammatory responses to injury (Hudetz, Patterson et al. 2009). A small prospective randomized study examined whether the administration of a single IV dose of ketamine compared to saline during anaesthetic for surgery under cardiopulmonary bypass would reduce the inflammatory response and incidence of postoperative delirium. Hudetz *et al* found in N=58 patients that the ketamine group had a significantly lower incidence of delirium (3% versus 31%), and while preoperative serum CRP was not significantly different between groups, postoperative CRP on day 1 was significantly higher in the placebo group, suggesting a greater peripheral inflammatory response. There was no difference in CRP between those who did or did not develop delirium, however. This is an interesting study, but the effect of ketamine may not be anti-inflammatory, since it may exert neuroprotective effects through other mechanisms and is also an NMDA receptor antagonist (Hudetz, Patterson et al. 2009). A further small randomized double-blind study has examined the role of ketamine in reducing early post-operative cognitive dysfunction, within one week of surgery, in N=56 elderly patients undergoing elective orthopaedic surgery. Patients were not tested for

Cortisol and inflammation in delirium and long-term cognitive decline after hip fracture delirium. The investigators found no difference in POCD nor in CRP level in the week after surgery (Lee, Kim et al. 2015).

1.9.8 Summary

In summary, there is good evidence for delirium prevention using multi-component interventions, which usually involve attention to detail and good medical and nursing care. There is less evidence that this approach proves successful once delirium is established. There is modest evidence that anti-psychotic medication is helpful in delirium, but it may be more promising when used in low doses prophylactically. Anti-cholinesterase inhibitors have not been proven in delirium and may be harmful. Melatonin may be helpful in delirium prevention, particularly in elderly medical patients. In anaesthesia and sedation, dexmedetomidine appears to be beneficial in reducing incidence of delirium, and whether this effect is through immune-modulating pathways needs to be further investigated. A small number of trials have examined different anti-inflammatory targets, with no clear beneficial results; the use of corticosteroids as anti-inflammatory agents may be counteracted by the risk of dexamethasone itself inducing delirium. There have been no interventions tested which address excess or prolonged cortisol production.

1.10 Summary and outline of research questions

This PhD aims to investigate the roles of deranged cortisol and inflammatory responses to stress in delirium and long-term cognitive decline in patients with hip fracture, who are very prone to delirium as outlined above. It is not currently known whether delirium is associated with hypercortisolaemia or attenuated circadian responses after 2-3 days, and indeed cortisol levels may be high for up to eight weeks post hip-fracture (Roberts, Barton et al. 1990). The relationship between prolonged high cortisol, inflammation and long-term cognitive decline is also not known. Patients with hip fracture commonly undergo fracture repair under spinal anaesthesia, which affords an excellent opportunity to sample cerebrospinal fluid in a group of patients vulnerable to delirium, with minimal upset for the patients themselves as the procedure is a necessary one. This allows for the examination of CSF levels of cortisol, inflammatory markers and markers of CNS damage in delirium. A power calculation was performed for the main analyses of the study, i.e. comparisons of cortisol and cytokine levels between patients with (~N=40) and without (~N=80) delirium. Setting alpha at p=0.05 we have > 80% power to detect medium-sized (Cohen's d=0.5) differences for these analyses with N=120 participants.

1.10.1 Aims and Objectives

(A1) To systematically review the literature on CSF biomarkers in delirium, in order to summarise existing knowledge and to provide a basis for future studies.

(A2) To determine if high cortisol levels (in saliva and serum) in the two weeks after hip fracture are associated with delirium in older patients.

(B1) To determine if high cortisol levels in the first two weeks after acute hip fracture predict persistent delirium and/or deterioration in thinking and memory at intervals up to one year.

(B2) To determine if high cortisol levels seen in acute delirium are sustained up to one year after acute hip fracture and, if so, to determine any relationships with cognition.

(B3) To determine if levels of cortisol in cerebrospinal fluid (CSF) sampled at the onset of spinal anaesthesia are associated with delirium and with longer−term cognitive decline.

(B4) To determine the relationships between delirium after hip fracture and peripheral and CSF markers of acute inflammation and examine whether these relate to cortisol levels.

(B5) To determine if CSF markers of acute central nervous system damage are associated with high salivary and CSF levels of cortisol.

2 Chapter 2: General Methods

2.1 Study populations

The primary study reported in this thesis includes a cohort of community-dwelling patients in Edinburgh with acute hip fracture (CDHIP). The cerebrospinal fluid studies reported in Chapters 4 and 5 involve collaboration with another research group in Oslo, Norway. The Oslo cohort was an elderly cohort (median age 85) with acute hip fracture enrolled to the Oslo Orthogeriatric Trial (Watne, Torbergsen et al. 2014), who had cerebrospinal fluid collected at spinal anaesthetic, and perioperative delirium assessment and many other measures collected in the same way as the CDHIP study cohort. As these studies are difficult to perform, and many of the salient features of the cohorts are the same, we combined these cohorts in order to increase statistical power to answer the research questions. The other study cohort will be described in the methods section and it will be clearly indicated in each chapter where it was included

2.2 CDHIP Study Recruitment

Patients with hip fracture were admitted via Accident and Emergency to the Orthopaedic wards of the New Royal Infirmary, Edinburgh. On the mornings and afternoons of assessment, I identified potential participants who met inclusion criteria by asking Nursing Staff on the ward or from the Trauma theatre list. Exclusion criteria were sought through discussion with Nursing Staff who could examine patient case-notes.

Inclusion criteria:

- Over the age of 60
- Acute hip fracture, before surgery
- For spinal anaesthesia
- Patients with apparently normal cognition and cognitive impairment included

Exclusion criteria:

- Nursing Home resident
- Inhaled / oral steroids in last 10 weeks
- Active malignancy, co−morbid disease such that prognosis is less than one year
- Significant Parkinson's disease
- Major communication difficulties: aphasia, English not first language

A lower age limit of 60 years was included given age-related risk of delirium, as young patients with acute hip fracture are unusual and less likely to develop delirium. All patients needed to have had acute hip fracture and were recruited preoperatively in order to have a baseline preoperative assessment of delirium and the opportunity to collect CSF and blood samples at the time of spinal anaesthetic. Patients planned for spinal anaesthetic were included, or those where there was no anaesthetic plan if they had not yet been reviewed, to allow the collection of CSF at spinal anaesthetic; if the anaesthetic plan later changed to general anaesthetic, patients were not excluded. Community-dwelling patients with known cognitive impairment were not excluded due to the higher risk of delirium, to avoid the possibility that only low rates of delirium would be detected in a cohort who was exclusively cognitively intact premorbidly.

Patients from Nursing Homes were excluded due to the high rates of frailty and dementia in this population, since the proposed 12 month follow-up with cognitive testing would most likely lead to high rates of attrition and missing data in these patients. Those on steroid medication were excluded as this can interfere with measurement of cortisol in biological samples. Those with a projected prognosis of less than one year due to known malignancy or co-morbid disease were excluded due to the planned 12 month follow-up, to limit rates of attrition. Those with significant Parkinson’s disease were excluded due to the difficulty in differential diagnosis of delirium or features such as hallucinations in these patients, and the likelihood that

there might be a specific aetiology such as L-dopa related delirium. Patients with major communication difficulties were excluded due to the challenges relating to cognitive testing in these patients.

Potential participants were approached in alphabetical order or, if this was not possible (e.g. patients were being washed or prepared for theatre) or in the interests of time, in the order of the theatre list. The first approach was made by a member of the patient's usual care team, who introduced me. I explained the study, checking at each stage that the patient could hear and understood the explanation. If no further exclusion criteria were discovered, I asked if the patient had any questions and if they would be interested in taking part in the study. If so, I provided them with the patient information sheet to read through. If the patient had poor eyesight or did not have their glasses, I read out the information sheet (Appendix 4). I asked if the patient had any further questions, or if they would like more time to decide. If the patient would like to take part, I assessed their capacity to give informed consent; much of this information could be gained from the interaction of explaining and discussing the study. I checked that the patient understood and could weigh, retain and communicate the following information about the study:

- they are being asked to help with a research project
- the project is looking at the causes of thinking and memory problems
- they will be asked to answer some questions on thinking and memory
- a sample of their spinal fluid and a blood sample will be collected at the time of surgery, and further samples of blood and saliva will be taken in the two weeks following their surgery
- I will look at the patient's casenotes and will also discuss their case with staff to find out how they have been doing
- with their permission, the information in the study will be passed to the hospital staff
- I will follow them up over the next year

- I will contact the participant's GP with their consent to inform him/her of their participation in the study and prior to follow-up contact
- the study is voluntary and they can withdraw at any time
- participation in the study will not change the management for their broken hip

If the patient demonstrated capacity to decide to take part in the study, he or she was taken through the consent form and asked to sign it (Appendix 4). If the patient was willing to take part but did not demonstrate capacity, I asked if I could return to discuss the study with the patient and their next of kin. Due to the time-pressures of hip fracture repair, this was sometimes not possible. If the patient's next of kin was happy for the patient to participate in the study, he or she was asked to give informed consent on their behalf.

Photocopies of the consent form were placed in the medical notes and given to the participant. A letter was written to the participant's GP informing him or her of their recruitment to the study, outlining what the study entailed.

Once the participant had a place on the trauma theatre list, I contacted the Anaesthetist for the list and asked that CSF and blood tests were taken during the onset of anaesthesia.

2.3 Preoperative Assessment

Participants were first assessed for delirium preoperatively. There follows a summary of the structure and tools used for delirium assessment, and then each tool is described in more detail. A full schedule of assessments is included in Appendix 1. Delirium assessment comprised a semi-structured interview with additional cognitive testing which took 10-15 minutes (Appendix 1). This allowed the completion of the Confusion Assessment Method (CAM) (Inouye, Vandyck et al. 1990), Delirium

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Rating Scale Revised ’98 (DRS-R-98) (Trzepacz, Mittal et al. 2001), Mini Mental State Examination (MMSE) (Folstein, Folstein et al. 1975) and a brief test of attention using the Edinburgh Delirium Test Box-1 (EDTB-1) (Brown, Fordyce et al. 2010). Level of arousal was assessed with the Richmond Agitation Sedation Scale (RASS) (Sessler, Gosnell et al. 2002) and an in-house assessment the Observational Scale of Level of Arousal (OSLA) (Tieges, McGrath et al. 2013). I also discussed any signs of delirium with nursing staff and sought evidence in the casenotes. This format was chosen to provide a brief but reliable assessment of delirium presence and severity using well-established and well-validated research tools based on DSM III and DSM IV criteria (Inouye, Vandyck et al. 1990, Trzepacz, Mittal et al. 2001). The assessment was tailored pragmatically to take account of patient fatigue, pain and other factors.

2.3.1 The Confusion Assessment Method

The CAM is a diagnostic algorithm which operationalizes the DSM III diagnostic criteria for delirium (Inouye, Vandyck et al. 1990). It assesses four features: (i) Acute onset and/or fluctuating course, (ii) Inattention, (iii) Disorganised thinking and (iv) Altered level of consciousness. Delirium is deemed to be present if the patient has features (i) and (ii), and either feature (iii) or (iv). The CAM algorithm is informed by a semi-structured interview. Assessment of attention and disorganised thinking is through interview and sometimes cognitive testing (e.g. digit span), some general cognitive testing (often by the Mini-Mental State Examination) and questioning of nursing staff and carers. With the requisite mental status testing the CAM typically takes 10-12 minutes to administer. A period of training is required to gain competence in administering the CAM. During this period, I assessed multiple patients in acute geriatric wards, alone and with an experienced rater (AMJM) until competency was achieved. I also read the training manual. A systematic review of research on the CAM highlighted the importance of training (Wei, Fearing et al. 2008) because sensitivity of the tool is poor without training (Lemiengre, Nelis et al. 2006, Sands, Dantoc et al. 2010).

2.3.2 The Delirium Rating Scale - Revised '98

The DRS-R98 is a detailed assessment of delirium symptomatology and severity, consisting of 13 items (sleep-wake cycle disturbance, perceptual disturbances, delusions, lability of affect, language and thought process abnormalities, motor agitation or retardation, orientation, attention, short and long-term memory and visuospatial ability) with 3 additional diagnostic items (temporal onset, symptom fluctuation and physical disorder). Each feature is scored from 0-2 or 0-3 according to both its presence and severity. The total possible score is 39 for the severity scale and 46 including the diagnostic items, and the cut-off for delirium is a score of >15.25 and >17.75 respectively (Trzepacz, Mittal et al. 2001). The assessment involves a semi-structured interview with questioning to elicit certain symptoms, plus assessment of attention and brief cognitive testing. Additional information from nursing staff, carers and casenotes is also gathered wherever possible. The DRS-R98 was employed here to provide a detailed assessment of delirium severity and symptomatology. I had training in its use prior to starting the study from an experienced rater (AMJM). Information from the MMSE was used to aid completion of short term memory and orientation items. For visuospatial ability, the intersecting pentagons item from MMSE was used in addition to a map of Scotland, where participants were asked to identify well known cities, in addition to information from nurses or casenotes on wandering.

2.3.3 The Mini-Mental State Examination

The MMSE is a widely used instrument, designed to test global cognition (Folstein, Folstein et al. 1975). It assesses orientation, registration and short-term recall, attention and calculation, language and praxis. The maximum score is 30, with a score below 24 usually indicating significant cognitive impairment. It takes around 5 minutes to complete, with face-to-face questioning and pen and paper tasks. The MMSE is not usually used for diagnosis of delirium, but when serially administered, it has been found to be responsive to change in an acute geriatric population (O'Keeffe, Mulkerrin et al. 2005). Individual test items were used to aid completion of orientation, memory and visuospatial components of the DRS-R98.

2.3.4 Assessment of level of arousal

2.3.4.1 Richmond Agitation Sedation Scale

The RASS is a well-known scale of level of sedation and arousal (Sessler, Gosnell et al. 2002). It is bidirectional, and was originally developed for assessment of agitated and sedated patients in critical care settings (Sessler, Gosnell et al. 2002). A score of zero indicates a normal level of arousal, positive scores indicate a restless or agitated state, and negative scores a drowsy or sedated state. It takes a few seconds to a minute to complete; the tester first observes the patient and if they are alert scores them from 0 to +4. If the patient is not alert the tester says the patient's name and asks them to open their eyes and look at the speaker. The degree to which the patient responds to verbal stimuli may then be recorded (-1 to -3). If there is no response to verbal stimuli, the tester then physically stimulates the patient by shaking their shoulder or rubbing their sternum. The degree of response to physical stimuli can then be recorded (-4 to -5) (Sessler, Gosnell et al. 2002).

2.3.4.2 The Observational Scale of Level of Arousal

I developed the OSLA for use in this study (Tieges, McGrath et al. 2013). This was because existing scales of arousal are not very detailed, and do not fully capture the spectrum of change in arousal level, particularly mild change, in elderly hospital patients. It is a bidirectional scale encompassing four subscales; eye opening, eye contact, posture and movement, shown in Table 2.1. An exploratory study in a different cohort with acute hip fracture (N=30) found that abnormal level of arousal was strongly associated with the presence of delirium. The area under the receiver operating curve for the OSLA was 0.89 (95% confidence interval 0.68-0.94), with a sensitivity of 0.87 and a specificity of 0.81 (Tieges, McGrath et al. 2013).

Table 2.1 The Observational Scale of Level of Arousal

Subscale	Criteria	Score
Eye opening	Open on arrival and remain so, under patient's control, outlasts stimulus	0
	Open on arrival but close if stimulus removed	1
	Open to voice but then outlasts stimulus	1
	Open to voice but close if stimulus removed	2
	Open to gentle physical stimulation eg squeezing hand, gentle shake to shoulder	3
	Open to pain only	4
	No eye opening	5
Eye contact	Spontaneously makes and holds eye contact appropriately	0
	Drowsy and makes eye contact to command but can't hold it very long	1
	Alert but eyes wandering, some appropriate eye contact	1
	Alert but eyes wandering, little or no appropriate eye contact	2
	Drowsy but makes brief eye contact	2
	Eyes will / are open but no eye contact	3
Posture	Sitting out in chair or up in bed, holding appropriate posture	0
	Slumped in chair/bed but attempts to sit upright & sustain posture on request	1
	Slumped in chair or bed and unable to sustain posture	2
	Lying in bed and unable or no response to request to sustain posture	3
Movement	Moves spontaneously and purposefully, no restless or agitated movements	0
	Occasional or mild restless or fidgety movements, no aggressive or vigorous movements	1
		1
	Reduced frequency of movement, mildly slowed up	2
	Frequent restless or fidgety movements, no aggressive or vigorous movements	2
	Moderately ↓ frequency & speed of movement, interferes with assessment or self-care	3
	Aggressive or vigorous, recent pulling out of lines	4
	Overtly combative, violent	4
	Severely ↓ frequency & speed of movement, few spontaneous movements	
Score range 0-15, 0 represents a normal level of arousal		

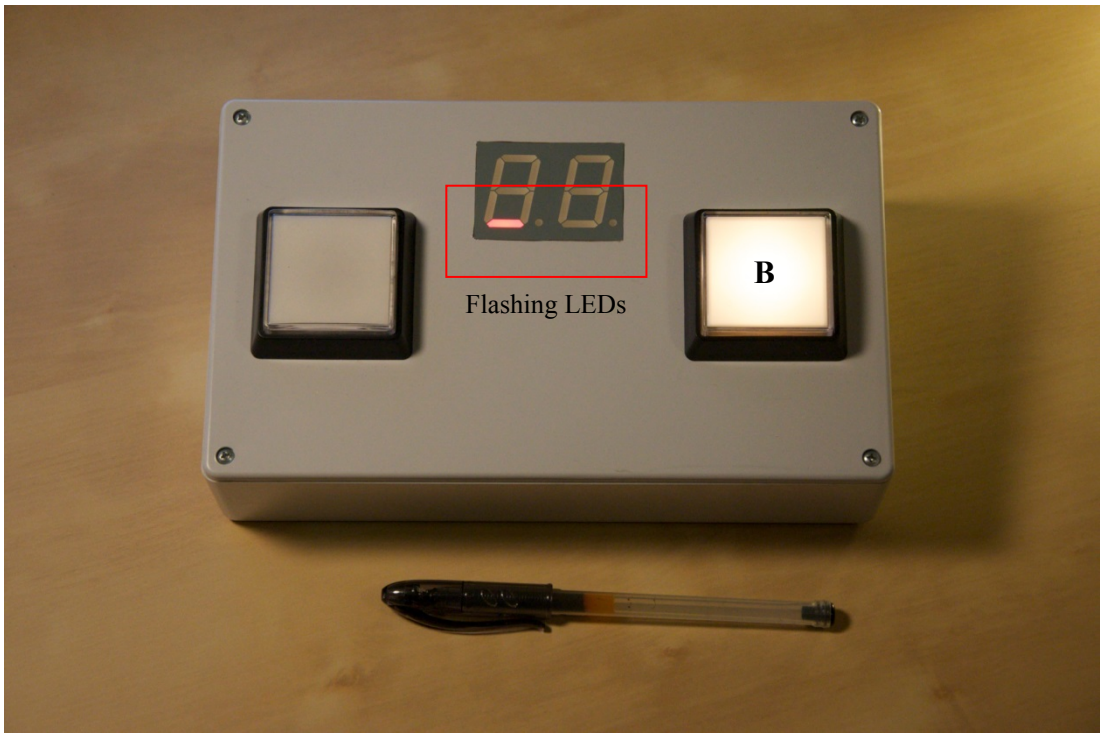
2.3.5 Assessment of attention

2.3.5.1 Edinburgh Delirium Test Box 1

The Edinburgh Delirium Test Box 1 (EDTB 1; figure 2.1) task 'focussed vigilance with distraction' was included as an objective test of attention (Brown, Fordyce et

Cortisol and inflammation in delirium and cognitive decline after hip fracture al. 2010). The participant is shown the test box, and directed to a large white button (button B); they are told that the button will light up, and asked to count how many times it lights up. They are told that there will be some red flashing lights on the central panel, and told to ignore them. The participant is then shown a series of white lights on the button B, of varying duration and frequency, and the number of lights they count is noted down. The task includes six trials. The first three are relatively short and simple, and the second three are more taxing. The task is scored from 0-6 according to whether or not the participant gives the right answer for the number of lights counted on button B.

Figure 2.1 - Edinburgh Delirium Test Box 1



2.3.6 Baseline information

Background medical information was collected from the participant's casenotes. This included date, type and side of fracture, date of admission and type of operation planned, past medical history, drug history on admission and changes made, baseline observations and examination findings, details of any preoperative acute medical problems and their treatment, social history including smoking and alcohol intake, type of accommodation, details of package of care, baseline mobility, previous occupation and years in education, baseline routine blood test results, electrocardiograph results and chest x-ray results. This information was also used to complete the Rockwood Brief Frailty Score (Rockwood, Stadnyk et al. 1999), the APACHE II Score (Acute Physiology and Chronic Health Evaluation II) (Knaus, Draper et al. 1985) and the Charlson Co-morbidity Index (Charlson, Pompei et al. 1987) (Appendix 1)

2.3.6.1 Rockwood Brief Frailty Score

The concept of frailty as a syndrome combining age, co-morbid illness, function and other factors such as nutrition and fitness, has grown significantly in recent years. A brief frailty score was used here, from Rockwood *et al* who have contributed significantly to the frailty literature (Rockwood, Stadnyk et al. 1999). The scale, based on the Geriatric Status Scale, classifies patients into one of four categories, from fitness to frailty; (0) those who walk independently, are independent in personal ADLs, are continent and not cognitively impaired, (1) bladder incontinence only, (2) one (or two if incontinent) of needing assistance with mobility or ADLs, cognitive impairment but not dementia, or bladder or bowel incontinence, and (3) two (three if incontinent) of totally dependent for transfers or one or more ADLs, double incontinence and dementia. Rockwood *et al* found that the scale had a dose response relationship between the categories of frailty and subsequent institutionalisation in a longitudinal study (Rockwood, Stadnyk et al. 1999). It is relatively quick and simple to complete.

2.3.6.2 Acute Physiology and Chronic Health Evaluation II Score

The APACHE II score was used here as a measure of illness severity. It uses basic physiological parameters and the presence of chronic co-morbidities to stratify acutely ill patients according to their risk of death from their current illness, and is commonly used for prognostic purposes in ICU (Knaus, Draper et al. 1985). A modified version of the APACHE II score was used which did not include arterial pH, since arterial blood sampling is not routine preoperatively in patients with hip fracture. There is a precedent for this small change in the delirium literature (Adamis, Treloar et al. 2007).

2.3.6.3 The Charlson Co-morbidity Index

The Charlson Co-morbidity Index (CCI) is a widely used measure of co-morbid disease burden (Charlson, Pompei et al. 1987). It was designed for use in longitudinal studies, to give prognostic information in order to control for the influence of co-morbid illness on outcomes. It gives a weighted score to different diagnoses, based on the influence each is likely to have on survival. For example, peripheral vascular disease scores 1, and a metastatic solid tumour scores 6. The scores are added up for each patient's co-morbid diseases. However, the CCI was initially developed in the 1980s and further validated in the 1990s (Charlson, Szatrowski et al. 1994), and specific treatment for some of the diseases has changed significantly since then. For example, ulcer disease and myocardial infarction have had their treatments revolutionised by widespread proton pump inhibitor use, H. Pylori eradication and primary percutaneous coronary intervention, and they score 1 point which is the same as dementia. AIDS scores 6 points, although the advances in retroviral therapy mean HIV disease has a much better prognosis than 30 years ago.

2.4 Measures of cortisol, inflammation and CSF biomarkers

2.4.1 Salivary cortisol

Diurnal salivary cortisol samples were collected using a Salivette® (Sarstedt, Germany) between 08.00-10.00 and 15.00-17.00. This involved the participant chewing on a small sterile cotton swab for 2-3 minutes until it was moist, which was then replaced in a sterile plastic container. Samples were collected preoperatively, and on days 4, 7 and 10-14 postoperatively. Diurnal saliva samples were also collected at each follow-up visit at 3, 6 and 12 months.

2.4.2 Cerebrospinal fluid and baseline serum sample

At spinal anaesthetic, the anaesthetist withdrew 2-3mls of CSF into a polypropylene universal container once the spinal needle was in position and before the administration of anaesthetic agent intrathecally. A paired venous blood sample was taken at the same time. If the participant had a general anaesthetic for medical reasons or by choice, a blood sample only was collected. Once these samples were ready, I was contacted and collected the samples on ice as quickly as possible, and processed them for storage. Samples were usually processed within one hour of collection, with the maximum time being approximately two hours.

2.4.3 Serum for morning cortisol and inflammatory cytokines

Further venous blood samples for cortisol and inflammatory markers were collected between 08.00-10.00 on Day 4 and the visit between Days 10-14. Morning blood samples were also collected at each follow-up visit.

2.5 Sample processing

2.5.1 CSF

CSF samples were centrifuged at 1000g for 10 minutes at 4ºC. The supernatant was stored in 100µl aliquots in labelled polypropylene eppendorfs at -80°C before analysis.

To check for blood contamination in the CSF, the pellet was resuspended in 100µl phosphate buffer saline followed by a cytospin. To perform the cytospin, a glass-slide was pre-labelled with a pencil. The glass-slide was placed in a cytospin-holder and a white filter paper placed on top, followed by a plastic funnel. The 100µl resuspended pellet was pipetted into the cytospin funnel. The cells were centrifuged at 300g for 3 minutes in a Shandon Cytospin II (Shandon, UK). The holder was carefully opened, the funnel and filter paper removed. The cells were air-dryed for a few minutes and then fixed with 100% methanol for 2 minutes at room temperature, followed by staining with Diff-quik Red solution (Gamidor Ltd, UK), for 1 minute at room temperature and then staining with Diff-quik Blue solution (Gamidor Ltd, UK), for 1 minute at room temperature. The slides were rinsed under running distilled-water, left to air-dry and then cover-slipped and mounted.

2.5.2 Blood

All blood samples were centrifuged at 3000g for 10 minutes at 4ºC. The serum was stored in 500µl aliquots in labelled polypropylene eppendorfs at -80ºC before analysis.

2.5.3 Saliva

The Salivette® was centrifuged at 1000g for 2 minutes and the supernatant frozen in a new polypropylene eppendorf at -80ºC.

2.6 Perioperative Assessments

Participants were interviewed, if they felt well enough and capable of interaction, on Days 1, 2, 3, 4, 7, and once between Days 10-14 postoperatively or until transfer or discharge from the ward. Each assessment lasted around 10-15 minutes and consisted of a semi-structured interview which allowed the completion of the CAM, DRS-R98, MMSE, RASS and OSLA, and a brief test of attention using the EDTB-1. I discussed any symptoms or signs of delirium with nursing staff. If the participant could not be interviewed, I informed nursing or medical staff of anything of concern and performed the delirium review by completing those assessments which could be performed through observation only. I reviewed the casenotes and recorded any medication changes, complications, physiological changes and most recent observations. I recorded delirium status and MMSE results in the casenotes for the benefit of the medical team.

During the perioperative period, I also completed tests of premorbid intelligence (the National Adult Reading Test (NART) (Nelson 1982)) and met with the participant's next of kin or nominated family member or friend to complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE). These measures were taken in order to gain as good a picture as possible of premorbid cognitive ability or impairment. The Katz and Lawton scales of activities of daily living are completed with information from the patient and / or their next of kin (Katz, Ford et al. 1963, Lawton and Brody 1969) to gain baseline information on functional level (Appendix 1). Length of hospital stay and destination from the trauma ward (i.e. home, orthogeriatric rehabilitation, death) were also recorded.

2.6.1 The National Adult Reading Test

The NART (Nelson 1982) is a test in which the subject is asked to pronounce a series of irregularly spelt words such as "chord", "subtle" and "labile". This suggests the subject is familiar with the word and indicates vocabulary level. Performance on the NART correlates highly with full scale IQ as measured by the Wechsler Adult

Intelligence Scale (Lezak 1995). The main use of the NART is to estimate 'premorbid' IQ, as scores correlate highly with IQ scores in childhood and adulthood (Crawford, Deary et al. 2001). The NART has also been shown to be stable in delirium in a population of patients post-CABG (Brown, Ferner et al. 2011).

2.6.2 The Informant Questionnaire on Cognitive Decline in the Elderly

The IQCODE is a widely used and well-validated questionnaire used to screen for and aid diagnosis of dementia, with other cognitive testing (Jorm 2004). It asks the informant to consider whether there has been any change in the past ten years in the subject's ability regarding a number of situations where they use their memory or intelligence, such as recalling conversations, learning how to use new things and handling money. The informant then indicates whether there has been "no change" or whether they are "a bit" worse or improved, or "much" worse or improved. An average score of 3.00 indicates no change in cognition. Studies of use of the IQCODE in medical hospital in-patients have established a cut off for evidence of prior cognitive impairment of 3.44 (Harwood, Hope et al. 1997). In a large (total N=657 for MCI group) Chinese study, a cut-off for mild cognitive impairment and for mild cognitive impairment without functional impairment of 3.19 (Li, Jia et al. 2012) has also been established.

2.6.3 Katz scale of activities of daily living

The Katz index of independence in activities of daily living (ADLs) is a widely used scale of a person's functional status, which was developed in the 1960s (Katz, Ford et al. 1963). It focuses on basic ADLs; bathing, dressing, toileting, transfers, continence and feeding (Appendix 5). It can be scored 0-6, with the person scoring one point for independence in each domain of function, 0 being dependent and 6 being independent. It can also be scored in a hierarchical sense from A to G, depending on which functions the person has become dependent in, A being independent in all functions and G being dependent in all six functions, since Katz *et al* found that independence was usually lost in a predictable order, first in bathing,

Cortisol and inflammation in delirium and cognitive decline after hip fracture then dressing etc. (Katz, Ford et al. 1963). In this study, the numerical scoring system was used.

2.6.4 Lawton scale of activities of daily living

The Lawton scale of instrumental activities of daily living assesses the person's ability to perform more complex independent living tasks such as shopping, housekeeping and managing finances (Appendix 5) (Lawton and Brody 1969). It is scored 0-8 based on the person's independence in the eight domains tested. The scale was devised in 1969, and traditionally women were scored 0-8 and men 0-5, omitting food preparation, housekeeping and laundry. In this study, men and women were all scored 0-8.

2.7 Follow-up Assessments at 3, 6 and 12 months

I first contacted the participant's General Practitioner, to check on their health status and confirm that it was still appropriate to contact the participant. I then telephoned the participant and arranged a date and time in the morning for the visit, with the participant's choice of a home visit or a visit to the Clinical Research Facility (CRF), NRIE with travel via taxi arranged. I explained what the visit would entail and asked the participant to fast on the morning of the visit prior to the blood test. I sent a letter confirming these details, and telephoned the participant 1-2 days prior to the visit for a final check.

The follow-up visit lasted 60-80 minutes. I first collected blood samples for fasting cortisol and inflammatory markers, and a saliva sample for cortisol. Once the participant had had some breakfast, I recorded information on medication changes, new medical history, current mobility and completed the Katz and Lawton functional scales. A delirium assessment was performed (a semi-structured interview taking 10-15 minutes which allows completion of the CAM, DRS-R-98, OSLA and RASS, the MMSE and EDTB-1 assessment).

The cognitive test battery was then completed, paying particular attention to participant fatigue, and this was cut short if the participant tired. The battery took 30-40 minutes to complete and included Verbal Fluency, Visual Reproduction Recall, Delayed Recall and Copy from the Wechsler Memory Scale III, HVLT-R list learning, Victoria Stroop, Digit Span Forwards and Backwards and the Digit Symbol Substitution Test. The battery was chosen to provide a broad but relatively brief assessment of cognition, with particular attention to executive function. Finally, the 15 item Geriatric Depression Scale, a widely-used screening tool for depression (Brown, Woods et al. 2007), the EQ-5D quality of life scale, a very brief but increasingly widely used instrument which has performed well in elderly populations (Brazier, Walters et al. 1996, Tidermark, Bergstrom et al. 2003) and Apathy Evaluation Scale, Clinician Version, the most widely-used and best validated

Cortisol and inflammation in delirium and cognitive decline after hip fracture assessment for apathy (Clarke, Van Reekum et al. 2007), were completed (Appendix 1).

2.7.1 Summary of cognitive test battery

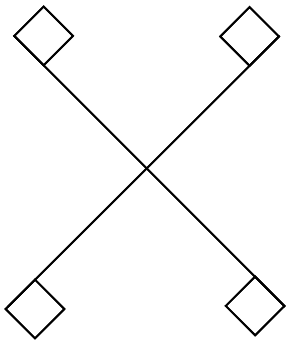
2.7.1.1 Verbal Fluency

The most commonly-used test of verbal fluency is the Controlled Oral Word Association Test (Lezak 1995). The examiner speaks out loud a letter from the alphabet and the subject is instructed to speak out loud as many words beginning with that letter as possible in one minute, excluding numbers, proper nouns and the same word with different suffixes. In this study, the letters F, A and S were used, followed by the number of animals the subject could name in one minute. This test has been shown to be sensitive to damage to the frontal lobe and is often considered a test of 'executive' or 'frontal lobe' function. Performance on the Controlled Word Association Test has been shown to decline with ageing and in dementia (Lezak 1995).

2.7.1.2 Visual Reproduction: Recall, Delayed Recall and Copy

This test is part of the Wechsler Memory Scale – Revised (Wechsler 1987). In this test the subject is shown a series of five geometric designs of increasing complexity. An example is shown in Figure 2.2. The participant is given five seconds to look at one design, then this is hidden and the subject is asked to draw the design. Thirty minutes later, the test is repeated and the participant is asked to draw as many of the designs as they can remember, in any order, without having been warned that they will be asked to reproduce the designs during the first part of the test. They are then shown the designs again and asked to copy them. The participant is given as much time as they need to copy the designs. Performance on Visual Reproduction declines with age and with dementia (Bak and Greene 1981, Wilson, Bacon et al. 1983, Lezak 1995).

Figure 2.2: Sample item from Visual Reproduction



2.7.1.3 HVLT-R list learning

The HVLT-R list learning test is a test of verbal learning and memory (Strauss, Sherman et al. 2006). The participant is told that they will be read a list of words, and they are asked to listen carefully because at the end they will be asked to tell the tester as many words as they can remember, in any order. The participant is then read a list of twelve words slowly and clearly. The words contain three groups of four related words e.g. emerald, sapphire, pearl and opal, with the three groups of words

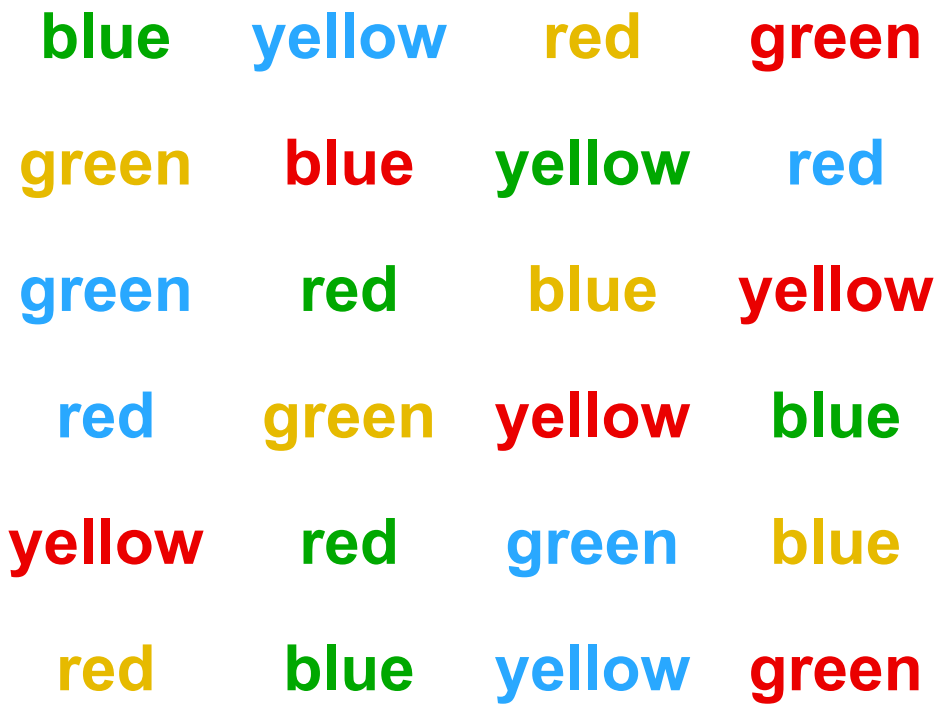
interspersed within the list. The words the participant can remember are noted down. There are two further trials in the same manner. The total recall score is the sum of the first three learning trials. To test delayed recall, without prior warning, 20-25 minutes later the participant is asked if they can remember the list of words they tried to remember before, and then asked to tell the tester as many words as they can remember. The participant is then read a longer list of words, some from the original list and some not, and asked to tell the tester whether or not each word was on the list. This longer list of words contains six semantically-related false positives (e.g. diamond, ruby) and six semantically-unrelated false positives (e.g. balloon, coffee). The number of total true positive answers (words from the original list correctly identified) minus the number of total false positive answers (both semantically related and unrelated) is termed the recognition discrimination index (RDI). Scores on the test deteriorate with dementia, in particular the number of false positives increases with even early Alzheimer's dementia due to decline in semantic memory, and it has been shown to be valid as a screening test for dementia (Strauss, Sherman et al. 2006).

2.7.1.4 Victoria Stroop

Stroop tests are tests of executive function, measuring selective attention and cognitive flexibility (Strauss, Sherman et al. 2006). The Victoria Stroop version is a commonly used, brief version of the task. The participant's colour vision is first checked by asking them to name the colours of four coloured dots. If they have no difficulty distinguishing the colours, they go on to the main test, which has three parts. In the first part, they are asked to name the colours of a series of dots, as quickly as they can (part D). The time taken to complete the task is timed, and any errors noted. The second part requires the participant to name the colour of a series of words as quickly as they can (part W), and again the time taken and any errors are noted. In the third part of the test, the participant is asked to name the colour that a series of words is printed in, as quickly as they can (part C). In this part of the test, the words are colour words printed in a different colour (Figure 2.3). Again, the time taken and any errors are noted. If the participant spontaneously corrects a mistake it

Cortisol and inflammation in delirium and cognitive decline after hip fracture is scored as correct. The difference score was calculated, which is the difference between the time taken to complete the interference card (part C) compared to the colour card (part D). This score increases with age, and there is a modest relation with educational level (Strauss, Sherman et al. 2006). The interference score was also calculated, which is the difference score divided by the time taken to complete the colour card.

Figure 2.3 Victoria Stroop Colours



2.7.1.5 Digit Span Forwards and Backwards

Digit span is a brief, simple test of attention span. The participant is read strings of digits, beginning with two digits, at a rate of one per second, and with a drop in pitch on the last digit. For digit span forwards, the participant must then repeat them back in turn, and for digit span backwards they must correctly reverse the string of numbers. Both tests are halted when the patient fails to correctly repeat or reverse the

same length digit string on two occasions. The test is scored according to the total number of correct answers and the longest digit string correctly repeated. Digit span backwards has been shown to have reasonable sensitivity and specificity for delirium versus dementia and cognitively unimpaired controls (O'Keeffe and Devlin 1994). Digit span has also been used to monitor delirium over time (Christensen, Bettin et al. 1996, Bettin, Maletta et al. 1998), and scores tend to improve, possibly reflecting the transient nature of attentional impairment in delirium, or possibly a learning effect.

2.7.1.6 Digit Symbol Substitution Test

The Digit Symbol Substitution Test (DSST) is part of the Wechsler Adult Intelligence Scale-Revised (Wechsler 1981). It was used as an indicator of attention and processing speed. Subjects are instructed to draw in symbols in blank boxes under numbers according to a code given at the top of the answer sheet. The score was the number of correct substitutions in 90 seconds. The DSST is highly sensitive to age (Wechsler 1981), showing declines from the age of 20, and also shows decline in dementia (Lezak 1995).

2.7.2 Geriatric Depression Scale

The Geriatric Depression Scale (Appendix 6) is a widely used screening questionnaire for depression in older adults. The 15-item short form was used. The patient is asked a series of questions about their mood and spirits, and asked to give a "yes" or "no" answer based on their experiences over the past week. A score of >5 positive answers is suggestive of depression, and a score of >10 is almost always indicative of depression.

2.7.3 EQ-5D

The EQ-5D (Appendix 7) is a short and simple questionnaire assessment of health status (Cheung, Oemar et al. 2009). There are two parts, the descriptive system and visual analogue scale. The descriptive system has five dimensions; mobility, self

care, usual activities, pain/discomfort and anxiety/depression. The respondent is asked to rate their health state according to three levels; no problems, some problems or severe problems. Each response corresponds to a single digit answer (1-3) which leads to a composite five digit number, e.g. 21121, which represents the participant's health state. The visual analogue scale asks the participant to indicate how good or bad their health is that day, with 0 being the worst state they can imagine and 100 being the best they can imagine, by drawing a line to the scale. This instrument is brief and easy to use in elderly patients, and has been shown to be responsive to change in patients with hip fracture and suitable for use in assessing quality of life outcomes in this population (Tidermark, Bergstrom et al. 2003).

At the end of the visit, I arranged a time to return to collect an afternoon saliva sample for cortisol. If the patient was well and mobile enough, they were provided with a Salivette®, instructions for taking the sample and a labelled Royal Mail Safebox™ to post the Salivette® to the CRF.

2.8 Ethical approval

The study was reviewed and approved by the Scotland A Research Ethics Committee, reference number 09/MRE00/28, and by NHS Research and Development, reference number 2009/R/ME/02.

2.9 Cerebrospinal fluid assays

2.9.1 Cortisol

Cortisol was measured in CSF with a commercially available high-sensitivity cortisol Enzyme-Linked Immunosorbent Assay (ELISA) (Salimetrics, Pennsylvania, USA). This ELISA is designed to measure cortisol in saliva, but was chosen as its detectable range for cortisol is low and in line with expected CSF values, and previous in-house experience was that it reliably detected cortisol in CSF samples in a pilot study (Pearson, de Vries et al. 2010). Samples were stored at -80°C in 100µl aliquots until the assay. The assay procedure is as described for the salivary cortisol section. CSF samples were not diluted and were assayed in singlicate.

2.9.2 Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone Sulphate (DHEAS)

Dehydroepiandrosterone and Dehydroepiandrosterone Sulphate were measured in CSF with separate commercially available Enzyme-Linked Immunosorbent Assays (ELISA) (Salimetrics, Pennsylvania, USA). These ELISAs were designed to measure DHEA and DHEAS in saliva, but they were chosen as their detectable range for both neurosteroids was low and in line with expected CSF values according to the CSF literature. Samples were stored at -80°C in 100µl aliquots until the assay. CSF samples were not diluted and were assayed in singlicate.

2.9.2.1 DHEA assay protocol

All reagents were brought to room temperature. A plate was provided pre-coated with polyclonal anti-DHEA antibodies. Standards were serially diluted from the 1000pg/ml DHEA concentrate to give a standard curve of 1000, 400, 160, 64, 25.6 and 10.2pg/ml. Fifty microlitres of standards, high and low controls and unknown samples were pipetted into the 96-well plate provided, and assay diluent into “blank” and “non-specific binding” wells. A 1:1500 dilution of the conjugate was prepared,

and 150µl immediately pipetted into each well. The plate was mixed for 5 minutes at 500rpm and incubated at room temperature for 3 hours. The plate was washed 4 times with wash buffer using a plate washer. Two-hundred microlitres of tetramethylbenzidine solution was added to each well. The plate was mixed for 5 minutes at 500rpm and incubated in the dark at room temperature for a further 25 minutes. Fiftymicrolitres of stop solution (sulphuric acid) was added to each well and the plate was mixed for a further 3 minutes at 500rpm. The plate was read on a plate reader at 450nm within 10 minutes of adding stop solution. Manufacturer reported sensitivity suggests a minimum detectable concentration of 5pg/ml. Intra-assay precision as expressed by % coefficient of variation (C.V.) is 5.3% and 5.8%, and inter-assay precision is 7.9% and 8.5%. The manufacturers report a 0.063% cross-reactivity with DHEAS and 0.0378% with androstenedione, but <0.004% with the other steroids tested.

2.9.2.2 DHEAS assay protocol

All reagents were brought to room temperature. A plate was provided pre-coated with rabbit anti-DHEAS antibodies. Standards were serially diluted from the 15300pg/ml DHEAS concentrate to give a standard curve of 15300, 5100, 1700, 566.7 and 188.9pg/ml. One hundred microlitres of standards, high and low controls and unknown samples were pipetted into the 96-well plate provided, and assay diluent into “blank” and “non-specific binding” wells. A 1:225 dilution of the conjugate was prepared, and 150µl immediately pipetted into each well. The plate was incubated at room temperature for 1 hour, constantly mixed at 500rpm on a plate shaker. The plate was washed 4 times with wash buffer using a plate washer. Two-hundred microlitres of tetramethylbenzidine solution was added to each well. The plate was mixed for 5 minutes at 500rpm and incubated in the dark at room temperature for a further 25 minutes. Fifty microlitres of stop solution (sulphuric acid) was added to each well and the plate was mixed for a further 3 minutes at 500rpm. The plate was read on a plate reader at 450nm within 10 minutes of adding stop solution. Manufacturer reported sensitivity suggests a minimum detectable concentration of <43pg/ml. Intra-assay precision as expressed by % coefficient of

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variation (C.V.) is 5.7% and 8.8%, and inter-assay precision is 7.4% and 7.7%. The manufacturers report a 0.0844% cross-reactivity with androstenedione and 0.0268% with transandrosterone, but <0.004% with the other steroids tested.

2.9.3 Inflammatory human cytokine panel

A high sensitivity Milliplex MAP human cytokine assay (HSCYTO 13-plex, Merck Millipore) was performed using a Luminex 200 reader (Biorad). This measured a panel of 13 cytokines, comprising IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, Interferon (IFN)-γ, Granulocyte macrophage colony stimulating factor (GM-CSF) and Tumour Necrosis Factor (TNF)-α. Seventy-six patients were age-matched, 50% who developed delirium at any stage during the perioperative period, and 50% who did not. Samples were from the Edinburgh CDHIP cohort and the Oslo cohort. CSF samples were assayed in singlicate and were not diluted. All reagents were brought to room temperature before use. The high sensitivity human cytokine standard was reconstituted with deionised water, vortexed and left for 10-15 minutes before making up a set of working standards by serial dilution, at 2000, 400, 80, 16, 3.2, 0.64 and 0.13pg/ml. A filter-bottomed 96-well plate was prewet before use by pipetting 200µl of wash buffer into each well and mixing on a plate shaker for 10 minutes at room temperature. The wash buffer was removed by vacuum. The bead bottle containing pre-mixed antibody beads was sonicated for 30 seconds and then vortexed for 1 minute. Twenty-five microlitres of the premixed bead solution was added to each well, shaking the bead bottle intermittently. The liquid was removed from the wells by vacuum. Next, 50µl of standard or control was added to the wells, and 50µl of assay buffer to the 0pg/ml background wells and the sample wells. Fifty microlitres of serum matrix solution was added to the background, standards and control wells. The CSF samples were centrifuged and then 50µl added to the sample wells. The plate was sealed and covered with a lid, and incubated with agitation on a plate shaker overnight at 4°C. The fluid was removed from the wells by vacuum, and the plate washed twice with wash buffer and vacuum filtration. Fifty microlitres of detection antibodies was added to each well, and the plate was sealed, covered with a lid and incubated at room temperature with agitation on a plate shaker for 1 hour.

Fifty microlitres of streptovidin-phycoerythrin was added to each well. The plate was sealed and covered again and incubated at room temperature with agitation for 30 minutes. The contents of the wells was removed by vacuum, the wells washed twice with wash buffer, the beads resuspended with 100µl of Luminex sheath fluid and agitated on a plate shaker for 5 minutes. The plate was read on a Luminex 200 machine. Manufacturer reported sensitivity suggests a minimum detectable concentration for each cytokine as follows: IL-1β 0.06pg/ml, IL-2 0.16pg/ml, IL-4 0.13pg/ml, IL-5 0.01pg/ml, IL-6 0.10pg/ml, IL-7 0.12pg/ml, IL-8 0.11pg/ml, IL-10 0.15pg/ml, IL-12(p70) 0.11pg/ml, IL-13 0.48pg/ml, IFNγ 0.29pg/ml, GM-CSF 0.46pg/ml and TNF-α 0.05pg/ml. Intra-assay precision, expressed as % C.V. was as follows: IL-1β 3.11%, IL-2 4.27%, IL-4 4.16%, IL-5 4.50%, IL-6 3.51%, IL-7 4.75%, IL-8 3.26%, IL-10 3.31%, IL-12(p70) 4.31%, IL-13 5.86%, IFN-γ 4.88%, GM-CSF 4.14% and TNF-α 3.49%. Inter-assay prescision was as follows: : IL-1β 2.16%, IL-2 7.48%, IL-4 9.12%, IL-5 14.27%, IL-6 4.48%, IL-7 6.24%, IL-8 6.48%, IL-10 11.84%, IL-12(p70) 5.08%, IL-13 11.99%, IFN-γ 7.79%, GM-CSF 5.03% and TNF-α 3.78% The manufacturers report no or negligible cross-reactivity between any other analytes.

2.9.4 S100B

Levels of S100B in CSF were measured using an in-house Enzyme-Linked Immunosorbent Assay (Green, Keir et al. 1997), at the National CJD Surveillance Unit, Western General Hospital, Edinburgh, by Alison Green and Mary Andrews. Assays were performed, in batches, within 6 months of sample collection to minimise marker degradation in storage and maintain uniformity of analysis. Sensitivity was 0.04ng/ml. The mean intra-assay precision of the S100B assay is 9.3% C.V. and 5.6% C.V., and inter-assay precision is 8.9% C.V. and 8.1% C.V. Cross-reactivity with S100A was less than 0.006% (Green, Keir et al. 1997).

2.9.5 Tau

Levels of tau (total) in CSF were measured using a commercially-available Enzyme-Linked Immunosorbent Assay (Life Technologies, Paisley, UK) at the National CJD

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2.10 Serum assays

2.10.1 Cortisol

Serum samples were thawed once and assayed in batches by me using a commercially available cortisol ELISA (MP Biomedicals, Germany). All reagents were brought to room temperature before use. Cortisol standards were provided at concentrations of 50, 20, 10, 4, 1 and 0μg/dl. Twenty-five microlitres of cortisol standard, control or unknown sample were pipetted into a 96-well streptavidin coated plate. Working cortisol enzyme reagent was made up by adding 0.7ml of cortisol enzyme reagent (a cortisol-horseradish peroxides conjugate in a protein-stabilising matrix with green dye) to a 7ml vial of steroid conjugate buffer. Next, 50μl of working cortisol enzyme reagent was added to each well. The microplate was swirled gently for 20-30 seconds to mix. Fifty microlitres of cortisol biotin reagent (anti-cortisol biotinylated mIgG conjugate in buffer, green dye and reagent) were added to each well. The microplate was swirled gently for 20-30 seconds to mix again. The plate was covered and incubated at room temperature for 1 hour. The plate was washed with wash buffer 3 times on a plate washer. Working substrate solution was made up by combining equal quantities of tetramethylbenzidine in buffer and hydrogen peroxide in buffer. One-hundred microlitres of this working substrate solution was added to each well. The plate was not swirled or shaken, and was incubated at room temperature for 15 minutes. Finally, 50μl of stop solution (1N HCl) was added to each well, and the plate read on a plate reader at 450nm, within 30 minutes. Manufacturer reported sensitivity indicates a minimal concentration of cortisol that can be distinguished from 0 of 0.25μg/dL (6.9nM/L). Intra-assay precision for high concentration cortisol was 6.1% C.V., and for low concentration 8.2% C.V. Inter-assay precision for high concentration cortisol was 7.3% C.V. and for low concentration 9.7% C.V. The manufacturers report 0.23% cross-reactivity with cortisone and 0.18% with corticosterone.

2.10.2 Inflammatory cytokines

Serum samples were thawed once and assayed in batches using a commercially available Fluorokine Multi-Analyte Profiling "Luminex" assay (R&D, Abingdon). I performed a test assay and the full set of assays was performed at the SuRF facility in the Queen's Medical Research Institute, University of Edinburgh, by Dr Pam Brown and Linda Ferguson. The cytokines and chemokines measured in each Luminex assay were Interleukin (IL)-1β, IL-1ra, IL-5, IL-6, IL-8, IL-10, Macrophage Inflammatory Protein (MIP)-1a, MIP-1b, Monocyte Chemotactic Protein (MCP)-1 and Tumour Necrosis Factor (TNF)-α. The manufacturers recommend a 4 fold dilution of serum samples. We tested a series of dilutions from 1 to 8-fold, and chose to use a 4 fold dilution. Samples were diluted in calibrator diluent. All samples were assayed in duplicate.

First, the two standard cocktails were reconstituted with calibrator diluent, and left for 30 minutes with gentle agitation. A dilution series was constructed with an additional final dilution to aid detection of low levels of cytokines. A filter-bottomed 96-well microplate was pre-wet with wash buffer, and then the buffer removed through the plate by vacuum. Each cytokine or chemokine microparticle concentrate vial was centrifuged and then vortexed, and 50µl of each microparticle concentrate was added to a vial containing 5ml of diluent. This microparticle cocktail was vortexed and 50ul added to each microplate well. Next, 50µl of standard or diluted sample was added to each well, ensuring that the plate was pipetted within 15 minutes. The plate was covered and protected from light. It was incubated at room temperature for 3 hours on an orbital shaker at 500rpm. The liquid was then removed by vacuum, and the plate washed 3 times with wash buffer, vacuuming each time. The biotin antibody cocktail was centrifuged for 30 seconds at 1000g, and then gently vortexed. This was diluted by adding 50µl of the concentrate to the 5.5ml vial of biotin antibody diluent. Next, 50µl of the diluted antibody cocktail was added to each well. The plate was covered with a foil plate sealer and incubated at room temperature for one hour on the shaker. The plate was washed again with wash buffer 3 times. The Streptavidin-PE concentrate vial was centrifuged for 30 seconds

at 1000g and then vortexed, and diluted 100x by adding 55µl to 5.5ml of wash buffer. Fifty microlitres of the 1x Streptavidin PE was added to each well, the plate covered with a foil plate sealer and incubated for 30 minutes at room temperature on the shaker. The plate was washed again 3 times, and the microparticles resuspended with 100µl of wash buffer. The plate was incubated for 2 minutes on the shaker and then read on a Biorad analyser.

The plates were run in batches, with a standard curve on each plate. Due to occasional problems with the standard curves, the best set of standard curves for each batch of plates was applied to all plates for that batch. The manufacturers say that batches of plates can be run with only one set of standard curves on the first plate, however we chose to include a standard curve on each plate and chose the most accurate from each batch.

Manufacturer reported sensitivity suggests a mean minimum detectable concentration for each cytokine as follows: IL-1β 0.27pg/ml, IL-1ra 4.05pg/ml, IL-5 0.33pg/ml, IL-6 0.36pg/ml, IL-8 0.39pg/ml, IL-10 0.13pg/ml, MCP-1 0.16pg/ml, MIP-1α 8.11pg/ml, MIP-1β 0.44pg/ml and TNF-α 0.60pg/ml. Intra-assay precision, expressed as % C.V. was as follows: IL-1β 4.5-5.5%, IL-1ra 2.8-4.4%, IL-5 4.8-6.4%, IL-6 4.3-4.7%, IL-8 4.6-7.8%, IL-10 5.2-6.4%, MCP-1 3.8-6.4%, MIP-1α 10.8-15.2%, MIP-1β 4.3-6.0% and TNF-α 3.7-4.8%. Inter-assay prescision was as follows: IL-1β 7.4-10.0%, IL-1ra 6.6-10.9%, IL-5 4.5-9.5%, IL-6 5.9-8.8%, IL-8 11.6-18.7%, IL-10 7.3-10.1%, MCP-1 5.6-11.1%, MIP-1α 4.8-16.6%, MIP-1β 5.0-15.9% and TNF-α 6.4-7.0% The manufacturers report <0.5% cross-reactivity between any other analytes.

2.11 Salivary cortisol assays

Saliva samples were thawed once and assayed in batches by me using a commercially available high-sensitivity cortisol ELISA designed for saliva samples (Salimetrics). All reagents were brought to room temperature. A plate was provided pre-coated with monoclonal anti-cortisol antibodies. Ready to use cortisol standards were provided with the kit at concentrations of 82.77, 27.59, 9.19, 3.06, 1.02 and 0.33 nmol/L. Twenty-five microlitres of standards, high and low controls and unknown samples were pipetted into a 96-well plate, and assay diluent into "blank" and "non-specific binding" wells. A 1:1600 dilution of the conjugate was prepared, and 200µl immediately pipetted into each well. The plate was mixed for 5 minutes at 500rpm and incubated for a further 55 minutes. The plate was washed 4 times with wash buffer using a plate washer. Two-hundred microlitres of tetramethylbenzidine solution was added to each well. The plate was mixed for 5 minutes at 500rpm and incubated in the dark for a further 25 minutes at room temperature. Fifty-microlitres of stop solution (sulphuric acid) was added to each well and the plate was mixed for a further 3 minutes at 500rpm. The plate was read on a plate reader at 450nm within 10 minutes of adding stop solution. Manufacturer reported sensitivity indicates a minimal concentration of cortisol that can be distinguished from 0 of <0.003g/dL (<0.083nM/L). Intra-assay precision for high level cortisol concentrations was 3.35% C.V., and for low concentration 3.65% C.V. Inter-assay precision for high concentration cortisol was 3.75% C.V. and for low concentration 6.41% C.V. The manufacturers report 0.130% cross-reactivity with cortisone and 0.214% with corticosterone.

2.12 Statistical Analysis

Statistical analysis was performed by me using SPSS version 19.0, except for the logistic regression modelling in Chapter 7 which was performed by Dr Dan Davis, and the trajectory analysis in Chapter 9 which was performed by Dr Mike Allerhand. Specific analyses are detailed in each chapter.

3 Chapter 3: A systematic literature review of cerebrospinal fluid (CSF) biomarkers in delirium

3.1 Introduction

Delirium is a serious, acute neuropsychiatric syndrome affecting 11-42% of general medical inpatients (Siddiqi, House et al. 2006). The main features are acute onset and fluctuating course, inattention, altered level of arousal with psychomotor agitation or retardation, perceptual disturbance, and sleep-wake cycle disturbance (APA 2000, MacLullich, Ferguson et al. 2008, Young and Inouye 2008). Delirium is associated with increased morbidity and mortality, and a greater risk of long-term cognitive decline (MacLullich, Beaglehole et al. 2009, Witlox, Eurelings et al. 2010). However, despite its importance, the mechanisms of delirium are under-researched and remain largely obscure.

There are many hypotheses of the pathophysiology of delirium. These include:

i) Direct brain insults such as hypoxia, ischaemia, metabolic derangement, cerebral infarction (MacLullich, Ferguson et al. 2008).
ii) Neurotransmitter imbalance such as cholinergic deficiency or dopamine excess, which may be induced by several classes of drugs (e.g. anticholinergics, antidepressants, L-dopa) (Flacker and Lipsitz 1999).
iii) Dysregulation of the hypothalamic pituitary adrenal axis in the elderly leading to sustained high cortisol levels (Olsson 1999, MacLullich, Ferguson et al. 2008).
iv) Hyper-responsiveness of brain immune cells to stimulation from peripheral inflammation, leading to increased neuroinflammation and exaggerated sickness behaviour syndrome (MacLullich, Ferguson et al. 2008). These potential mechanisms are not mutually exclusive.

Whatever the peripheral or indeed central precipitants are for delirium, understanding the central nervous system (CNS) changes associated with the syndrome is crucial. Examining cerebrospinal fluid (CSF) for evidence of these changes is potentially

Cortisol and inflammation in delirium and cognitive decline after hip fracture highly informative. However, there are obvious ethical and practical difficulties in obtaining CSF specimens in patients with delirium and such studies remain relatively rare compared with other CNS disorders of comparable prevalence and severity. In recent years there has been a blossoming of studies examining CSF in delirium, often collecting CSF using the excellent opportunity afforded by spinal anaesthetic administration for high risk surgical procedures.

This systematic literature review asked the following research questions::

1) What is the current published literature examining CSF in delirium?

2) What does this tell us about delirium pathophysiology?

3) What would be a rational basis for future studies?

This systematic literature review was originally published in Dementia and Geriatric Cognitive Disorders in 2011 (Hall, Shenkin et al. 2011), and has been updated for the purposes of this thesis. Permission to reproduce it in this thesis is included in Appendix 8.

3.2 Methods

Because all studies were observational in design I devised a protocol based on the MOOSE (Meta-analysis Of Observational Studies in Epidemiology) (Stroup, Berlin et al. 2000) guidelines.

3.2.1 Study identification

Two reviewers (RJH and SDS) sought studies published to the 5th of January 2015 that examined CSF in delirium. Studies were identified using a comprehensive textword and MeSH-based electronic search of MEDLINE, EMBASE, PsycINFO, Web of Science (including conference proceedings), PubMed and the Evidence Based Medicine reviews database (including the Cochrane database of systematic reviews and the Cochrane central register of controlled trials). We used terms including Delirium or Acute Confusional State, and Cerebrospinal Fluid or Lumbar Puncture. For the full search strategy see Appendix 2. A Google™ search was also performed using the terms "CSF" and "delirium". Additional search terms which encompassed the main families of biomarkers were also included in the searches in MEDLINE and EMBASE but these retrieved very few additional citations, none of which were relevant, and therefore the simpler strategy was used. Additionally we hand searched the bibliographies of relevant articles (Stroup, Berlin et al. 2000), performed a forward citation search in Web of Science for all studies examined, and contacted experts in the field via the European Delirium Association.

3.2.2 Study selection

Inclusion criteria were (i) that delirium was diagnosed using one of the following diagnostic criteria for delirium or acute confusional state: *Diagnostic and Statistical Manual of Mental Disorders (DSM Third Edition; DSM Third Edition Revised;* or *DSM Fourth Edition)* (APA 2000), *International Classification of Diseases* (1992) or validated measures based on these criteria such as the Confusion Assessment Method (Inouye, Vandyck et al. 1990), (ii) that we could extract data on biomarker findings in CSF in delirium and (iii) studies investigating CSF in neuropsychiatric systemic lupus erythematosus (SLE) were included only where delirium was thought to be

Cortisol and inflammation in delirium and cognitive decline after hip fracture secondary to a cause other than neuropsychiatric SLE. Exclusion criteria were (i) case reports, (ii) studies involving delirium tremens or hepatic encephalopathy, or where neuropsychiatric SLE was the primary cause of delirium, due to the specific aetiologies and clinical features of those conditions.

3.2.3 Data extraction and synthesis

RJH and SDS independently performed the search and extracted the data, which was checked by AMJM. We recorded (if available) (a) study design and provenance, (b) number and characteristics of patients and controls and comparability of patient and control groups, (c) method used to diagnose delirium, (d) delirium severity, (e) aetiology of delirium, (f) method of obtaining CSF (*i.e.* lumbar puncture or spinal anaesthetic), (g) biomarkers studied, (h) methods used for analysis, (i) main findings of the studies, with numerical data as far as possible, (j) statistical significance, (k) statistical methods, and (l) whether biomarker findings were predictive of prognosis or other outcomes.

3.2.4 Assessment of study quality

Two authors (RJH and SDS) independently assessed all manuscripts that met the inclusion criteria. There is no gold standard validated method for assessing the quality of observational studies (Sanderson, Tatt et al. 2007), but there is consensus that the three most fundamental domains are 1) appropriate selection of participants 2) appropriate measurement of variables and 3) appropriate control of confounding, as well as considering design specific biases. These quality criteria are discussed in relation to each study below.

3.3 Results

A total of 4,870 citations were identified (Appendix 3), and following review of the title and/or abstract 81 articles were retrieved for detailed analysis. Ninety-four further articles were retrieved by hand-searching of reference lists; many of these related to neuropsychiatric SLE and were not suitable for inclusion. One Japanese paper (Tanaka, Mitsushio et al. 1993) and related abstract (Tanaka 1993) were retrieved serendipitously from the bibliography of an unrelated delirium article. One hundred and seventy-seven articles were retrieved for detailed evaluation: One-hundred and fifty-eight were excluded (see Appendix 3). Eight of the remaining 19 articles report longitudinal data on four biomarkers examined in one study population (Koponen, Stenback et al. 1989, Koponen, Stenback et al. 1989, Koponen, Reinikainen et al. 1990, Koponen and Riekkinen 1990, Koponen, Sirvio et al. 1991, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994). We have included the four articles that report the most complete data set for each biomarker (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994). Therefore 15 articles are discussed, reporting data from eight research groups; more recently, several of these studies have involved collaborations between these groups (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994, Ramirez-Bermudez, Ruiz-Chow et al. 2008, Caplan, Kvelde et al. 2010, Pearson, de Vries et al. 2010, MacLullich, Edelshain et al. 2011, Witlox, Kalisvaart et al. 2011, Hall, Ferguson et al. 2013, Westhoff, Witlox et al. 2013, Cape, Hall et al. 2014, Poljak, Hill et al. 2014, Watne, Hall et al. 2014, Xie, Swain et al. 2014)(Table 3.1-3.3).

3.3.1 Overview

The articles included were four papers reporting data from a prospective cohort study conducted by Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994), and 11 papers from seven other prospective cohort studies, many of them collaborative (Ramirez-Bermudez, Ruiz-Chow et al. 2008, Caplan, Kvelde et al. 2010, Pearson, de Vries et

Cortisol and inflammation in delirium and cognitive decline after hip fracture al. 2010, MacLullich, Edelshain et al. 2011, Witlox, Kalisvaart et al. 2011, Hall, Ferguson et al. 2013, Westhoff, Witlox et al. 2013, Cape, Hall et al. 2014, Poljak, Hill et al. 2014, Watne, Hall et al. 2014, Xie, Swain et al. 2014). A total of 353 patients with delirium and 463 controls were studied, although some are included in more than one study. Some studies used lumbar puncture to obtain CSF, but several of the more recent studies have collected CSF at the onset of spinal anaesthetic. Around 60 different biomarkers were studied, plus proteomics analysis has now been performed. Each of the articles relating to Koponen's study reported only one biomarker at various time-points. Most research groups focused on different biomarkers, but there is now some overlap for some biomarkers, and the differing results in individual studies are therefore discussed. Each unique result was considered in the context of the quality of that research study and the supporting evidence provided, and studies could not compared to each other for meta-analysis.

3.3.2 Additional information on individual studies

A narrative review outlining each study and summarising the main findings is presented below. Further detailed results are in Table 3.1-3.3.

Table 3.1 Baseline characteristics of included studies

Study / provenance	Design / setting	Biomarker(s) studied	Causes of delirium	Cases	Controls
Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994) Finland 1990, 1994	Prospective cohort study, with external control group Psychogeriatric Hospital	Somatostatin-like immunoreactivity (SLI), Beta Endorphin-like immunoreactivity, 5-Hydroxyindole-acetic acid (5-HIAA), Acetyl-cholinesterase (AChE)	Stroke (n=15), Infection (n=9), Metabolic (n=8), Epileptic fit (n=6), Medication (n=6), Life change in dementia (n=5), Myocardial infarction or ischaemia (n=4), Carcinoma (n=4), Functional psychosis (n=2), Subdural haematoma (n=2), Intracerebral haemorrhage (n=2), Trauma (n=2), Cerebral tumour (n=1), Aneurysm of basilar artery and hydrocephalus (n=1)	n=67 for SLI *(41.8%male)* n=69 for other biomarkers *(42% male)* n=56 at 2 weeks n=33 at 1 year n=16 at 4 years (11 LP)	n=13 *(38.5% male)* for SLI, 5-HIAA, AChE n=19 *(31.6% male)* for BLI
Ramirez-Bermudez *et al* (Ramirez-Bermudez, Ruiz-Chow et al. 2008) Mexico 2008	Prospective cohort study General Hospital	Homovanillic acid (HVA)	Primarily acute central nervous system (CNS) infection	n=31 *(54.9% male in whole group)*	n=20
Pearson *et al* (Pearson, de Vries et al. 2010) Scotland	Prospective cohort study General Hospital	Cortisol	Hip fracture and consequent surgery	n=7 *(25% male in whole group)*	n=13

2010					
Caplan *et al* (Caplan, Kvelde et al. 2010) Australia 2010	Prospective cohort study General Hospital	Lactate, Neuron-specific enolase (NSE), S100B, Glucose, Protein	Heterogeneous, acute medical illness	n=20 *(% male not stated)*	n=20
MacLullich *et al* (MacLullich, Edelshain et al. 2011) Scotland and the Netherlands 2011	Prospective cohort study General Hospital	TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12p70	Hip fracture and consequent surgery	n=15 *(% male not stated)*	n=21
Witlox *et al* (Witlox, Kalisvaart et al. 2011) The Netherlands 2011	Prospective cohort study nested within a randomised controlled trial General Hospital	β-amyloid1-42, tau, hyperphosphorylated tau	Hip fracture and consequent surgery	n=30 *(33% male)*	n=46
Westhoff *et al* (Westhoff, Witlox et al. 2013)	Prospective cohort study nested within a randomised controlled trial	Epidermal Growth Factor (EGF), eotaxin, fibroblast growth factor 2 (FGF-2), FMS-like tyrosine kinase 3 ligand (Flt-3L), Fractalkine,	Hip fracture and consequent surgery	n=23 *(31.1% male)*	n=38

The Netherlands 2013	General Hospital	granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), growth-related oncogene (GRO), Interferon(IFN)-α2, IFN-γ, IL-1α, IL-1β, IL-2, soluble IL-2 receptor-α, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8,IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, Interferon gamma-induced protein 10 (IP-10), monocyte chemotactic protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP) 1α and 1β, platelet-derived growth factors (PDGF) AA and AB/BB, regulated and normal T-cell expressed and secreted (RANTES), soluble CD40 ligand, transforming growth factor (TGF)-α, tumor necrosis factors (TNF) α and β, and vascular endothelial growth factor (VEGF)			
Hall *et al* (Hall, Ferguson et al. 2013) Scotland 2013	Prospective cohort study General Hospital	S100B	Hip fracture and consequent surgery	n=19 *(37% male)*	n=36
Watne *et al* (Watne, Hall et al. 2014)	Prospective cohort study, part of cohort nested within randomised	Anticholinergic activity	Hip fracture and consequent surgery	n=72 *n=52 Oslo (25% male)*	n=79 *n=47 Oslo*

Norway and Scotland 2014	controlled trial General Hospital			*n=20 Edinburgh (40% male)*	*n=32 Edinburgh*
Cape *et al* (Cape, Hall et al. 2014) Scotland and the Netherlands 2014	Prospective cohort study General Hospital	IL-1β, IL-1ra, IGF-1, Glial Fibrillary Acidic Protein (GFAP), IFN-γ	Hip fracture and consequent surgery	n=19 *(26% male)*	n=24
Xie *et al* (Xie, Swain et al. 2014) USA 2014	Prospective cohort study General Hospital	β-amyloid-40 β-amyloid-42 Total tau β-amyloid-40/tau ratio β-amyloid-42/tau ratio	Hip or knee arthroplasty surgery	n=31 *(% male not stated)*	n=122
Poljak *et al* (Poljak, Hill et al. 2014) Australia and Scotland 2014	Prospective cohort study General Hospital	Proteomics	Heterogeneous, acute medical illness (Australia) Hip fracture and consequent surgery (Scotland)	n=17 *(60% male)*	n=8 normal control n=17 Alzheimer's dementia

Table 3.2 Summary of results at baseline

Study	N delirious at LP	Main findings in delirious patients at baseline	Cases Mean (SD) or median (range) or *(IQR)*	Controls Mean (SD) or median (range) or *(IQR)*	*P value*
Koponen *et al* (Koponen, Leinonen et al. 1994)	67/67	↓ SLI in whole delirious group ↓ SLI in delirious group with no central nervous system (CNS) disease	26.2pg/ml (8.8) 25.9pg/ml (6.4)	37.6pg/ml (9.8) 37.6pg/ml (9.8)	<0.001 <0.001
Koponen *et al* (Koponen and Riekkinen 1990)	69/69	↓ BLI in whole delirious group ↓ BLI in delirious group with no CNS disease	12.5pg/ml (3.0) 12.4pg/ml (3.8)	15.2pg/ml (2.8) 15.2pg/ml (2.8)	<0.001 <0.05
Koponen *et al* (Koponen, Lepola et al. 1994)	69/69	↑ in 5-HIAA vs. controls in those with delirium and no CNS disease Association between 5-HIAA level and lifespan after delirium (proportional hazards modeling)	168.9nmol/L (56.9)	118.1nmol/L (34.9)	<0.05 0.007 $z = 2.93$
Koponen *et al* (Koponen, Sirvio et al. 1994)	69/69	↑ AChE in delirious patients who died during follow-up vs. rest of cohort ↑ AChE in those requiring prolonged institutional care Association between AChE and lifespan after delirium (proportional hazards modeling)	22.0nmol/ml/min (8.0) 21.0nmol/ml/min (5.9)	18.1nmol/ml/min (5.0) 17.8mnol/ml/min (4.5)	0.005 0.05 0.019 $z = 2.39$
Ramirez-Bermudez *et al*	Not stated; LP within 24	HVA not significantly different in delirium vs non-delirium	300.8nM (36-1915.7)	231.65nM (36-1062.3)	0.108

(Ramirez-Bermudez, Ruiz-Chow et al. 2008)	hrs of neuro-psychological assessment	HVA ↑ in those with: Hallucinations Delusions Antipsychotic treatment HVA ↓ with HIV infection Trend towards ↑ HVA with agitation	 436.54nM (36-1915.7) 490.5nM (36-1915.7) 436.54nM (233.34-1915.7) 118.72nM (36-496.85)	 192.39nM (36-1062.3) 157.4nM (36-1062.3) 192.39nM (36-1443.23) 314.25nM (36-1915.7)	 0.018 0.001 0.002 0.006 0.066 X^2 5.45
Pearson *et al* (Pearson, de Vries et al. 2010)	7/20	↑ CSF cortisol in delirium vs controls ↑ Plasma cortisol in delirium vs controls	63.9nmol/L *(40.4-102.1)* 968.8nmol/L *(886.2-1394.4)*	31.4nmol/L *(21.7-43.3)* 809.4nmol/L *(544.0-986.4)*	0.029 0.036
Caplan *et al* (Caplan, Kvelde et al. 2010)	20/20	In delirium vs. controls with Alzheimer's dementia: ↑ CSF lactate ↓ CSF NSE ↑ CSF protein S100B no different Higher CSF lactate in delirium subgroup who died in hospital vs. those discharged	 1.87mmol/L (0.31) 4.84ng/ml (2.02) 0.62g/L (0.33) 604.8pg/ml (163.0) 2.15mmol/L (0.17)	 1.48mmol/L (0.23) 8.98ng/ml (2.98) 0.44g/L (0.15) 697.4pg/ml (306.9) 1.77 mmol/L (0.26)	 <0.001 <0.001 0.036 0.33 0.029
MacLullich *et al* (MacLullich, Edelshain et al.	Not stated	↑ CSF IL-8 in delirium vs. Controls ↑ Serum IL-6 in delirium vs. controls TNF-α, IL-1β, IL-10 and IL-12p70 not detected	69.8pg/ml *(47.9-125.6)* 42.4pg/ml *(28.9-438.4)*	39.6pg/ml *(28.0-64.6)* 24.3pg/ml *(0-217.2)*	0.003 0.013

2011)					
Witlox *et al* (Witlox, Kalisvaart et al. 2011)	0/76	β-amyloid1-42 no different Tau no different Hyperphosphorylated tau no different	631.0pg/ml *(500.3-985.3)* 306.0pg/ml *(231.0-389.0)* 71.5pg/ml *(51.0-80.5)*	755.0pg/ml *(566.5-1030.8)* 324.5pg/ml *(245.3-522.5)* 70.0pg/ml *(58.5-96.0)*	0.21 0.75 0.55
Westhoff *et al* (Westhoff, Witlox et al. 2013)	0/61	↓ Flt-3L in delirium vs. controls G-CSF no different IFN-α2 no different IFN-γ no different IL-1α no different ↓ IL-1ra in delirium vs. controls ↓ IL-6 in delirium vs.controls IL-8 no different IL-15 no different IP-10 no different MCP-1 no different MCP-3 no different PDGF-AA no different RANTES no different	16.54pg/ml *(12.2-20.2)* 23.15pg/ml *(14.7-33.9)* 24.41pg/ml *(17.7-34.1)* 1.61pg/ml *(1.6-2.4)* 0.78pg/ml *(0.1-1.3)* 2.90pg/ml *(2.9-6.9)* 0.21pg/ml *(0.1-0.7)* 25.25pg/ml *(19.4-37.6)* 2.79pg/ml *(1.4-3.5)* 334.21pg/ml *(241.0-590.0)* 301.79pg/ml *(241.6-389.5)* 19.4pg/ml *(19.4-25.2)* 6.67pg/ml *(5.4-9.0)* 0.08pg/ml *(0.1-3.5)*	20.16pg/ml *(18.2-29.5)* 31.26pg/ml *(14.7-55.8)* 31.70pg/ml *(21.1-41.1)* 1.61pg/ml *(1.6-2.4)* 0.28pg/ml *(0.2-2.3)* 6.90pg/ml *(2.9-6.9)* 0.99pg/ml *(0.2-2.2)* 28.40pg/ml *(22.7-42.5)* 3.47pg/ml *(1.4-4.5)* 270.84pg/ml *(183.1-350.6)* 312.67pg/ml *(257.2-422.6)* 19.4pg/ml *(12.2-25.2)* 8.04pg/ml *(5.6-10.3)* 3.50pg/ml *(0.1-3.5)*	0.021 0.233 0.136 0.336 0.170 0.032 0.005 0.308 0.060 0.064 0.634 0.375 0.267 0.290

		sCD40-L no different TGF-α no different EGF, eotaxin, FGF-2, Fractalkine, GM-CSF, GRO, IL-1β, IL-2, soluble IL-2 receptor-α, IL-3, IL-4, IL-5, IL-7, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, MDC, MIP-1α, MIP-1β, PDGF AB/BB, TNF-α, TNF-β, and VEGF detected in <50% of CSF samples	0.06pg/ml *(0.06-1.1)* 1.99pg/ml *(1.4-2.6)*	1.14pg/ml *(0.06-1.1)* 1.99pg/ml *(1.4-3.2)*	0.263 0.411
Hall *et al* (Hall, Ferguson et al. 2013)	8/45	↑ LogS100B in preoperative delirium LogS100B no different in delirium at any stage	-0.156 (0.238) -0.239 (0.184)	-0.306 (0.162) -0.306 (0.184)	0.035 0.218
Watne *et al* (Watne, Hall et al. 2014)	38/151	Anticholinergic activity no different in Oslo cohort Anticholinergic activity no different in Edinburgh cohort	0.39pmol/mL *(0.11-0.82)* 0.36pmol/mL *(0.18-0.67)*	0.48pmol/mL *(0.24-0.98)* 0.31pmol/mL *(0.10-0.75)*	0.26 0.93
Cape *et al* (Cape, Hall et al. 2014)	8/43	CSF IL-1β higher in incident delirium vs. prevalent delirium vs. never delirium CSF IL-1ra higher in prevalent delirium vs. incident delirium vs. never delirium GFAP no different in delirium	Prev 0.84pg/ml *(0.49-1.57)* Inc 1.74pg/ml *(1.02-1.74)* Prev 70.75pg/ml *(65.63-73.01)* Inc 31.06pg/ml *(28.12-35.15)* Prev 0.81ng/ml *(0.33-1.31)* Inc 0.61ng/ml *(0.46-0.76)*	Never 0.66pg/ml *(0-1.02)* Never 33.98 pg/ml *(28.71-43.28)* Never 0.45ng/ml *(0.31-0.86)*	0.03 0.04 0.58

		IGF-1 and IFN-γ not detected			
Xie *et al* (Xie, Swain et al. 2014)	0/153	β-amyloid-40/tau ratio no different β-amyloid-42/tau ratio no different ↑ Delirium incidence in lowest quartile of Aβ40/tau ratio vs. other 3 quartiles ↑ Delirium incidence in lowest quartile of Aβ42/tau ratio vs. other 3 quartiles	12.2 *(8.1-14.8)* 1.3 *(0.7-1.9)* 32% 32%	12.6 *(9.6-16.1)* 1.4 *(1.0-2.1)* 17% 17%	0.241 0.192 0.048 0.048
Poljak *et al* (Poljak, Hill et al. 2014)	17/42	Apolipoproteins, chromogranins and secretogranins downregulated in delirium α-2-macroglobulin, fibrinogen, α-1-antitrypsin, α-1-antichymotrypsin, transferrin, complement component 3 and haptoglobin upregulated in delirium Upregulated α-1-glycoprotein in delirium, with ↑ levels in delirium vs. Alzheimer's dementia confirmed by ELISA	277.85μg/ml (19.51)	207.44μg/ml (22.81)	0.026

Table 3.3 Summary of results at follow-up

Studies: Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994) Biomarkers: Somatostatin-like immunoreactivity (SLI), Beta-endorphin-like immunoreactivity (BLI), 5-Hydroxyindoleacetic acid (5-HIAA), Acetylcholinesterase (AChE)					
Timepoint	N delirious at lumbar puncture	Main findings	Cases Mean (SD)	Controls Mean (SD)	*P value*
2 weeks	14/66	↓ SLI in whole delirious group	25.0pg/ml (9.1)	37.6pg/ml (9.8)	<0.001
		↓ SLI in delirious group with no central nervous system (CNS) disease	25.2pg/ml (6.8)	37.6pg/ml (9.8)	<0.01
		SLI unchanged in group with resolved delirium	25.4pg/ml (8.8)	25.3pg/ml (9.6)	NS
		↓ BLI in whole delirious group	12.1pg/ml (4.3)	15.2pg/ml (2.8)	<0.01
		BLI in delirious group with no CNS disease comparable with controls	14.4pg/ml (6.2)	15.2pg/ml (2.8)	NS
		BLI unchanged in group with resolved delirium	12.2pg/ml (3.5)	11.8pg/ml (3.3)	NS
	14/58	5-HIAA unchanged in group with resolved delirium	161.9nmol/L (121.2)	176.9nmol/L (100.0)	NS
	14/58	AChE unchanged in group with resolved delirium	21.8nmol/ml/min (8.0)	21.2 nmol/ml/min (6.2)	NS
1 year	0/33	↓ SLI in whole delirious group	24.7pg/ml (10.7)	37.6pg/ml (9.8)	<0.001
		SLI in delirious group with no CNS disease comparable with controls	41.6pg/ml (7.5)	37.6pg/ml (9.8)	NS

		SLI correlated with level of daily functioning ↓ BLI in whole delirious group BLI in delirious group with no CNS disease comparable with controls 5-HIAA and mini-mental state examination (MMSE) positively correlated	 10.8pg/ml (3.2) 13.4pg/ml (4.8) 	 15.2pg/ml (2.8) 15.2pg/ml (2.8) 	<0.01, r = -0.51 <0.001 NS <0.01 r = 0.43
4 years	0/11	↓ SLI in delirious group SLI correlated with level of daily functioning	22.9pg/ml (5.8)	37.6pg/ml (9.8)	<0.001 <0.01, r = -0.75

Table 3.4 Results for 5-Hydroxyindoleacetic acid (5-HIAA), Koponen *et al* (Koponen, Lepola et al. 1994)

Timepoint	Subgroup means for 5-HIAA, nmol/L mean (SD)				
	Multi-infarct dementia	Alzheimer's dementia	Parkinson's disease	No CNS disease	Whole group
Baseline	174.6 (87.7) N=39, p<0.05 vs. Controls	173.6 (238.0) N=14	150.1 (56.8) N=3	168.9 (56.9) N=13, p<0.05 vs. controls	172.3 (125.8) N=69
2 weeks	190.9 (137.4) N=36	101.6 (6.9) N=12	131.6 (31.3) N=3	154.6 (50.3) N=7	165.0 (117.1) N=58
1 year	146.4 (75.0) N=17	106.9 (46.8) N=8	145.3 (94.8) N=3	184.6 (84.6) N=5, p<0.05 vs. controls	142.5 (73.2) N=33

Control group: 5-HIAA = 118.1 nmol/L (SD 34.9), n=13

Table 3.5 Results for Acetylcholinesterase (AChE), Koponen *et al* (Koponen, Sirvio et al. 1994)

Timepoint	Subgroup means for AChE nmol/ml/min, mean (SD)				
	Multi-infarct dementia	Alzheimer's dementia	Parkinson's disease	No CNS disease	Whole group
Baseline	22.6 (8.2) N=39	21.1 (7.0) N=14	18.9 (2.1) N=3	17.9 (4.4) N=13, p<0.01 vs. controls	21.3 (7.4) N=69
2 weeks	21.9 (6.6) N=36	19.2 (6.3) N=12	17.8 (3.6) N=3	19.0 (3.8) N=7	20.8 (6.2) N=58
1 year	19.2 (5.8) N=17, p<0.05 vs. controls	17.7 (5.1) N=8, p<0.05 vs. controls	18.5 (0.1) N=3	18.0 (3.1) N=5, p<0.01 vs. controls	18.0 (3.1) N=33, p<0.001 vs. Controls
4 years	18.4 (5.9) N=7	17.7 (2.8) N=2		24.5 (0.2) N=2	19.4 (5.3) N=11, p<0.05 vs. controls

Control group: AChE = 24.3nmol/ml/min (SD 6.2), N=13

3.3.2.1 Somatostatin, Beta-endorphin, 5-Hydroxyindoleacetic acid, Acetylcholinesterase; Koponen et al

Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994) undertook a prospective cohort study aiming to characterise the neurochemical features and long-term cognitive and functional outcomes of an aetiologically heterogeneous group of older patients with delirium admitted to a psychogeriatric hospital in Finland. Participants were selected appropriately, since they consecutively recruited 70 older patients, (30 (42.9%) men), with a mean age of 75 years (range 60-88 years). Diagnosis of delirium was based on DSM-III criteria. Patients with delirium tremens were excluded. Based on clinical assessment and CT brain scan, patients were categorised according to whether they met criteria for probable Alzheimer's Dementia (20%, n=14), multi-infarct dementia (57%, n=40), Parkinson's Disease (4%, n=3) or whether they had no observable CNS disease (19%, n=13). Patients underwent a morning fasting lumbar puncture (LP) on the next working day following their index admission, two weeks after admission, and at one year and four years. There was no information on how long patients had been delirious prior to their admission and subsequent index lumbar puncture. Data were only reported for some of the biomarkers from initial assessment through to the four year assessment. There was a significant rate of attrition, with 66 patients surviving to the LP at two weeks (6% mortality), 33 of 44 surviving patients participating in one year follow-up (37% mortality), and 16 of 24 surviving patients participating in four year follow-up (66% mortality), of whom five refused to have an LP. There was no information on the reasons for other loss to follow-up. The control group used was drawn from a Healthy Ageing study at Kuopio University. A group of 19 controls was used for the beta-endorphin study (Koponen and Riekkinen 1990) and 13 controls for the somatostatin, 5-HIAA and acetylcholinesterase studies (Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994). Although age- and sex- matched, this set of controls was community dwelling and had no evidence of dementia and no psychoactive drug use, and therefore as a group differed from the delirium group which had a high proportion (n=53/69, 76.8%) of patients with probable dementia. Other issues identified in the quality assessment were that there

was no evidence that the researcher analysing the CSF samples was blinded to the results, and there was no power calculation, although statistical methods used were appropriate.

Koponen *et al* (Koponen, Leinonen et al. 1994) measured somatostatin-like immunoreactivity (SLI) in CSF by radioimmunoassay. Koponen found a reduction in mean SLI level for the whole delirious group at index, two weeks, one year and four years. They also found that SLI correlated with cognitive and functional performance: falling SLI correlated with worsening cognition (Mini-Mental State Examination, MMSE) from two weeks to four years, and with the level of daily functioning at one and four years. In the group with vascular dementia, SLI rose acutely in those with an acute stroke as the probable cause of their delirium; this may represent a response to injury or cerebral ischaemia (Koponen, Leinonen et al. 1994). In those whose delirium abated within the first two weeks there was no change in the level of SLI. In the group with no observable CNS disease who could be seen as the closest to a *de novo* delirium episode, SLI was significantly low up to two weeks, rising to a level comparable with the control group at one and four years. In summary, loss of somatostatin signaling appears to have a role in dementia and transient loss may be implicated in delirium.

Beta-endorphin like immunoreactivity (BLI) was measured by radioimmunoassay. The group found lower levels of mean BLI in the delirious group at index, two weeks and one year. BLI was lower in those with "silent" or hypoactive and mixed delirium, severe cognitive decline and vascular dementia but not Alzheimer's dementia. However, the numbers for some of these subgroup means were quite small (N's range from four to 40 at baseline and three to 12 at one year) and it is unclear whether these were *a priori* or *post hoc* analyses. There was also a positive correlation between MMSE and BLI within the whole delirious group at the acute stage only, and in subgroups with hyperkinetic delirium, moderate cognitive decline and in those receiving psychoactive drugs. There was no change in BLI in those whose delirium had abated at the two-week point. However, in the group with no CNS disease there was a transient and apparently reversible fall in BLI at index, with

a rise to levels comparable to controls at one year. In summary, there may be a fall in beta-endorphin signaling in delirium, but it is difficult to conclude that these changes are related to delirium rather than dementia.

HPLC (high performance liquid chromatography) with amperometric detection was used to measure 5-HIAA, the main metabolite of serotonin (Koponen, Lepola et al. 1994). Mean 5-HIAA was higher only in delirious subgroups with vascular dementia or no apparent CNS disease (Table 3.4). The levels stayed high at two weeks and one year in both groups. There was no reduction in 5-HIAA levels in those whose delirium abated. There was a positive correlation between 5-HIAA and MMSE in the whole delirious population at one year, (i.e. higher 5-HIAA associated with better score on MMSE). Interestingly there was also an association between the index 5-HIAA level and lifespan after delirium (z=2.93, p=0.007), and patients needing prolonged institutional care had a lower 5-HIAA level acutely (p=0.01).

High levels of 5-HIAA in the group with vascular dementia may be related to release from ischaemic neurons, and many cases of delirium in the group with vascular dementia were attributed to acute stroke (Koponen, Lepola et al. 1994). An alternative possibility might be small vessel disease. The findings in the group with no CNS disease support a possible role for excess serotoninergic activity or turnover in delirium, or perhaps a vascular aetiology for some episodes of delirium in patients with vascular dementia. There also seem to be prognostic implications of the association between low 5-HIAA in acute delirium and shorter lifespan after delirium and the need for prolonged institutional care.

A modification of a colorimetric method was used to measure Acetylcholinesterase (AChE), the enzyme with a major role in metabolising acetylcholine (Koponen, Sirvio et al. 1994). There was no association between AChE and delirium in the delirious group as a whole. However, there was a fall in levels of AChE between the two-week and one year time-points in the delirium subgroups with Alzheimer's dementia, multi-infarct dementia and hyperkinetic delirium (Table 3.5). There was also a small but non-significant reduction in levels of AChE in the group with no

Cortisol and inflammation in delirium and cognitive decline after hip fracture CNS disease. There was no change in AChE level in those whose delirium abated. Higher baseline AChE levels predicted a need for prolonged institutional care, shorter life span and death during follow-up. In summary, lower AChE was associated with later dementia, but higher AChE was associated with poorer outcomes. The discrepancy between these results makes it difficult to conclude whether high or low levels of the enzyme AChE is more indicative of delirium and poor outcomes.

3.3.2.2 Homovanillic acid; Ramirez-Bermudez et al

Ramirez-Bermudez *et al* (Ramirez-Bermudez, Ruiz-Chow et al. 2008) conducted a prospective cohort study with the aim of determining if CSF HVA (homovanillic acid, a dopamine metabolite) levels are raised in delirium. They recruited 51 patients with acute neurological symptoms requiring lumbar puncture over a three year period at a general hospital in Mexico. No exclusion criteria were cited, nor the number eligible who did not take part, and there is no evidence that sampling bias was minimised or that a power calculation was used. The mean age of the cohort was 36.4 years, with the range 18-85 years, and 54.9% were male. This cohort had a high proportion of CNS infections (43/51 patients). The other eight patients had other neurological diagnoses. There was also a relatively high rate of HIV infection (16/51 patients). These aetiological factors may have confounded the results. The investigators used the Spanish translation of the Delirium Rating Scale (DRS) (Trzepacz, Baker et al. 1988, Secin, Esponda et al. 1998), and delirium was diagnosed based on DSM-IV criteria by a trained clinical neuropsychiatrist. Although the assessment included informant history, no comment was made on premorbid cognition. The authors stated that age was not significantly different between the groups. There was no other external control group. Lumbar puncture was performed within 24 hours of neuropsychological assessment. Only the results pertaining to CSF HVA levels were reported in the included paper. However, a conference abstract from these authors reported that they had also measured neurochemical metabolites of serotonin, nitric oxide and the amino acids GABA, glutamate, glutamine and glycine but that levels of these were not significantly

Cortisol and inflammation in delirium and cognitive decline after hip fracture different in those with delirium or psychotic features (Ramirez-Bermudez, Camilo et al. 2008). No further published details of these additional measurements appear to be available.

Ramirez-Bermudez et al (Ramirez-Bermudez, Ruiz-Chow et al. 2008) used HPLC (high performance liquid chromatography) with electrochemical detection to measure HVA. Although HVA was not significantly different between those with and without delirium, HVA was found to be higher with psychotic features such as hallucinations and delusions (Table 3.2). HVA was also significantly related to the total DRS score and to scores on subtests rating psychotic features, cognitive dysfunction and symptom fluctuation. In subgroup analyses, those without anti-psychotic exposure (n=38) had higher HVA correlated with delusions. In those with acute viral encephalitis (n=23), HVA was higher in those with psychotic features. In those who were HIV negative (n=35), HVA was higher with psychotic features, hallucinations and delusions. Regardless of delirium status, HVA levels were also significantly higher in HIV negative patients and in those who had received anti-psychotic medication. These results suggest excess dopamine signaling may be involved in psychotic features, and perhaps in delirium pathogenesis overall (Ramirez-Bermudez, Ruiz-Chow et al. 2008). However, this was a cohort study of patients requiring lumbar puncture for clinical reasons, therefore those who developed delirium and those who did not were not comparable at baseline, and causation cannot be inferred.

3.3.2.3 Cortisol; Pearson et al

Pearson *et al* (Pearson, de Vries et al. 2010) undertook a prospective cohort study in patients over the age of 60 undergoing spinal anaesthetic for repair of a hip fracture, with the aim of determining if CSF cortisol levels are elevated in delirium. The setting was a University hospital in Scotland. They recruited 27 patients preoperatively, however because of a change to general anaesthetic and blood contamination of CSF, 20 patients were included in the analyses. No power calculation was stated and they did not attempt to minimise sampling bias. The cohort was mainly female (75%) and had a mean age of 80.6 years (range 62-93

years). The age of patients with delirium was not significantly different from controls (81.4 years SD 7.2 vs. 80.5 years SD 8.7, p=0.88). There was no other comment on the comparability of cases and controls although further information on co-morbid disease was provided in table format. No external control group was used. Patients were assessed for delirium preoperatively with a validated assessment battery which informed completion of the Confusion Assessment Method (Inouye, Vandyck et al. 1990) which is based on DSM-IIIR criteria. There was no assessment made of prior cognition. CSF specimens were taken at the onset of spinal anaesthetic, before administration of the anaesthetic agent. Cortisol levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) and in a paired plasma sample by Cortisol-^{125}I Radioimmunoassay. The researcher was not blinded to the participants' diagnoses. The investigators found CSF cortisol levels to be higher in those with delirium than those without (median (interquartile range) 63.9 (40.4-102.1) nmol/L in delirium vs. 31.4 (21.7-43.3) nmol/L in controls), p=0.029). Plasma cortisol was also significantly higher in cases than controls (Pearson, de Vries et al. 2010). This suggests an association between elevated CSF cortisol and delirium (McIntosh, Bush et al. 1985, Kudoh, Takese et al. 2005), although causation could not be inferred in this cross-sectional, observational study. As the authors comment (Pearson, de Vries et al. 2010), dementia, severity of co-morbid disease and physiological stress are potential confounding factors which should be assessed in future studies.

3.3.2.4 Markers of neuronal cell death; Caplan et al, Hall et al

Caplan *et al* (Caplan, Kvelde et al. 2010) undertook a prospective cohort study in a Geriatric Medicine Unit in a General Hospital in Australia. Their patient group had persistent delirium of longer than five days duration, where further investigation including lumbar puncture was deemed to be clinically appropriate and was agreed by the patient and/or their proxy. Twenty patients with heterogeneous delirium were recruited. Although no power calculation is cited, they attempted to minimise sampling bias. The aim was to compare CSF, blood and clinical markers of delirium and neuronal cell death between patients with delirium and an external control group of outpatients with known Alzheimer's Dementia but no delirium (n=20). There was

Cortisol and inflammation in delirium and cognitive decline after hip fracture no inpatient control group. The two groups were relatively well-matched: age was not significantly different (mean 81.6 (SD 7.7) in the delirium group and 81.1 (SD 6.1) in the dementia group), and Instrumental Activities of Daily Living (IADL), Charlson Index (a measure of co-morbidity), Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and Geriatric Depression Score were not significantly different. However, the fact that the average IQCODE in the delirium group was the same as in the dementia group (3.7 in both groups) suggests a high prevalence of dementia in the delirium group. Delirium was diagnosed using the Confusion Assessment Method and Delirium Index. The Acute Physiology, Age, Chronic Health Evaluation (APACHE III) score, used for risk prediction in the severely ill, was significantly higher in the delirium group. Lumbar puncture was performed during the delirium episode or on an outpatient basis for the dementia group. CSF lactate, protein and glucose were measured at the local area laboratory and S100B and neuron-specific enolase were measured by ELISA, but no comment is made on whether there was blinding to participants' diagnoses.

The investigators found higher CSF lactate in the inpatient group with delirium compared to the outpatient group with Alzheimer's dementia (1.87mmol/L vs. 1.48mmol/L, $p<0.001$), lower neuron-specific enolase (NSE) (4.84ng/ml vs. 8.98ng/ml, $p<0.001$), higher protein, and no difference in S100B and glucose (Caplan, Kvelde et al. 2010). In all participants, CSF lactate correlated positively with APACHE III score, CAM and Delirium Index. NSE correlated negatively with CAM and Delirium Index. CSF lactate correlated with a greater deterioration in scores of daily function (IADL, and trend to correlation with Barthel index). Those within the delirium group who died in hospital had higher CSF lactate than those discharged home, and those who required institutional care had an intermediate level. The finding that CSF lactate was higher in persistent delirium, and was also associated with poorer outcomes, suggested that delirium may be associated with neuronal injury, or failure of normal aerobic energy metabolism such as ischaemia. However, here the group with delirium was acutely unwell, compared to the group with dementia who were stable outpatients, therefore differences may be due to acute illness rather than delirium. A third group with acute illness but without delirium

Cortisol and inflammation in delirium and cognitive decline after hip fracture would have strengthened the study, as the group comment. The finding of high protein is relatively non-specific, and may indicate inflammation, trauma, infection or blood contamination (Seehusen, Reeves et al. 2003). The somewhat surprising finding of lower NSE in delirium in the context of high lactate was explained by the investigators as possibly due to lactate suppressing NSE. This could occur via glutamate stimulation suppressing glycolysis in neurons, which instead gain energy from astrocytic lactate, as per the shuttle hypothesis (Caplan, Kvelde et al. 2010), but these findings require replication in other studies.

Hall *et al* conducted a prospective cohort study in patients with acute hip fracture who underwent surgery under spinal anaesthetic (Hall, Ferguson et al. 2013). The study is included in this thesis in chapter 6. The hypothesis under study was that CSF S100B would be elevated in patients with delirium. Forty-five patients had sufficient CSF sample to be included, with 66/108 patients having had CSF collected. No power calculation is cited and there was no comment on minimising sampling bias. There was no external control group. Age was not different between groups. The delirium group was more likely to be cognitively and functionally impaired. Six patients had dementia according to IQCODE, and all developed delirium. Patients were assessed for delirium preoperatively, daily postoperatively to day 4, and on postoperative days 7 and 10-14, and delirium was diagnosed with positive CAM or DRS-R98 >17.75. S100B was measured using an in-house ELISA at the National CJD Surveillance Unit in Edinburgh. S100B concentrations were log-transformed, and the authors found that levels were higher in those with acute delirium (n=8), although there was no difference between those with and without delirium at any stage during the perioperative period (n=19). These results suggest that active delirium may be associated with astroglial activation and CNS damage.

3.3.2.5 Cytokines; MacLullich et al, Westhoff et al, Cape et al

MacLullich *et al* undertook a prospective cohort study in patients over the age of 60 undergoing hip fracture repair under spinal anaesthesia, with the aim of investigating whether CNS pro-inflammatory cytokines are elevated in delirium (MacLullich, Edelshain et al. 2011). This was on a background of prior studies reporting elevated

Cortisol and inflammation in delirium and cognitive decline after hip fracture serum pro-inflammatory cytokines such as IL-6 and IL-8. Thirty-six patients were recruited in University-affiliated medical centres in Edinburgh and Amsterdam. No power calculation is cited, and there was no comment on whether they attempted to minimise sampling bias. The mean age was 81.8 years in the group with delirium and 82.5 years in the group without, which was not significantly different. Charlson co-morbidity indices were not significantly different between the groups, but a documented history of dementia (n=7) was associated with a higher incidence of delirium. There was no external control group. Patients were assessed for delirium preoperatively and 3-4 days postoperatively using the CAM. There was no assessment of inter-rater reliability between the two centres. CSF specimens were taken at the onset of spinal anaesthesia prior to the administration of anaesthetic agent. Cytokine analysis of CSF samples, and paired serum samples from the Edinburgh patients (n=16), was performed using a Cytometric Bead Array immunoassay in the Edinburgh laboratory, although the researcher was not blinded to the participants' diagnoses. This measured TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12p70. The investigators found IL-8 to be detected in 33/36 CSF samples, and IL-6 in 3/36 samples. The other cytokines were not detected. CSF IL-8 was significantly higher in cases than controls (median 69.8pg/ml vs. 39.6pg/ml, p=0.003). Cases also had higher serum IL-6 levels (median 42.4pg/ml vs. 24.3pg/ml, p=0.013). Dementia was not associated with raised IL-8, and when the investigators excluded patients with known dementia from the analysis, the pattern of results did not change. These results suggest an association between delirium and elevated CNS IL-8, although causation cannot be implied in this cross-sectional, observational study.

Westhoff *et al* performed an exploratory study in patients with acute hip fracture, with the hypothesis that those with a dysfunctional immune response would be more vulnerable to postoperative delirium (Westhoff, Witlox et al. 2013). The investigators monitored for delirium using the CAM, with severity scoring using the DRS-R98 until day 5 postoperatively, or longer if delirium was still present at that time. There was no power calculation is cited, and there was no comment on whether they attempted to minimise sampling bias. Of N=122 patients enrolled, N=61 patients were included in the analysis due to a change to general anaesthesia, the

Cortisol and inflammation in delirium and cognitive decline after hip fracture exclusion of those with preoperative delirium and logistical reasons. There was no external control group. They collected blood preoperatively to measure CRP, IL-6 and ESR (erythrocyte sedimentation rate). CSF and a paired serum sample was collected at the onset of spinal anaesthetic, and a panel of 42 cytokines and chemokines measured using a Luminex assay (see Table 3.1 for full list). Some of the cytokines and chemokines were not detectable in the majority of samples, and they compared levels of the 16 markers where there was sufficient (>50% of samples) detected between those who did and did not develop delirium postoperatively using Mann Whitney U test. There was no difference in the majority of the detected inflammatory markers, however the authors found that those who developed postoperative delirium had lower levels of CSF IL-6, IL-1ra and Flt-3L (FMS-like tyrosine kinase 3 ligand) preoperatively. Of note they detected but found no difference in levels of IL-8. However, the delirium group had higher levels of serum IL-6 preoperatively. They found no difference in CRP and ESR levels (Westhoff, Witlox et al. 2013). There is no comment on whether there was any correlation between serum and CSF levels of IL-6, but there is also no information on how IL-6 was measured in serum, since if this was by a different assay technique they would be harder to compare.

Cape *et al* (Cape, Hall et al. 2014) conducted a collaborative prospective cohort study in patients with acute hip fracture, and measured several inflammatory markers and a marker of astroglial activation (Glial Fibrillary Acidic Protein, GFAP). The hypotheses were that interleukin(IL)-1β, insulin-like growth factor-1 (IGF-1), interferon (IFN)-γ and GFAP would be elevated and IL-1 receptor antagonist (IL-1ra) would be reduced in delirium. Participants were drawn from two Edinburgh studies, including the study described in this thesis (although this pilot study is not included as a results chapter), and a study in Amsterdam. They were recruited opportunistically, and no power calculation is cited nor attempt to minimise sampling bias. Forty-three patients with hip fracture were included, and CSF was collected during spinal anaesthetic. Serum was also collected in Edinburgh, paired with the serum sample. There was no external control group. Patients were assessed for delirium according to the CAM preoperatively and at least once 3-4 days

Cortisol and inflammation in delirium and cognitive decline after hip fracture postoperatively. CSF analysis was performed in Edinburgh, and there was no blinding to diagnosis. All the biomarkers were measured using individual commercially-available ELISAs. IFN-γ and IGF-1 were not detected in CSF. IL-1β was detected in CSF and serum at low concentrations, IL-1ra was detected in CSF and serum and GFAP was detected in CSF only. The investigators compared delirium present preoperatively (prevalent) to postoperative (incident) to never delirium. They found that IL-1β was elevated in those with incident delirium, and IL-1ra was elevated in prevalent delirium. GFAP was not significantly different between groups. In serum, no differences were seen in levels of IL-1β, IL-1ra or IGF-1 between those with or without delirium at any stage, and IFN-γ and GFAP were not detected in serum. The CSF:serum ratio of IL-1β was higher in the delirium group. The authors performed a logistic regression analysis to adjust for the presence of prior dementia, and the relationship between CSF IL-1β and delirium remained significant. This study suggests that there is a role for the IL-1 family in delirium, although since it is observational, no causal link can be identified. The higher CSF:serum ratio of IL-1β, with no correlation between the two, suggests that the source of IL-1β may be within the CNS.

3.3.2.6 Dementia neuropathology markers; Witlox et al, Xie et al

Witlox *et al* conducted a prospective cohort study in patients with acute hip fracture, to examine levels of β-amyloid1-42, tau and phosphorylated tau in CSF in delirium. These markers are pathological indicators of cognitive impairment, and the hypothesis was that β-amyloid1-42 would be low and tau and phosphorylated tau would be elevated in patients who developed delirium, due to the high risk of delirium in those with cognitive impairment (Witlox, Kalisvaart et al. 2011). They recruited N=122 patients with acute hip fracture to a trial of taurine vs. placebo in reducing 1-year morbidity and mortality of patients with hip fracture. They were able to collect CSF samples from N=76 patients, N=30 of whom developed delirium postoperatively. None had full syndromal delirium preoperatively. No power calculation is cited, and there was no comment on whether they attempted to minimise sampling bias. There was no external control group. The delirium group

Cortisol and inflammation in delirium and cognitive decline after hip fracture was older (mean age 84.7 years vs. 82.4 years in no delirium group, p=0.04). The delirium group were more likely to be cognitively and functionally impaired according to the IQCODE, Barthel index and Lawton IADL score, and they were prescribed more regular medication. The investigators found no significant difference in levels of any of the dementia markers between those who did or did not develop delirium postoperatively, although the levels of all three markers were different between groups in the directions hypothesised. They also examined the ratios between β-amyloid1-42 to tau and to phosphorylated tau, and there was no difference in either ratio between groups. Therefore, although those with evidence of prior cognitive impairment in this group of older patients with acute hip fracture were more likely to develop delirium, it was not associated with these well-established markers of dementia neuropathology.

Xie *et al* (Xie, Swain et al. 2014) performed a prospective cohort study in older patients undergoing elective orthopaedic surgery, to investigate whether there is an association between the ratio of β-amyloid-40 or β-amyloid-42 to tau protein in CSF at baseline and both the incidence and severity of postoperative delirium (Xie, Swain et al. 2014). Given that tau is usually elevated in dementia and β-amyloid-42 proteins are usually lowered, they hypothesised that a lower β-amyloid-42/tau ratio preoperatively would be associated with greater delirium severity, as would a lower β-amyloid-40/tau ratio, and that both ratios would be associated with greater delirium incidence. They performed a power calculation and had estimated that a sample size of N=150 should be sufficient for their primary hypothesis. They enrolled N=244 participants into the study and were able to analyse samples from N=153. CSF was collected at the onset of spinal anaesthesia, and participants had standardised perioperative care. They were assessed for delirium on the first and second postoperative days, using the CAM and MDAS; delirium developing later in the postoperative course may therefore have been missed. Although the investigators excluded patients with known dementia or other neurological or psychiatric disease, they did not include an informant interview such as IQCODE, or baseline cognitive testing to assess for undiagnosed dementia; the authors qualify this by describing the study as a proof of concept study. Thirty-one participants (20%) were diagnosed as

Cortisol and inflammation in delirium and cognitive decline after hip fracture delirious postoperatively using the CAM. There was no significant difference in CSF β-amyloid-42/tau ratio or β-amyloid-40/tau ratio between groups with and without delirium. When they examined quartiles of the β-amyloid/tau ratios, they found a higher incidence of delirium in those in the lowest quartile for both ratios. They also found that the median MDAS score in the group within the lowest quartile β-amyloid-40/tau ratio was higher than the median score in the other three quartiles, and there was a trend towards elevation of the median MDAS score in the group within the lowest quartile of β-amyloid-42/tau ratio. Using linear regression, they also found an inverse association between MDAS score and both β-amyloid/tau ratios, which remained significant after adjusting for age and gender. This study suggests that lower β-amyloid/tau ratio is associated with increased risk of developing delirium postoperatively; it seems likely that this would be related to the presence of incipient or active dementia, and future studies should take this into account.

3.3.2.7 Anticholinergic activity; Watne et al

Watne *et al* (Watne, Hall et al. 2014) performed a prospective cohort study in patients with acute hip fracture. Patients were recruited at two general hospitals in Oslo and Edinburgh. The Edinburgh patients (n=52) are from the study this thesis is based on, but the results are not included in this thesis. The patients in the Oslo study (n=99) were part of a randomised controlled trial of orthogeriatric vs. standard orthopaedic care. The hypothesis for this study was that higher anticholinergic activity in both CSF and serum would be associated with an increased risk of perioperative delirium. There was no power calculation cited and no comment on minimising sampling bias. There was no external control group. Patients had CSF and a paired serum sample collected at spinal anaesthetic. Anticholinergic activity was measured in CSF and serum with a muscarinic radio receptor bioassay by an operator who was blinded to all clinical data. Patients were assessed for delirium preoperatively and postoperatively daily for five days or until delirium resolution (Oslo) or preoperatively and on postoperative days 1-4, 7 and once between days 10-14 (Edinburgh). The CAM was used at both sites, but delirium severity was assessed

Cortisol and inflammation in delirium and cognitive decline after hip fracture using the Memorial Delirium Assessment Scale (MDAS) in Oslo and the DRS-R98 in Edinburgh. In order to standardise delirium severity measurements at both sites, MDAS scores were retrospectively calculated for the Edinburgh patients. Delirium was diagnosed in 52/99 patients in Oslo and 20/32 patients in Edinburgh. The patients from the Oslo study had higher rates of prior dementia and functional impairment. The results from the two centres were analysed separately, and in both centres, CSF and serum anticholinergic activity were not significantly different in delirium. Levels of serum anticholinergic activity were much higher in the Oslo patients than in the Edinburgh patients. In patients without prefracture cognitive impairment, there was a trend towards a correlation between delirium severity and CSF anticholinergic activity in both centres, which was significant in the pooled dataset. The results of this study do not support the hypothesis that anticholinergic activity in the CNS is involved in delirium pathophysiology, but in those without prior cognitive impairment, higher levels of anticholinergic activity may lead to more severe delirium.

3.3.2.8 Proteomics; Poljak et al

Poljak *et al* performed the first CSF proteomics study in delirium. Patients were recruited in two centres, Sydney and Edinburgh (Poljak, Hill et al. 2014). The patients in the Sydney study were elderly patients who had prolonged delirium secondary to various causes, which lasted for at least five days, and they had a lumbar puncture performed for further investigation and CSF was also collected for research purposes. This is the same cohort as Caplan *et al* (Caplan, Kvelde et al. 2010). The patients in the Edinburgh study had acute hip fracture, and CSF was collected at spinal anaesthetic. These patients are from the study described in this thesis, but the results presented in the Poljak paper are not discussed further as part of the thesis. Four groups were compared; patients with active delirium from the Sydney study, patients with active preoperative delirium from the Edinburgh study, patients with acute hip fracture from the Edinburgh study who did not develop delirium, and outpatients with Alzheimer's dementia. CSF was analysed using iTRAQ reagents, two-dimensional liquid chromatography-tandem mass

Cortisol and inflammation in delirium and cognitive decline after hip fracture spectrometry. An ELISA of α-1-acid glycoprotein-1 was also performed to confirm one of the positive results. Two-hundred and seventy-three proteins were identified, of which sixteen were dysregulated in eight or more delirium subjects, across both cohorts. Apolipoproteins, chromogranins and secretogranins were downregulated in the majority of delirium subjects. Several proteins involved in the inflammatory response were also upregulated in the majority of those with delirium. These included α-1 acid glycoprotein, α-2-macroglobulin, fibrinogen, α-1-antitrypsin, α-1-antichymotrypsin, transferrin, complement component 3 and haptoglobin. Only one isoform of α-1 acid glycoprotein was upregulated, and it was not upregulated in either of the control groups, so it has the potential to be a specific biomarker for delirium. This was confirmed by ELISA measurement of α-1 acid glycoprotein, with higher levels found in delirium (N=12, 277.85μg/ml) compared to Alzheimer's dementia (N= 18, 207.44μg/nl), p=0.026.

3.4 Discussion

This chapter updates the first systematic literature review of studies examining CSF biomarkers in delirium, which was originally published in 2011 (Hall, Shenkin et al. 2011). The quality of all studies is reported in a narrative fashion as there is no accepted gold standard tool for assessing quality and susceptibility to bias in observational studies (Sanderson, Tatt et al. 2007), and there are potential concerns that scoring systems may be spuriously precise (von Elm 2007). All studies were prospective studies, designed to investigate CSF biomarkers in delirium, which used appropriate statistical methods. Only one study reported a power calculation (Xie, Swain et al. 2014), and two studies blinded the researcher analysing the CSF samples to the clinical status of the patient (Ramirez-Bermudez, Ruiz-Chow et al. 2008, Watne, Hall et al. 2014). Several of the studies' results could have been influenced by bias or confounding, and these are reported with their results above. Sample sizes were generally small, although more recent collaborative studies collecting CSF at the onset of spinal anaesthetic have been larger. A diverse range of populations was studied even within the nine main study cohorts. Within Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994, Koponen, Lepola et al. 1994, Koponen, Sirvio et al. 1994) and Caplan *et al*'s (Caplan, Kvelde et al. 2010) heterogeneous elderly delirious populations and the elderly cohorts with hip fracture and attendant co-morbidities studied by the Scottish, Dutch and Norwegian research groups (Pearson, de Vries et al. 2010, MacLullich, Edelshain et al. 2011, Witlox, Kalisvaart et al. 2011, Hall, Ferguson et al. 2013, Westhoff, Witlox et al. 2013, Cape, Hall et al. 2014, Watne, Hall et al. 2014), there is likely to be a high proportion of unrecognised dementia. The patients studied by Ramirez-Bermudez *et al* (Ramirez-Bermudez, Ruiz-Chow et al. 2008) are a more homogeneous, younger cohort (but which is less representative of the majority of inpatients with delirium). Where there is potential concomitant dementia, it is very difficult to determine whether observed changes are related to acute delirium or confounded by underlying dementia pathology (Inouye and Ferrucci 2006). A further potential confounding factor in these heterogeneous groups of patients is the underlying aetiologies of delirium. These are common problems in studies of delirium pathophysiology. It is important

that control groups are age-matched, to control for physiological changes with age; age-specific reference ranges for most biomarkers in CSF have not been determined, and many of these systems including the hypothalamic-pituitary-adrenal axis and immune system change with age (Murakami, Nakagawa et al. 1999, Otte, Hart et al. 2005, Ginaldi, Mengoli et al. 2008). Also, unless a biomarker is known only to originate from the CNS, co-measurement of the biomarker in a paired serum sample helps to establish whether it has been produced predominantly in the CNS or whether high peripheral levels may have led, in some cases, to increased passage into the CNS, for example in the event of blood-brain-barrier compromise in sepsis (Davies 2002, Ballabh, Braun et al. 2004). Serum values were reported by Pearson *et al* (Pearson, de Vries et al. 2010), Caplan *et al* (Caplan, Kvelde et al. 2010), MacLullich *et al* (MacLullich, Edelshain et al. 2011), Watne *et al* (Watne, Hall et al. 2014), Cape *et al* (Cape, Hall et al. 2014) and Westhoff *et al* (Westhoff, Witlox et al. 2013). Interestingly, the direction of change in markers measured in both CSF and serum was sometimes at odds. Serum and CSF values are sometimes difficult to compare, and the difference in protein composition of the two fluids can lead to technical difficulties with certain assays.

The paucity of studies and their heterogeneous nature make it difficult to draw firm conclusions relating to CSF neurochemical disturbances in delirium which might shed light on pathophysiological processes. These studies suggest, however, the following possibilities in delirium: (1) a transient and reversible fall in both somatostatin and beta endorphin signaling, (2) increased serotoninergic signaling, (3) increased dopamine signaling involved in psychotic features, (4) high acetylcholinesterase as a predictor of poor outcome, (5) increased cortisol levels, (6) increased lactate and protein, (8) low neuron-specific enolase, (9) changes in inflammatory cytokines and chemokines are unclear; increased IL-1β in one study and no different in another, increased IL-8 release in one study and no different in another, reduced IL-6 levels, increased IL-1ra in one study and reduced in another, (10) increased S100B in one study and no different in another, (11) reduced Flt-3L levels, (12) increased α-1-glycoprotein levels, (13) down-regulated apolipoproteins, chromogranins and secretogranins, upregulated proteins involved in inflammation

including α-2-macroglobulin, fibrinogen, α-1-antitrypsin, α-1-antichymotrypsin, transferrin, complement component 3 and haptoglobin; (14) β-amyloid, tau and hyperphosphorylated tau, β-amyloid-40/tau ratio, β-amyloid-42/tau ratio, anticholinergic activity, GFAP, TNF-α, TGF-α, IL-10, IL-12p70 G-CSF, IFN-α2, IFN-γ, IL-1α, IL-15, IP-10, MCP-1, MCP-3, PDGF-AA, RANTES and sCD40-L currently show no associations.

I now briefly discuss the relevance of these findings to the main hypotheses for delirium pathogenesis discussed earlier. For a putative model of causal pathways in delirium suggested by the results of this review, please see Figure 3.2.

i) Direct brain insults

None of the studies directly tested this theory since the aetiology of delirium is usually heterogeneous. Since many direct brain insults lead to a disruption of brain energy supply and metabolism, the finding of Caplan *et al* (Caplan, Kvelde et al. 2010) of raised CSF lactate suggests that a disruption of the brain's normal aerobic metabolism, either through low-grade ischaemia or a combination of factors, may be involved in delirium. Whether this is a cause or effect of delirium, or indeed a measure of how unwell the delirious patients are compared to controls, is unclear. Lactate is produced during ischaemia, when the lack of oxygen necessitates the switching of glycolytic metabolism from aerobic to anaerobic (Karkela, Pasanen et al. 1992, Petzold 2007), and high levels have also been found in CSF in dementia (Parnetti, Reboldi et al. 2000) and after out of hospital cardiac arrest (Karkela, Pasanen et al. 1992). Somewhat surprisingly, one study found low neuron-specific enolase in the CSF of patients with delirium. Neuron-specific enolase is a glycolytic enzyme released following neuronal cell damage. The investigators suspected that the low levels could have resulted from lactate suppressing neuron-specific enolase, possibly via glutamate stimulation suppressing glycolysis in neurons, which instead gain energy from astrocytic lactate, as described in the astrocyte-neurone-lactate shuttle hypothesis (Fillenz 2005). The marker of CNS injury and astrogliosis S100B was found to be elevated in those with active delirium after hip fracture (Hall, Ferguson et al. 2013), but in a different study it was not found to be different in

Cortisol and inflammation in delirium and cognitive decline after hip fracture medical patients with active delirium compared to patients with dementia who were not acutely unwell (Caplan, Kvelde et al. 2010).

ii) Neurotransmitter imbalance

Several of the studies lend support to this hypothesis. Serotonin, a monoamine neurotransmitter, has roles in mood, cognition and wakefulness, and excess or deficiency have been hypothesised to be involved in delirium (Flacker and Lipsitz 1999). The results of Koponen *et al* support an excess (Koponen, Lepola et al. 1994) of serotonin, as does serotoninergic syndrome, similar clinically to delirium, where high levels of cerebral serotonin are caused by certain drugs (White 2002). Findings of an imbalance in levels of precursor amino acids for serotonin and other neurotransmitters also suggest a downstream role for serotonin (van der Mast, Fekkes et al. 1991, Pandharipande, Morandi et al. 2009).

The association of high dopamine metabolites with psychotic features (Ramirez-Bermudez, Ruiz-Chow et al. 2008) supports a role in these symptoms of delirium, if not the whole syndrome. Dopamine has a role in motor activity, attention, memory, thought and perception (Trzepacz 2000), all domains which may be affected in delirium. The usefulness of D2 receptor inhibitors such as haloperidol in the treatment of psychotic symptoms of delirium (Flacker and Lipsitz 1999) lends further support.

Disrupted cholinergic signaling with low acetylcholine has long been held to be one of the pathophysiologic mechanisms in delirium (Flacker and Lipsitz 1999), and it has a well established role in Alzheimer's dementia (Talesa 2001, Birks 2006). The association of higher acetylcholinesterase with poorer outcomes after delirium may be related to a more severe initial delirium with greater acetylcholine release from degenerating neurons, or perhaps a tendency to higher levels of the enzyme initially leading to more severe delirium and poorer outcomes later on. Interestingly, low plasma acetylcholinesterase levels have been found in delirium in older medical in-patients (White, Calver et al. 2005). Watne *et al* did not find any association with anticholinergic activity in CSF, suggesting that acetylcholine deficiency is not a

Cortisol and inflammation in delirium and cognitive decline after hip fracture major mechanism in delirium pathophysiology, at least in the heterogeneous delirium seen in elderly patients after hip fracture (Watne, Hall et al. 2014).

The results for the neuropeptide hormones somatostatin and beta-endorphin measured by Koponen *et al* (Koponen and Riekkinen 1990, Koponen, Leinonen et al. 1994) lend some support to a disruption in neurotransmission, but also possibly to a dysregulation in hypothalamic-pituitary signaling. Somatostatin is a hypothalamic peptide hormone with roles in the CNS in locomotion, sedation and excitation, sleep, learning and memory (Vecsai and Widerlov 1990, Koponen, Leinonen et al. 1994). A transient fall in levels may therefore be associated with acute changes in these domains, which may account for some features of delirium. Somatostatin forms part of the Growth Hormone Releasing Hormone - Growth Hormone (GH) – Insulin-like Growth Factor 1 (IGF-1) axis, whereby somatostatin inhibits GH release from the pituitary. GH induces IGF-1 production and the pair work in synergy in target tissues (Murray, Kim et al. 2004). IGF-1 has neurotrophic and anti-inflammatory properties and has been found to reduce tau-phosphorylation and protect neurons from β-amyloid (Broadhurst and Wilson 2001), and low serum IGF-1 is associated with incident and prevalent delirium (Wilson, Broadhurst et al. 2005, Adamis, Lunn et al. 2009).

Beta-endorphin is an endogenous opioid peptide (O'Donahue and Dorsa 1982, Wollemann and Benyhe 2004) released with ACTH from the anterior pituitary in response to stress (O'Donahue and Dorsa 1982, Zager and Black 1985). In addition to its analgesic effect, it has a role in learning and memory (Zager and Black 1985), and possibly in sensory processing and selective attention. The locus coeruleus and limbic areas (important for these processes) have dense beta-endorphinergic innervation (Koponen, Stenback et al. 1989). A transient fall may therefore disrupt vulnerable attentional processes and learning and memory. Interestingly, a study by McIntosh *et al* found a prolonged rise in plasma levels of beta-endorphin and cortisol in a small cohort of patients with postoperative delirium (McIntosh, Bush et al. 1985). Decreased beta-endorphin levels have been found in CSF in Parkinson's disease and Alzheimer's and multi-infarct dementia (Sulkava, Erkinjuntti et al. 1985,

Jolkkonen, Soininen et al. 1987), but normal levels have also been found in Alzheimer's dementia (Raskind, Peskind et al. 1986).

iii) Dysregulation of the Hypothalamic-Pituitary-Adrenocortical axis

The findings of Pearson *et al* (Pearson, de Vries et al. 2010) support the hypothesis that delirium is associated with elevated cortisol levels. Cortisol, the effector hormone of the hypothalamic-pituitary-adrenal (HPA) axis, forms part of the primary response of the body to stressful or traumatic insults (Olsson 1999). Ageing and dementia are associated with an increased magnitude and duration of cortisol response to stress (MacLullich, Ferguson et al. 2008). Prolonged hypercortisolaemia, seen in Cushing's disease, ageing and Alzheimer's dementia is associated with atrophy of the hippocampus (Olsson 1999). The hippocampus is involved in long-term potentiation, which is involved in the formation of synaptic memory, and an intact hippocampus is important in negative feedback in the HPA axis (Olsson 1999, MacLullich, Ferguson et al. 2008). Prolonged hypercortisolaemia exacerbates the effects on neurons of hypoxia, ischaemia and hypoglycaemia and can increase vulnerability to cell damage, with synaptic loss and dendritic atrophy . (Trzepacz 2000, Tata and Anderson 2010). Higher plasma cortisol levels have been found in perioperative delirium (Kudoh, Takese et al. 2005). Intriguingly, recent studies have found that in some circumstances, elevated glucocorticoids can have pro-inflammatory effects in the CNS (Munhoz, Sorrells et al. 2010).

iv) Hyper-responsiveness of brain immune cells to stimulation from peripheral inflammation

Investigation of markers of inflammation in the central nervous system in delirium has produced conflicting results. The findings of MacLullich *et al* (MacLullich, Edelshain et al. 2011) support the idea that peripheral inflammatory insults may lead to an exaggerated central inflammatory response with increased sickness behaviour (Perry, Cunningham et al. 2007, MacLullich, Ferguson et al. 2008). Hip fracture is one such peripheral inflammatory insult, and the CSF levels of IL-8 found in the study were greater that those found peripherally, suggesting possible magnified CNS production. IL-8 is a generally pro-inflammatory chemokine, with a role in

neutrophil chemotaxis. It is produced systemically and in the CNS. In vitro, IL-8 suppresses cultured septal cholinergic neurotransmission (Puma, Danik et al. 2001), and cerebral microinfusion of IL-8 into rats leads to anorexia and pyrexia (Platasalaman and Borkoski 1993). IL-8 may also have protective effects, including a neuroprotective function, possibly by an antioxidant response pathway or by blocking apoptosis (Hanson, Bostick et al. 2006, Hussein, Daoud et al. 2010). Westhoff *et al* did not find any difference in levels of CSF IL-8 however, and also found CSF IL-6, which is a key player in the acute phase inflammatory response, to be lower (Westhoff, Witlox et al. 2013). The majority of the cytokines and chemokines they detected in their study were not different between those who did or did not develop delirium. Westhoff *et al* also found lower CSF IL-1ra levels in those about to develop delirium, whereas Cape *et al* found higher CSF IL-1ra levels in those with active delirium. IL-1ra is the endogenous antagonist of the key pro-inflammatory cytokine IL-1β, and is usually released after IL-1β to limit the inflammatory response (Allan, Tyrrell et al. 2005). It it thought to be neuroprotective, with higher levels being correlated with better outcomes after brain injury (Bartfai, Sanchez-Alavez et al. 2007). The differing results found may indicate that a lower baseline level increases the risk of going on to develop delirium whereas during active delirium the higher levels may indicate either an endogenous response to an initial spike of IL-1β or a neuroprotective response to the insult of delirium or the inflammatory and traumatic response. IL-1β has proved difficult to measure, and was not detected sufficiently in two studies (MacLullich, Edelshain et al. 2011, Westhoff, Witlox et al. 2013), and at low levels in a third (Cape, Hall et al. 2014). The higher levels of CSF IL-1β found in those about to develop delirium suggest that it might an early player in the central inflammatory response in delirium, and the higher ratio of CSF:serum IL-1β in delirium demonstrated by Cape *et* al is also in keeping with a central hyper-responsiveness of the brain's immune cells to insult in delirium (Cape, Hall et al. 2014).

A CNS inflammatory response is further suggested by the findings of the first proteomics analysis of CSF in delirium, with several proteins involved in the inflammatory response upregulated in many of those with delirium (Poljak, Hill et al.

2014). One of these markers, α-1-glycoprotein, was elevated in those with delirium but not in those with dementia, and has the potential to be a specific marker for delirium.

v) Relationship with dementia

Dementia has been shown to be a strong risk factor for delirium, and patients who have delirium are much more likely to develop subsequent dementia (Juliebo, Bjoro et al. 2009, Witlox, Eurelings et al. 2010). However, the two studies that examined whether levels of established CSF markers for dementia were associated with risk of delirium after hip fracture or arthroplasty surgery have been negative, with only the lowest quartile of Aβ40/tau and Aβ42/tau ratios associated with an increased rate of incident delirium (Witlox, Kalisvaart et al. 2011, Xie, Swain et al. 2014). Indeed, dementia is not the only risk factor for delirium, and not all dementia is of Alzheimer pathology.

3.5 Limitations

The results of this review highlight the scarcity of studies examining CSF in delirium. The cross-sectional designs preclude clear conclusions on the important question of whether any changes in biomarkers are the cause or the effects of the delirium. Results are reported on approximately 60 biomarkers but data may have been collected on others, or further data on some of the biomarkers reported here, and not published, leading to a publication bias.

This review was limited to studies published in the English language. We did not seek unpublished data. It is always possible to miss articles either with the search strategy or in searching the resulting citation lists. However, two individuals independently screened 4,870 citations derived from a search of six relevant and diverse databases, and we carefully hand-searched bibliographies and performed forward citation searches of all short-listed articles. We are therefore confident that no significant articles have been missed, and contact with leaders in the field of delirium research did not identify any other studies. We believe that the insistence

Cortisol and inflammation in delirium and cognitive decline after hip fracture that delirium be diagnosed by formal criteria is a strength of this review. Most of the included papers used DSM criteria or the CAM method which is based on DSM criteria.

3.6 Implications for future research

There is a clear need for further studies in this area, although this research faces several significant challenges. Studies attempting to understand the pathophysiology of delirium are limited by the heterogeneity of the clinical syndrome, the subjects' background (particularly preexisting, including undiagnosed, cognitive impairment), the cause and associated severity of illness, and the high associated mortality. Research is further limited by the care that must be taken in research with people who often do not have capacity to consent to participation in such studies (Holt, Siddiqi et al. 2008), and in particular when an invasive investigation such as CSF examination is proposed. CSF sampling at spinal anaesthesia in orthopaedic or other surgery where delirium is prevalent affords a good opportunity to obtain CSF with minimal additional upset for the patient because the procedure is clinically necessary (Pearson, de Vries et al. 2010). It is important though to avoid being too restrictive in performing lumbar puncture for research purposes in delirium. Although this is an undeniably challenging procedure, the high prevalence and severity of delirium warrants this approach. Furthermore, delirium is a CNS disorder, albeit often precipitated by peripheral disease or injury. CSF analysis is commonly done for research purposes in patients with other CNS disorders (eg. schizophrenia, depression, bipolar disorder, dementia, multiple sclerosis, epilepsy, traumatic brain injury and Creutzfeldt-Jakob disease (van Everbroeck, Boons et al. 2005, Petzold 2007, Schwarz and Bahn 2008, Zetterberg 2008, Regenold, Phatak et al. 2009, Awad, Hemmer et al. 2010, Gudmundsson, Skoog et al. 2010)), and this has led to significant advances of the fundamental knowledge of these disorders. Therefore, with sufficient care and selection of patients, studies of the CSF in delirium need not be restricted to opportunistic use of clinical samples or only where spinal anaesthesia is being performed. Proactive engagement with patient advocacy groups and research

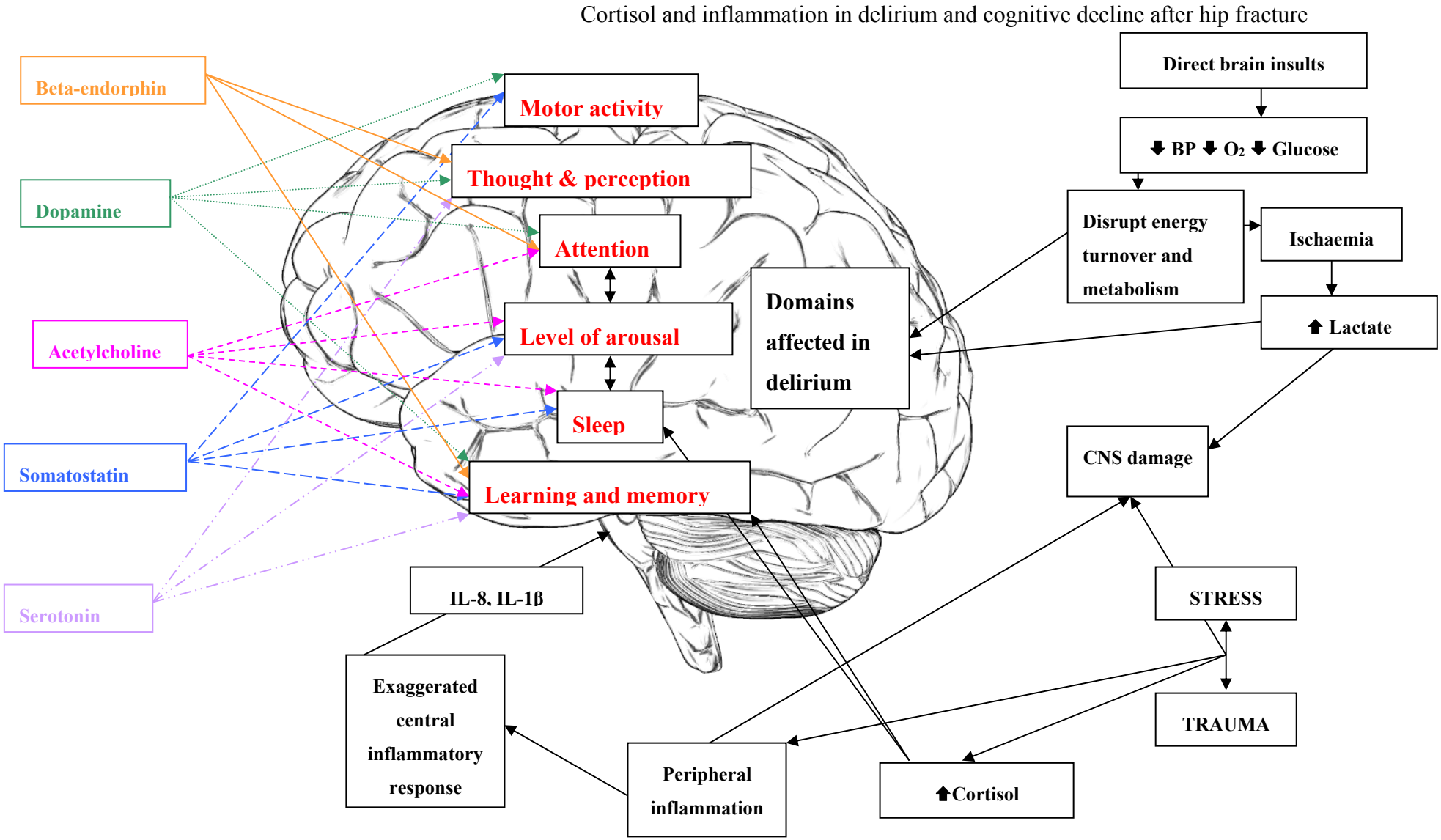
ethics committees will be necessary to help develop this crucial third route to obtaining CSF for delirium research.

Future studies need to be informed by prior research, use power calculations to ensure they will answer predefined hypotheses, clearly report the baseline characteristics of all subjects, and ensure blinding wherever possible. Ideally more generalisable populations of medical and surgical patients should be approached, with careful, standardised assessment of delirium and its severity and aetiology, and as much information on prior cognitive status as possible.

Future research should be guided by, but not limited to, biomarkers that have already been shown to be associated with delirium, and should also take cues from the main hypotheses of delirium pathophysiology and seek evidence of disrupted endocrine and inflammatory responses to stress as well as neurotransmitter upset. The exciting results of the recent proteomics analysis, suggesting potential new avenues for inquiry, also need to be further investigated. Of note, particularly in the case of neurotransmitters, a solitary CSF measurement provides only a snapshot which does not take into account the regional and minute-to-minute changes which may be taking place in different parts of the brain and spinal cord (Hyland 2008). Analyses may also be limited by technical aspects, such as neurotransmitters which degrade too quickly to be accurately measured in vivo e.g. acetylcholine (Bender 1939, Frolich, Dirr et al. 1998).

Given the morbidity and mortality associated with delirium and our increasing knowledge of its link with longer-term cognitive decline, information on potentially disordered neurotransmission, CNS inflammation and damaging prolonged hypercortisolaemia needs to be married with information on markers of CNS damage at the site of injury. Improved understanding of delirium pathophysiology, particularly with evidence of the mechanism of damage to the CNS will likely provide better strategies for prevention and treatment of delirium in the future.

Figure 3.2


Cortisol and inflammation in delirium and cognitive decline after hip fracture
Beta-endorphin
Dopamine
Acetylcholine
Somatostatin
Serotonin
Motor activity
Thought & perception
Attention
Level of arousal
Sleep
Learning and memory
Domains affected in delirium
Direct brain insults
⬇ BP ⬇ O2 ⬇ Glucose
Disrupt energy turnover and metabolism
Ischaemia
⬆ Lactate
CNS damage
STRESS
TRAUMA
⬆Cortisol
Peripheral inflammation
IL-8, IL-1β
Exaggerated central inflammatory response

4 Chapter 4: Cerebrospinal fluid levels of cortisol and DHEA, DHEAS in delirium after hip fracture

4.1 Introduction

Delirium is an acute neuropsychiatric syndrome which is common and associated with multiple adverse outcomes (Witlox, Eurelings et al. 2010). Delirium pathophysiology has been challenging to study and is poorly understood. There has been interest for some time in the role of the Hypothalamic-Pituitary-Adrenal (HPA) axis response to stress in delirium, and the role of cortisol (Olsson 1999, MacLullich, Ferguson et al. 2008). Multiple different stressors such as trauma, surgery, acute illness and even psychological stress may precipitate delirium (Fong, Tulebaev et al. 2009). Ageing and dementia may both be associated with flattening of cortisol circadian rhythm, elevated evening cortisol levels (Ferrari, Cravello et al. 2001), and an increased and prolonged cortisol response to stressors due to impaired negative feedback, and consequently "shut-off" of the HPA axis (Olsson 1999, Ferrari, Cravello et al. 2001). Delirium may also be associated with impaired negative feedback of the HPA axis, as demonstrated by positive dexamethasone suppression tests in patients with delirium related to lower-respiratory tract infection (O'Keeffe and Devlin 1994) and delirium in dementia (Robertsson, Blennow et al. 2001).

Previous studies of serum cortisol in delirium are conflicting (Marcantonio, Rudolph et al. 2006). Several studies have demonstrated elevated serum cortisol in postoperative delirium following abdominal surgery (Kudoh, Takese et al. 2005) and cardiac surgery (Mu, Wang et al. 2010). However, a recent study by Bisschop *et al* found elevated serum cortisol in elderly patients with delirium after hip fracture, but this relationship was no longer significant after adjusting for prior cognitive impairment (Bisschop, de Rooij et al. 2011). Pearson *et al* previously undertook a pilot study examining cerebrospinal fluid (CSF) cortisol in delirium after hip fracture, and found it to be elevated in delirium (Pearson, de Vries et al. 2010).

Dehydroepiandrosterone (DHEA) and its sulphate form, Dehydroepiandrosterone Sulphate (DHEAS) work in interplay with cortisol. These neurosteroids have immune-enhancing roles (Butcher, Killampalli et al. 2005), in contrast with cortisol, for example protecting hippocampal neurones from cortisol neurotoxicity (Kimonides, Spillantini et al. 1998). Their

production naturally declines with age (Ferrari, Cravello et al. 2001, Chehab, Ouertani et al. 2007) including in CSF (Murakami, Nakagawa et al. 1999), and as cortisol production stays static and can rise, this can lead to a relative cortisol excess (Ferrari, Cravello et al. 2001, Chehab, Ouertani et al. 2007). These age-related changes may be further exaggerated by physical trauma; Butcher *et al* have found an elevated serum cortisol:DHEAS ratio in elderly patients with hip fracture, compared to age-matched controls and young fracture patients (Butcher, Killampalli et al. 2005).

Studies examining DHEA levels in dementia have produced variable findings (Maninger, Wolkowitz et al. 2009). Previous CSF studies in dementia have revealed low CSF DHEAS in multi-infarct dementia (Azuma, Nagai et al. 1999), and high DHEA but low DHEAS in Alzheimer's dementia and Vascular dementia (Kim, Hill et al. 2003). There are no previous studies specifically examining DHEA(S) in delirium. Measurement of DHEAS forms part of the assessment of allostatic load, which measures a combination of acute and chronic stressors and the body's adaptation to these, low DHEAS suggesting a greater allostatic load. Rigney *et al* found increased allostatic load in a group of older hospital in-patients with delirium however no comment was made on DHEAS levels (Rigney 2010).

This collaborative study aimed to examine the levels of cortisol, DHEA and DHEAS in the CSF of elderly patients, with and without delirium following acute hip fracture.

The research questions were as follows:

1) Is CSF cortisol increased in delirium?

2) Are CSF DHEA and DHEAS reduced in delirium?

3) Is the ratio of CSF cortisol:DHEA(S) elevated in delirium?

4.2 Methods

4.2.1 Participant recruitment

Participants with acute hip fracture aged over 60 were recruited in an opportunistic manner by researchers in two related studies. The first was in the Orthopaedic Trauma Unit at the Royal Infirmary of Edinburgh. The second was in the Emergency Department of Oslo University Hospital and was part of a trial of orthogeriatric versus standard orthopaedic care following hip fracture (Oslo Orthogeriatric Trial, OOT) (Watne, Torbergsen et al. 2014). Details of subjects in each study are as follows:

Edinburgh study (CDHIP): Inclusion criteria were age over 60 and preoperative with acute hip fracture. Exclusion criteria were: nursing home resident, significant Parkinson's disease, major communication difficulties (aphasia, English not first language), those taking inhaled or oral steroids in the last 10 weeks, moribund on admission. I approached and recruited all participants.

Oslo study (OOT): Fractures as a result of high energy or multi-trauma were excluded. As with the Edinburgh study, those taking inhaled or oral steroids in the last 10 weeks (retrospective exclusion for the purposes of the present analysis), or who were moribund on admission were excluded. Participants were approached and recruited by members of the Orthopaedic trauma team.

On both sites, written informed consent was obtained from participants or their nominated next of kin if capacity to give informed consent was lacking. Ethical approval was obtained from Scotland A Research Ethics Committee (reference no. 09/MRE00/28) and the Regional Committee for Ethics in Medical and Health Research, Norway.

4.2.2 Cognitive assessment

In both sites, participants were assessed for delirium preoperatively, using a semi-structured interview with cognitive testing, review of casenotes and discussion with nursing or medical staff to complete the Confusion Assessment Method (Inouye, Vandyck et al. 1990) (CAM). The specific additional assessments in each site were as follows:

Edinburgh: Conscious level was assessed with the Observational Scale of Level of Alertness and Richmond Agitation Sedation Scale, there was additional assessment of attention (Edinburgh Delirium Test Box 1 (Brown, Fordyce et al. 2010)) and cognitive testing was with the Mini-Mental State Examination (Folstein, Folstein et al. 1975) with assessment of delirium severity using the Delirium Rating Scale Revised-98 (Trzepacz, Mittal et al. 2001). I performed all in-patient delirium assessments.

Oslo: Additional cognitive testing and assessment of delirium severity was with the Memorial Delirium Assessment Scale (Breitbart, Rosenfeld et al. 1997). Patients were classified as delirious if they scored positive on the CAM and fulfilled DSM IV criteria. Assessments were performed by a Geriatrician and small team of research nurses.

In Edinburgh, participants were again assessed for delirium daily from postoperative days 1-4 and on days 7 and once between days10-14 or until transfer to a rehabilitation unit or discharge from hospital if these occurred sooner. In Oslo, participants were assessed daily until postoperative day 5 or until delirium-free.

4.2.2.1 Diagnosis of delirium

In both sites, patients were classified as delirious if they fulfilled DSM-IV criteria, informed by the CAM assessment process.

4.2.2.2 Delirium severity

In order to examine any relationship between delirium severity and biomarker level, due to the different delirium severity scales used by both centres, MDAS scores were retrospectively coded for the Edinburgh participants, using the appropriate component scores, following consultation with experts in the field. The peak MDAS score for each delirium case was then calculated, and the median of these peak scores (a score of ≥15) used as a cut-off between mild and severe delirium, as no published cut-off scores for mild vs. severe delirium could be found in common usage for the MDAS.

The Informant Questionnaire on Cognitive Decline in the Elderly (Jorm and Jacomb 1989) (IQCODE), a well-validated and widely used measure of pre-morbid cognition, was completed during a meeting with next of kin. I completed these in Edinburgh and Leiv Otto

Watne in Oslo. A cut-off score of ≥3.44 (average score) was used to indicate evidence of probable prior dementia (Harwood, Hope et al. 1997).

4.2.3 Assessment of function and co-morbidity

An assessment was made of participants' functional level at baseline, using the Katz basic activities of daily living score (Katz, Ford et al. 1963) in Edinburgh and the Barthel index (Mahoney and Barthel 1965) in Oslo. Participants were deemed to be independent in basic activities of daily living if they had a Barthel index score of ≥19/20 or a Katz score of ≥5/6. An assessment was made of illness severity and co-morbidity using the APACHE II score (Knaus, Draper et al. 1985), Charlson co-morbidity index (Charlson, Pompei et al. 1987) and the number of regular medications taken on admission. A modified APACHE II score was used, omitting arterial pH (arterial blood gas measurement is not routine in preoperative hip fracture patients), and haematocrit (not routinely reported in Oslo).

4.2.4 Sample collection

At the time of the operation to repair the hip fracture, if this was performed under spinal anaesthetic, the Anaesthetist collected a sample of CSF after placement of the spinal needle and prior to the injection of anaesthetic agents. A paired serum sample was collected at the same time as the CSF sample, in a serum separator tube. The CSF sample was placed in a polypropylene universal container and both samples put on ice. Samples were centrifuged as soon as possible (CSF: 1000 rpm, 4 ^{o}C for 10 minutes, serum 3000 rpm, 4 ^{o}C for 10 minutes) and stored in aliquots in polypropylene eppendorfs at -80 ^{o}C until assayed (see sections 2.5.1 and 2.5.2).

4.2.5 Cerebrospinal fluid and serum assays

Cortisol, DHEA and DHEAS were measured in CSF by individual commercially-available Enzyme-Linked Immunosorbent Assays (Salimetrics, Newmarket, Suffolk) (see sections 2.9.1 and 2.9.2). Cortisol was measured in serum with a commercially available Enzyme-Linked Immunosorbent Assay (MP Biomedicals, USA) (see section 2.10.1). I performed all CSF and serum assays in Edinburgh.

4.2.6 Statistical analysis

Normality of distribution of the results was checked with histogram and Kolgomorov-Smirnov test. Non-parametric cortisol levels were compared with Mann-Whitney U test for

univariate comparisons and with the Kruskal-Wallis test between those who developed prevalent, incident or who never developed delirium. Log_{10}-transformed DHEA and DHEAS levels were compared between groups with Student's t test and ANOVA. Demographic characteristics of the patient group were compared using Student's t test or Mann-Whitney U test for parametric or non-parametric numerical variables, and Pearson Chi-Squared for categorical variables. Correlations between neurosteroids were performed with Spearman's rho to see whether DHEA and DHEAS were correlated, as would be expected, and to see whether cortisol was inversely correlated with DHEA(S). In order to adjust for the effects of potential confounders, logistic regression models were built for biomarkers found to be significantly different in the univariate analysis, using as co-variates those variables found to be significantly different between groups (Table 3.1). Study site was also included in the model to ensure that it did not introduce bias. Finally, prediction logistic regression models were constructed to see if the level of the biomarkers at the time of surgery predicted delirium in the postoperative period. In the prediction models, participants who developed delirium prior to surgery were excluded. All statistical analyses were performed with the statistical package SPSS version 19.0, and $p<0.05$ was considered significant.

4.3 Results

Figure 4.1 shows the participants included and excluded at both sites. Cerebrospinal fluid samples were available for 167 participants across the two sites (N=66 Royal Infirmary of Edinburgh, N=101 Oslo University Hospital). Following exclusion of participants with insufficient data on delirium status (N=9) and participants who had received steroid therapy preoperatively (N=10), 148 participants remained, 42 men and 106 women. Delirium developed at any stage in 68 (45.5%) participants. Prevalent delirium (defined as present prior to surgery and sample collection) was diagnosed in 36 (24.8%) participants, and incident delirium (developing postoperatively where the participant was not delirious preoperatively) in 30 (20.7%) participants. In three participants, no data was available on preoperative delirium status and they were excluded from analyses comparing prevalent vs. incident vs. never delirium. Forty-nine participants had evidence of prior dementia, based on a known diagnosis of dementia and/or an IQCODE average score >3.44. Table 4.1 provides additional information on the functional status of the combined sample and other factors. The delirium group was more likely to be functionally and cognitively impaired, be taking more medications on admission and have a greater degree of illness severity as measured by the modified APACHE II score (Table 4.1). Table 4.2 provides a summary of the demographic characteristics of the Edinburgh and Oslo cohorts. Patients in Oslo were more functionally dependent and more likely to have prior dementia; this was most likely due to the exclusion in Edinburgh of patients in institutional care. The Oslo cohort had more prevalent delirium. Patients in Edinburgh were on more medication, and waited longer for their operation. This may be due to local operating practice, as hip fracture operations are performed “out of hours” in Oslo but not in Edinburgh (Table 4.2).

Figure 4.1

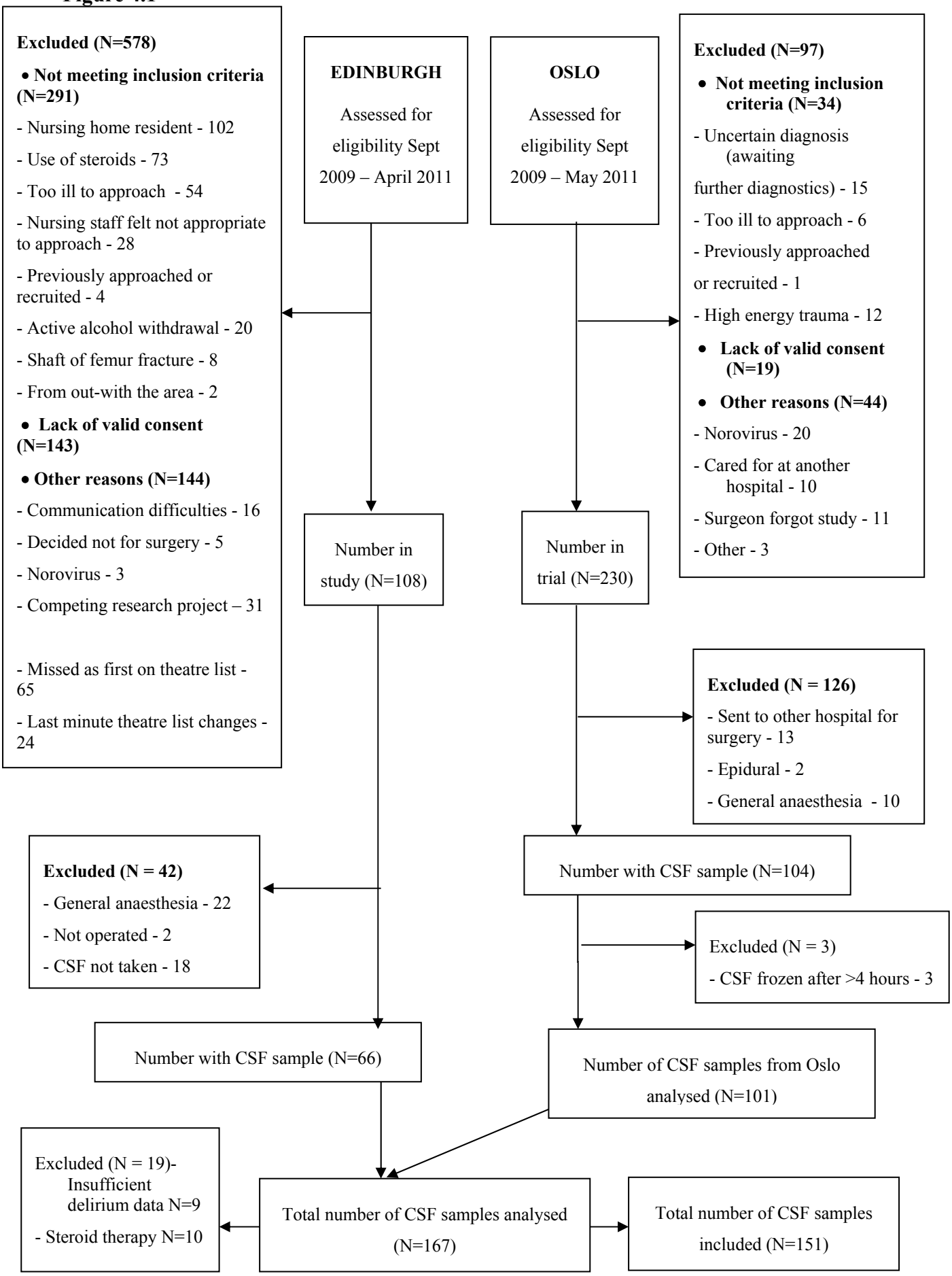

Excluded (N=578)
• Not meeting inclusion criteria (N=291)
- Nursing home resident - 102
- Use of steroids - 73
- Too ill to approach - 54
- Nursing staff felt not appropriate to approach - 28
- Previously approached or recruited - 4
- Active alcohol withdrawal - 20
- Shaft of femur fracture - 8
- From out-with the area - 2
• Lack of valid consent (N=143)
• Other reasons (N=144)
- Communication difficulties - 16
- Decided not for surgery - 5
- Norovirus - 3
- Competing research project – 31
- Missed as first on theatre list - 65
- Last minute theatre list changes - 24
EDINBURGH
Assessed for eligibility Sept 2009 – April 2011
OSLO
Assessed for eligibility Sept 2009 – May 2011
Excluded (N=97)
• Not meeting inclusion criteria (N=34)
- Uncertain diagnosis (awaiting further diagnostics) - 15
- Too ill to approach - 6
- Previously approached or recruited - 1
- High energy trauma - 12
• Lack of valid consent (N=19)
• Other reasons (N=44)
- Norovirus - 20
- Cared for at another hospital - 10
- Surgeon forgot study - 11
- Other - 3
Number in study (N=108)
Number in trial (N=230)
Excluded (N = 126)
- Sent to other hospital for surgery - 13
- Epidural - 2
- General anaesthesia - 10
Number with CSF sample (N=104)
Excluded (N = 42)
- General anaesthesia - 22
- Not operated - 2
- CSF not taken - 18
Excluded (N = 3)
- CSF frozen after >4 hours - 3
Number with CSF sample (N=66)
Number of CSF samples from Oslo analysed (N=101)
Excluded (N = 19)- Insufficient delirium data N=9
- Steroid therapy N=10
Total number of CSF samples analysed (N=167)
Total number of CSF samples included (N=151)

Table 4.1 Baseline medical, surgical and functional demographics of the combined study sample

	Delirium, N=68 Median (Interquartile range)	**No delirium, N=80** Median (Interquartile range)	**p value**	**Test statistic**
Age	84.5 years (IQR 79.3-88.0)	83.0 years (IQR 70.3-88.0)	0.084[a]	**2272[c]**
Female	N=46 (67.6%)	N=60 (75.0%)	0.323[b]	**0.978[d]**
IQCODE score	Total 59.0 (IQR 50.0-75.0)	Total 48.0 (IQR 48.0-53.0)	<0.001[a]	**1121[c]**
	Average 3.69 (IQR 3.13-4.69)	Average 3.00 (IQR 3.00-3.31)	<0.001[a]	**1131[c]**
Alcohol Units/week	0 (IQR 0-1)	0 (IQR 0-2)	0.077[a]	**2256[c]**
Type of accommodation	House or flat N=48 Sheltered housing N=3 Nursing home N=17	House or flat N=71 Sheltered housing N=6 Nursing home N=3	0.001[b]	**14.37[d]**
Independent in basic ADLs	N=30 (44.1%)	N=66 (82.5%)	<0.001[b]	**23.76[d]**
Katz basic ADL score /6 (Edinburgh)	6 (IQR 5-6)	6 (IQR 6-6)	0.034[a]	**401.5[c]**
Barthel basic ADL score /20 (Oslo)	16 (IQR 13-18)	19 (IQR 18-20)	<0.001[a]	**334.5[c]**
Number of medications	5 (IQR 2.25-7)	3.5 (IQR 1.25-5)	0.013[a]	**2075[c]**
Modified APACHE II score	9 (IQR 8-10.75)	8 (IQR 6-9)	<0.001[a]	**1756[c]**
Charlson Co-morbidity Index	1 (IQR 0-2)	1 (IQR 0-2)	0.037[a]	**2205[c]**
Type of fracture	Intracapsular N=43 Extracapsular N=23 Periprosthetic N=2	Intracapsular N=51 Extracapsular N=27 Periprosthetic N=2	0.986[b]	**0.028[d]**
Type of surgery	Hemiarthroplasty N=35 Internal fixation N=32 Total hip replacement N=0 No operation N=1	Hemiarthroplasty N=39 Internal fixation N=36 Total hip replacement N=5 No operation N=0	0.138[b]	**5.515[d]**
Waiting time for operation[e]	32.0 hours (IQR 17.6-43.5)	25.0 hours (IQR 19.0-39.6)	0.414[a]	**2407[c]**

[a]Mann-Whitney-U test, [b]Pearson Chi-square

[c]Mann-Whitney-U test statistic, [d]Pearson Chi-square test statistic

[e]Time from triage in Accident and Emergency to operation start time.

Table 4.2 Baseline medical, surgical and functional demographics of the Edinburgh and Oslo cohorts

	Edinburgh, N=66 Median (Interquartile range)	**Oslo, N=82** Median (Interquartile range)	**p value**	**Test statistic**
Age	83 years (IQR 76.8-87)	84.5 years (IQR 78.5-88.3)	0.299[a]	**2437[c]**
Female	N=44 (66.7%)	N=62 (75.6%)	0.230[b]	**1.439[d]**
Delirium[e]	Prevalent N=9 Incident N=16 Never N=41	Prevalent N=27 Incident N=14 Never N=38	0.017[b]	**8.147[d]**
IQCODE score	Total 48 (IQR 48-52) Average 3 (IQR 3-3.25)	Total 57 (IQR 48-74.8) Average 3.56 (IQR 3-4.67)	<0.001[a] <0.001[a]	**1286[c]** **1291[c]**
Alcohol Units/week	0 (IQR 0-5)	0 (IQR 0-1)	0.008[a]	**2061[c]**
Type of accommodation	House or flat N=61 Sheltered housing N=5 Nursing home N=0	House or flat N=58 Sheltered housing N=4 Nursing home N=20	<0.001[b]	**18.68[d]**
Independent in basic ADLs*	N=60 (90.9%)	N=36 (43.9%)	<0.001[b]	**35.45[d]**
Number of medications	5 (IQR 3-7.25)	3.5 (IQR 1-6)	0.004[a]	**1969[c]**
Modified APACHE II score*	8 (IQR 6.75-10)	8 (IQR 7-10)	0.167[a]	**2353[c]**
Charlson Co-morbidity Index	1 (IQR 0-2)	1 (IQR 0-2)	0.080[a]	**2275[c]**
Type of fracture	Intracapsular N=42 Extracapsular N=20 Periprosthetic N=4	Intracapsular N=52 Extracapsular N=30 Periprosthetic N=0	0.067[b]	**5.397[d]**
Type of surgery	Hemiarthroplasty N=33 Internal fixation N=29 Total hip replacement N=4 No operation N=0	Hemiarthroplasty N=41 Internal fixation N=39 Total hip replacement N=1 No operation N=1	0.328[b]	**3.446[d]**
Waiting time for operation[f]	37 hours (IQR 23-46)	23 hours (IQR 14-35)	<0.001[a]	**1573[c]**

[a]Mann-Whitney-U test, [b]Pearson Chi-square

[c]Mann-Whitney-U test statistic, [d]Pearson Chi-square test statistic

[e]Preoperative delirium status missing in 3 Oslo patients

[f]Time from triage in Accident and Emergency to operation start time.

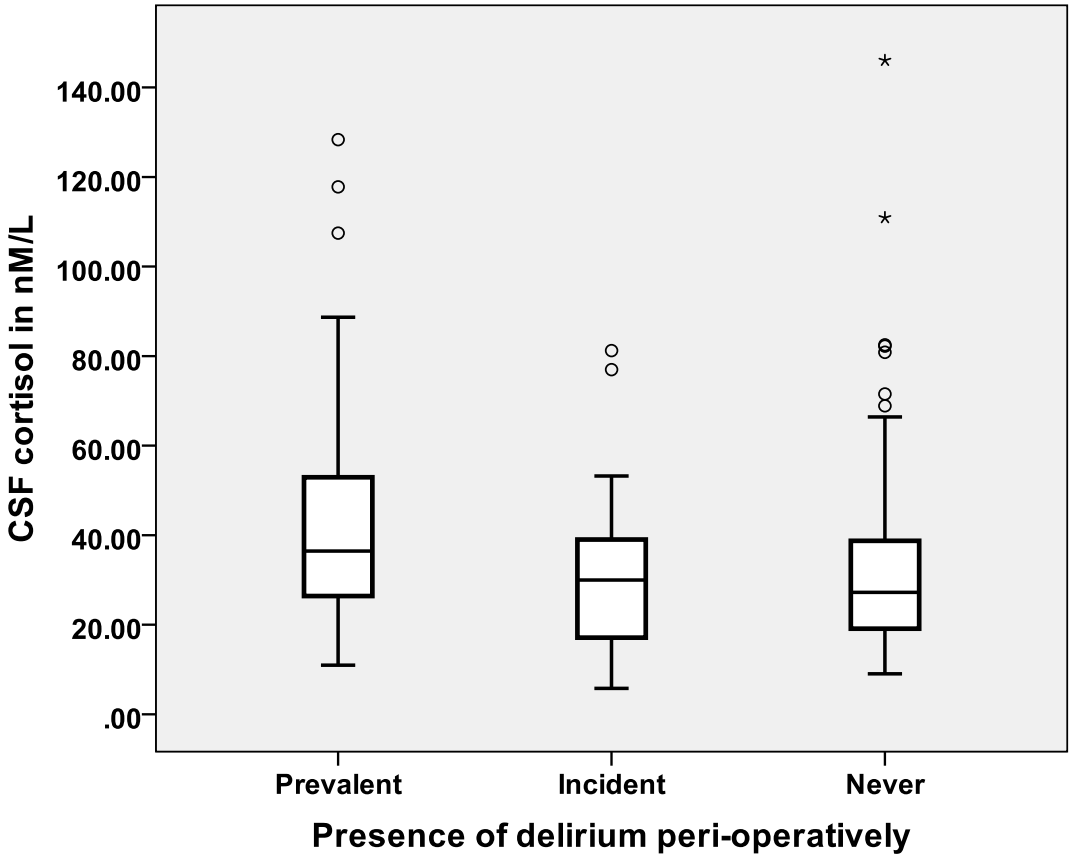
4.3.1 CSF cortisol

There was a trend to higher CSF cortisol in the group who ever developed delirium [median 33.3 nM/L (IQR 22.6-42.8) vs. 27.2 nM/L (18.9-39.0), Mann-Whitney-U test p=0.054]. Cerebrospinal fluid cortisol was significantly elevated in those with prevalent delirium [median 36.5 nM/L, (IQR 26.3-53.1)] compared to those who developed incident delirium [median 30.0 nM/L (IQR 16.8-39.2)] and those who never developed delirium [median 27.2 nM/L (IQR 18.8-39.3)], Kruskal-Wallis p=0.023 (Table 4.3, Figure 4.2). Post hoc pairwise comparisons revealed that this was due to the difference between prevalent delirium and never delirium, adjusted p=0.023.

A sub-group analysis revealed that in participants with evidence of prior dementia, there was no difference in CSF cortisol levels between delirium groups (Table 4.3). However, in the group without prior dementia, those with prevalent delirium [median 39.6 nM/L (IQR 35.4-53.3)] had a significantly elevated level of CSF cortisol compared to those with incident delirium [median 22.9 nM/L (IQR 13.6-33.7)] and those who never developed delirium [median 27.3 nM/L (IQR 18.3-41.5)], Kruskal-Wallis p=0.006 (Table 4.3). Post hoc pairwise comparisons revealed that this was due to the difference between prevalent delirium and never delirium (p=0.017) and the difference between prevalent delirium and incident delirium (p=0.008).

There was no difference in CSF cortisol level when participants with and without prior dementia were compared (Table 4.3). There was also no difference in CSF cortisol level between participants with mild or severe delirium (mild group (N=30) median 34.8 nM/L (IQR 24.9-47.8) vs. severe group (N=37) median 31.1 nM/L (IQR 22.6-46.9), Mann-Whitney U test p=0.880).

Figure 4.2



4.3.2 Serum cortisol

Matched preoperative serum samples were available for N=72 patients (N=63 Edinburgh patients, N=9 Oslo patients). There was no difference in serum cortisol levels in those with prevalent or incident delirium and those without delirium (Table 2).

Table 4.3 Cerebrospinal and serum cortisol levels according to delirium and dementia status

	Delirium Median (IQR)				Prior dementia Median (IQR)		
	Prevalent	Incident	Never	P value	Yes	No	P value
CSF Cortisol nM/L	36.5 (26.3-53.1) N=36	30.0 (16.8-39.1) N=30	27.2 (18.8-39.3) N=79	0.023[a]	30.8 (23.0-42.8) N=49	27.7 (19.5-40.9) N=91	0.186[b] MWU 1927
Serum Cortisol nM/L	513.9 (469.7-605.1) N=12	446.3 (397.9-625.2) N=17	484.5 (354.5-629.4) N=43	0.729[a]	497.4 (386.1-670.8) N=12	481.2 (387.9-605.6) N=55	0.659[b] MWU 303.0
CSF Cortisol nM/L, Prior dementia	30.9 (22.8-42.7) N=23	36.3 (17.1-52.1) N=15	27.2 (22.0-33.8) N=9	0.453[a]			
CSF Cortisol nM/L, No dementia	39.6 (35.4-53.3) N=13	22.9 (13.6-33.7) N=14	27.3 (18.3-41.5) N=64	0.006[a]			

[a]Kruskal-Wallis test

[b]Mann-Whitney U test

4.3.3 CSF Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone Sulphate (DHEAS)

There was no difference found in either CSF Log_{10} DHEA or Log_{10} DHEAS between the groups with prevalent or incident delirium and those without delirium (Table 4.4). There was also no difference in CSF Log_{10} DHEA or Log_{10} DHEAS between those with mild or severe delirium (data not shown). There was also no difference in CSF Log_{10} DHEA or Log_{10} DHEAS in those with and without prior dementia (Table 4.4). Subgroup analysis did not demonstrate any difference between delirium groups in those with and without prior dementia (Table 4.4). There was also no significant difference in the Log_{10} molar ratio of cortisol : DHEA + DHEAS between those who did and did not develop delirium (Table 4.4).

Table 4.4 Cerebrospinal fluid DHEA and DHEAS levels according to delirium and dementia status

	Delirium Mean (SD)				Prior dementia Mean (SD)		
	Prevalent	Incident	Never	p value	Yes	No	p value
CSF DHEA pg/ml Log_{10}	1.31 (0.61) N=33	1.28 (0.79) N=29	1.28 (0.78) N=73	0.983[a]	1.35 (0.63) N=47	1.27 (0.81) N=84	0.558[b] t=0.587
CSF DHEA pg/ml Log_{10} prior dementia	1.37 (0.54) N=22	1.37 (0.78) N=14	1.22 (0.66) N=9	0.817[a]			
CSF DHEA pg/ml Log_{10} no prior dementia	1.19 (0.76) N=11	1.20 (0.82) N=15	1.29 (0.80) N=64	0.866[a]			
CSF DHEAS pg/ml Log_{10}	2.20 (0.41) N=34	2.29 (0.51) N=29	2.30 (0.56) N=73	0.635[a]	2.26 (0.51) N=48	2.31 (0.53) N=84	0.614[b] t=-0.506
CSF DHEAS pg/ml Log_{10} prior dementia	2.22 (0.43) N=22	2.29 (0.60) N=14	2.25 (0.62) N=9	0.923[a]			
CSF DHEAS pg/ml Log_{10} no prior dementia	2.18 (0.38) N=12	2.29 (0.44) N=15	2.31 (0.56) N=64	0.706[a]			
Log_{10} Molar ratio CSF Cortisol:DHEA(S)	1.83 (0.39) N=34	1.59 (0.60) N=29	1.63 (0.62) N=73	0.157[a]	1.71 (0.51) N=48	1.63 (0.60) N=84	0.439[b] t=0.777

[a]One-way ANOVA

[b]Student's *t* test

4.3.4 Correlations between neurosteroids

CSF cortisol correlated highly with serum cortisol (Spearman's rho 0.746, p<0.001). There was no significant correlation between CSF cortisol and either CSF Log_{10} DHEA or DHEAS, but serum cortisol level correlated with CSF Log_{10} DHEA (Spearman's rho 0.323, p=0.010). CSF Log_{10} DHEA correlated with Log_{10} DHEAS (Spearman's rho 0.572, p<0.001).

4.3.5 CSF cortisol logistic regression analysis: adjusting for potential confounders

The demographic variables shown to be different between groups with and without delirium at any stage were independence in ADLs, number of medications, Charlson Co-morbidity

Index, modified APACHE II score and IQCODE score (Table 4.2). Study site was also included to ensure it did not introduce bias. A logistic regression model was built to adjust for the effects of these co-variates on the association between CSF cortisol level and delirium status, using an Enter method. Delirium at any stage was the dependent variable. The Wald statistic, odds ratios with 95% confidence intervals and p values for the logistic regression model are shown in Table 4.5, along with pseudo-R^2 values. A second model was built using the same co-variates but including cortisol as a dichotomous variable, split on the median (29.3nmol/L) (Table 4.6). In both models, modified APACHE II score and total IQCODE score remained significantly associated with delirium status. CSF cortisol as a continuous variable was not associated with ever delirium after adjusting for the co-variates, but a CSF cortisol level above the median was still associated with delirium. There was no evidence of collinearity in either model.

Table 4.5 Logistic regression model for CSF cortisol as a continuous variable

Co-variate	CSF cortisol model			
	Wald	OR[a]	95% CI[b] of OR[a]	p value
Study site	1.18	0.56	(0.20-1.59)	0.277
Independence in ADL[c]s	0.83	1.68	(0.55-5.17)	0.363
Number of medications	0.99	0.92	(0.79-1.08)	0.319
Charlson Comorbidity Index	0.04	0.97	(0.70-1.35)	0.852
Modified APACHE II	8.92	0.76	(0.64-0.91)	0.003
Total IQCODE score	13.93	0.89	(0.84-0.95)	<0.001
CSF cortisol (nmol/L)	0.11	1.00	(0.98-1.01)	0.746
Constant	16.72	8443		<0.001

This table shows the results of the multivariate logistic regression analysis for CSF cortisol as a continuous variable, built to adjust for potential confounding variables on delirium status. The dependent variable was delirium at any stage.

[a]OR: Odds ratio, [b]CI: Confidence interval. [c]ADLs: activities of daily living.

Pseudo-R^2 values 0.31 (Cox and Snell), 0.42 (Nagelkerke)

Table 4.6 Logistic regression model for CSF cortisol split on the median

Co-variate	CSF cortisol model			
	Wald	OR[a]	95% CI[b] of OR[a]	p value
Study site	0.66	0.64	(0.22-1.86)	0.415
Independence in ADL[c]s	0.20	1.30	(0.41-4.06)	0.655
Number of medications	1.51	0.90	(0.76-1.06)	0.219
Charlson Comorbidity Index	0.01	0.98	(0.70-1.37)	0.911
Modified APACHE II	8.21	0.77	(0.64-0.92)	0.004
Total IQCODE score	14.61	0.89	(0.84-0.95)	0.000
CSF cortisol above the median (yes/no)	3.94	0.42	(0.18-0.99)	0.047
Constant	19.03	39423		0.000

This table shows the results of the multivariate logistic regression analysis for CSF cortisol split on the median, built to adjust for potential confounding variables on delirium status. The dependent variable was delirium at any stage.

[a]OR: Odds ratio, [b]CI: Confidence interval. [c]ADLs: activities of daily living.

Pseudo-R^2 values 0.33 (Cox and Snell), 0.44 (Nagelkerke)

4.3.6 Logistic regression analysis: prediction of postoperative delirium

Logistic regression models were constructed to test whether levels of cortisol, DHEA, DHEAS or the Log_{10} Cortisol: (DHEA+DHEAS) ratio predicted the development of postoperative delirium. Participants who developed delirium preoperatively were excluded and the development of postoperative delirium was the dependent variable. Co-variates were chosen from the results of the univariate analysis and risk factors for delirium from the literature. A Forward (Wald) selection method was used. Total IQCODE score and modified APACHE II score were the only co-variates that predicted the development of postoperative delirium, and none of the biomarkers predicted delirium (Tables 4.7a – 4.7d). There was no evidence of collinearity.

Table 4.7a Logistic regression model for prediction of postoperative delirium by CSF cortisol

Co-variate	Wald	OR[a]	p value
Modified APACHE II	5.82	0.80	0.016
Total IQCODE score	11.77	0.91	0.001
Constant	18.19	2854.7	<0.001

Variables not in equation

Co-variate	Score	p value
Age	0.640	0.424
Gender	0.545	0.460
Independence in ADLs[b]	0.845	0.358
Number of medications	2.295	0.130
CCI[c]	0.038	0.846
Alcohol intake (units/week)	3.711	0.054
Fracture type	0.061	0.804
Operation type	0.827	0.363
Waiting time for surgery	1.396	0.237
CSF cortisol	0.392	0.531

These tables shows the results of the multivariate logistic regression analysis for CSF cortisol as a continuous variable, built to determine whether CSF cortisol level was predictive of delirium development during the postoperative period. The dependent variable was delirium developed postoperatively. The percentage of patients who would be correctly identified as delirious was 74.8%

[a]OR: Odds ratio, [b]ADLs: activities of daily living, [c]CCI: Charlson co-morbidity index

Pseudo-R^2 values 0.17 (Cox and Snell), 0.24 (Nagelkerke)

Table 4.7b Logistic regression model for prediction of postoperative delirium by CSF DHEA

Co-variate	Wald	OR[a]	p value
Modified APACHE II	5.25	0.81	0.022
Total IQCODE score	10.53	0.91	0.001
Constant	16.34	1772.6	<0.001

Variables not in equation

Co-variate	Score	p value
Age	1.044	0.307
Gender	0.392	0.531
Independence in ADLs[b]	0.691	0.406
Number of medications	1.589	0.208
CCI[c]	0.020	0.889
Alcohol intake (units/week)	3.632	0.057
Fracture type	0.064	0.800
Operation type	1.277	0.259
Waiting time for surgery	1.440	0.230
CSF DHEA	2.253	0.133

These tables shows the results of the multivariate logistic regression analysis for CSF DHEA as a continuous variable, built to determine whether CSF DHEA level was predictive of delirium development during the postoperative period. The dependent variable was delirium developed postoperatively. The percentage of patients who would be correctly identified as delirious was 74.2%

[a]OR: Odds ratio, [b]ADLs: activities of daily living, [c]CCI: Charlson co-morbidity index

Pseudo-R^2 values 0.16 (Cox and Snell), 0.22 (Nagelkerke)

Table 4.7c Logistic regression model for prediction of postoperative delirium by CSF DHEAS

Co-variate	Wald	OR[a]	p value
Modified APACHE II	5.25	0.81	0.022
Total IQCODE score	10.53	0.91	0.001
Constant	16.34	1772.6	<0.001

Variables not in equation

Co-variate	Score	p value
Age	1.044	0.307
Gender	0.392	0.531
Independence in ADLs[b]	0.691	0.406
Number of medications	1.589	0.208
CCI[c]	0.020	0.889
Alcohol intake (units/week)	3.632	0.057
Fracture type	0.064	0.800
Operation type	1.277	0.259
Waiting time for surgery	1.440	0.230
CSF DHEAS	0.224	0.636

These tables shows the results of the multivariate logistic regression analysis for CSF DHEAS as a continuous variable, built to determine whether CSF DHEAS level was predictive of delirium development during the postoperative period. The dependent variable was delirium developed postoperatively. The percentage of patients who would be correctly identified as delirious was 74.2%

[a]OR: Odds ratio, [b]ADLs: activities of daily living, [c]CCI: Charlson co-morbidity index

Pseudo-R^2 values 0.16 (Cox and Snell), 0.22 (Nagelkerke)

Table 4.7d Logistic regression model for prediction of postoperative delirium by CSF Log_{10} Cortisol: (DHEA+DHEAS) ratio

Co-variate	Wald	OR[a]	p value
Modified APACHE II	5.25	0.81	0.022
Total IQCODE score	10.53	0.91	0.001
Constant	16.34	1772.6	<0.001

Variables not in equation

Co-variate	Score	p value
Age	1.044	0.307
Gender	0.392	0.531
Independence in ADLs[b]	0.691	0.406
Number of medications	1.589	0.208
CCI[c]	0.020	0.889
Alcohol intake (units/week)	3.632	0.057
Fracture type	0.064	0.800
Operation type	1.277	0.259
Waiting time for surgery	1.440	0.230
CSF Log10 Cortisol: (DHEA+DHEAS) ratio	0.148	0.700

These tables shows the results of the multivariate logistic regression analysis for CSF Log10 Cortisol: (DHEA+DHEAS) ratio as a continuous variable, built to determine whether CSF Log10 Cortisol: (DHEA+DHEAS) ratio was predictive of delirium development during the postoperative period. The dependent variable was delirium developed postoperatively. The percentage of patients who would be correctly identified as delirious was 74.2%

[a]OR: Odds ratio, [b]ADLs: activities of daily living, [c]CCI: Charlson co-morbidity index

Pseudo-R^2 values 0.16 (Cox and Snell), 0.22 (Nagelkerke)

4.4 Discussion

This relatively large study provides further evidence to support a role for elevated central nervous system (CNS) cortisol in the pathogenesis of delirium. Cortisol was found to be elevated in CSF where delirium was current, but there was no elevation in baseline serum cortisol. There was no evidence to support the hypotheses that levels of CSF DHEA and DHEAS are reduced or that the ratio of cortisol:DHEA(S) is elevated.

A role for CNS cortisol in delirium pathogenesis is plausible. The relationship between delirium and cortisol is complex with potentially bidirectional change and the influence of illness severity and pre-existing cognitive impairment. Both psychological and physical stress will undoubtedly provoke a cortisol response and are known to precipitate delirium (MacLullich, Ferguson et al. 2008). Delirium itself may also be stressful, due to consequent disorientation, sleep disturbance and psychotic experiences, which could also cause cortisol levels to rise.

The major source of CNS cortisol has always thought to have been diffusion from the blood, with a small amount of local synthesis (Bellavance and Rivest 2014). Recently in rodent models, substantial local regeneration from inert corticosterone by 11β-HSD-1 in the brain has been demonstrated, (Yau, Wheelan et al. 2015). However, the degree of local regeneration or recycling between active and inactive isoforms in humans is less clear. A recent study in eight healthy adult men found no detectable interconversion between cortisol and cortisone (Kilgour, Semple et al. 2015). Glucocorticoid transporters are present in different brain regions but do not play a major role in the access of cortisol to the brain (Mason, Pariante et al. 2010). Levels in CSF are usually around 5-10% of those in the periphery, and sudden peripheral rises in cortisol in response to stress may lead to disproportionate rises in CSF cortisol due to the limited binding capacity of corticosteroid-binding globulin. Here there was elevated CSF cortisol in active delirium but no such elevation in the periphery (in the presence of a good correlation between CSF and serum cortisol, suggesting the relationship between them was maintained). This may have been due to a disproportionate rise in CSF cortisol in response to stress, greatest in those with delirium, perhaps exacerbated by the fall in corticosteroid-binding globulin with older age (Herbert, Goodyer et al. 2006). It could also have plausibly been due to increases in blood-brain barrier permeability due to the acute stresses of hip fracture, consequent immobilisation and surgery,

since stress has been shown to promote prolonged blood-brain barrier disruption in various CNS disorders (Tomkins, Kaufer et al. 2001). However, it may also have been due to smaller numbers in the serum substudy, where the highest levels were seen in the group with prevalent delirium. The outcome of ongoing exposure of the brain to elevated cortisol levels in delirium is likely to be detrimental at various levels, leading to increased neuronal vulnerability to cell damage and death from various insults, including glutamate excess, hypoxia, hypoglycaemia and oxidative stress (Kimonides, Spillantini et al. 1998, Herbert, Goodyer et al. 2006).

CSF cortisol was found to be significantly elevated in active (prevalent) delirium, but not in postoperative (incident) delirium compared to those without delirium, and CSF cortisol levels at the time of operation did not predict the development of postoperative delirium. These results suggest that either delirium needs to be established to see a rise in cortisol, that the stress of delirium further activates the HPA axis, or that postoperative delirium has a different mix of pathological mechanisms to the (potentially more heterogeneous) delirium seen preoperatively. These mechanisms might include postoperative dehydration, or the effects of anaesthesia or other perioperative drugs. It is unknown whether delirium has any effect on CNS 11-βHSD-1, as this has never been studied. The ratio of cortisol : corticosone in CSF in those with and without delirium could be measured in the future, as for example increased activity of the enzyme in delirium as a contributor towards increased CNS cortisol would represent a potential therapeutic target.

As would be expected from the literature, prior dementia was associated with development of delirium in this cohort, with higher IQCODE scores in the delirium group and prior dementia remaining significantly associated with delirium in the logistic regression analysis. However, an intriguing finding of the present study is that CSF cortisol remained elevated in those with prevalent delirium after excluding patients with evidence of prior dementia; in fact, the sub-group analysis showed that those patients with no prior dementia and active delirium had particularly high CSF cortisol levels. This relationship disappeared for those with prior dementia. One might have predicted that it would be the group with delirium super-imposed on dementia who would have the greatest rise in CSF cortisol, as there is evidence of impaired negative feedback of the HPA axis in dementia (Robertsson, Blennow et al. 2001). Perhaps delirium is most stressful for those with a usually normal “operating system”, or perhaps it takes the greatest, disordered, rise in cortisol to provoke delirium in a healthy

brain. These results in CSF differ from the findings in serum of Bisschop *et al* (Bisschop, de Rooij et al. 2011), where serum cortisol was found to be elevated in delirium after hip fracture, but not after controlling for prior cognition. This may be due to differing cortisol levels or kinetics in the CNS and the periphery, or that dementia causes or associates with more 11β-HSD1 in the CNS, as is the case in aged, cognitively impaired rodents (Holmes, Carter et al. 2010).

The levels of cortisol demonstrated here in CSF were lower than those found by Pearson *et al* (Pearson, de Vries et al. 2010) in delirium after hip fracture. This may be due to the Pearson study comprising a more unwell or delirious cohort, or the difference in subject numbers. The CSF cortisol levels in the present study were slightly higher than elderly patients with cognitive impairment and healthy elderly controls (Johansson, Johansson et al. 2011), and 3-4 fold lower than in bacterial meningitis (Beran, Dzupova et al. 2011). Different assay techniques may mean that individual studies are difficult to compare, however.

The hypothesis that CSF levels of DHEA and DHEAS would be low in delirium was not supported by this study. CSF DHEA and DHEAS were strongly correlated, which supports the validity of the assay. The study also did not find a difference in the cortisol : DHEA(S) ratio, and cortisol was not correlated with DHEA nor DHEAS, suggesting there may not be a significant relationship between the neurosteroids in this setting. The untransformed median levels of DHEAS found in CSF here were lower than those demonstrated in stroke and vascular dementia (Azuma, Nagai et al. 1999), and levels of DHEA were also lower than those found in Alzheimer's dementia and controls (Naylor, Hulette et al. 2008), although different assay techniques may impact on this.

Some limitations of the study must be acknowledged. Delirium was not assessed for every day or more than once per day in the postoperative period, in order not to over-burden participants; however, this may have missed some brief episodes of delirium. Some participants who were assessed preoperatively as not delirious may also have developed delirium in the time between assessment and surgery; every effort was made to assess patients close to surgery, but this was not always possible. The cohort comprises patients from studies at two centres. Although both cohorts are elderly hip fracture patients, differing exclusion criteria due to the objectives of the parent studies have meant that the Oslo cohort is slightly "frailer". There was however no difference in any of the biomarkers between

groups, and study site was not shown to have caused any significant bias in the logistic regression analysis. I believe that combining both cohorts greatly increases the power of the study and the conclusions drawn.

This study confirms the earlier finding of elevated CSF cortisol in prevalent delirium (Pearson, de Vries et al. 2010), in a much larger cohort of patients with acute hip fracture. It suggests that elevated CNS levels are associated with active delirium, but do not predict postoperative delirium. High levels of CSF cortisol seem still to be associated with delirium after controlling for the influence of prior dementia, but more work is needed in this complex area, and further work is needed examining CSF cortisol levels in delirium in different populations. The findings of this study also provide support for a potential means for delirium prevention or treatment, for example using 11β-Hydroxysteroid dehydrogenase type-1 inhibitors to block intracellular amplification of glucocorticoid levels.

5 Chapter 5: Exploratory multiplex analysis of CSF levels of inflammatory cytokines in delirium after hip fracture

5.1 Introduction

Delirium is an acute and distressing neuropsychiatric disorder. It is highly prevalent in multiple hospital settings, but particularly in older patients, affecting 11-42% of medical in-patients, 4-53% of hip fracture patients and 10-80% of intensive care patients (Siddiqi, House et al. 2006, Bruce, Ritchie et al. 2007, Ouimet, Kavanagh et al. 2007). There are several associated adverse outcomes, including an increased risk of institutionalisation (Siddiqi, House et al. 2006) and of subsequent dementia (MacLullich, Beaglehole et al. 2009, Witlox, Eurelings et al. 2010), and it is associated with increased mortality independent of age, gender, co-morbid illness and baseline dementia (Witlox, Eurelings et al. 2010). The pathophysiology of delirium has been challenging to study and remains poorly understood, and there are currently no formal specific treatments for delirium.

There has been interest in the potential role of an abnormal inflammatory response in delirium (Perry, Cunningham et al. 2007, MacLullich, Ferguson et al. 2008). This has been discussed in detail in section 1.6. Inflammatory processes are complex and involve both cellular and humoral components. Peripheral infection is a common precipitant of delirium, although the pathway from a seemingly benign peripheral infection in an elderly patient, without significant systemic upset, to the neuropsychiatric symptoms of delirium is unclear. Sepsis is also a common precipitant of ICU delirium in young, fit patients as well as older patients (Ebersoldt, Sharshar et al. 2007). Trauma, surgery and other insults also lead to an inflammatory response, as discussed in more detail in section 1.10.4.

Animal modelling has shown that a rodent with a "fragile" brain such as an older rodent or one with neurodegenerative disease such as the ME7 prion disease model mouse, has "primed" microglia (Godbout, Chen et al. 2005, Cunningham, Campion et al. 2009). These primed microglia, the brain's resident macrophages, are in a state of enhanced readiness compared with non-primed microglia, and when these ME7 animals are given a peripheral injection of the bacterial endotoxin lipopolysaccharide, to mimic peripheral infection, they respond with an exaggerated, prolonged sickness behaviour and a transient deterioration in

cognition, and a greater central nervous system (CNS) inflammatory response (Godbout, Chen et al. 2005, Cunningham, Campion et al. 2009).

Direct administration of cytokines used for cancer chemotherapy, such as Interferon (IFN)-α (Capuron, Ravaud et al. 2004) and Interleukin (IL)-2 (Rosenberg, Lotze et al. 1989, Walker, Wesnes et al. 1996, Walker, Walker et al. 1997), can lead to acute mental status change, such as disorientation, somnolence, reduced reaction times (Rosenberg, Lotze et al. 1989, Walker, Wesnes et al. 1996, Walker, Walker et al. 1997) and depressive symptoms (Capuron, Ravaud et al. 2004).

Raised serum levels of IL-6 and IL-8 have been found in patients with delirium following acute hip fracture (van Munster, Korevaar et al. 2008). There have been very few previous CSF studies of inflammation in delirium (Hall, Shenkin et al. 2011). Katsumata *et al* found elevated CSF IL-6 in those with delirium secondary to neuropsychiatric manifestations of Systemic Lupus Erythematosus (Katsumata, Harigai et al. 2007). A previous pilot study (n=36) found elevated CSF IL-8 in patients with delirium following acute hip fracture (MacLullich, Edelshain et al. 2011). More recently, Westhoff *et al* have found that in patients with acute hip fracture without preoperative delirium, those who went on to develop delirium postoperatively had lower levels of CSF IL-6, IL-1ra and Flt-3L (FMS-like tyrosine kinase 3 ligand) than those who did not (Westhoff, Witlox et al. 2013). A comprehensive review of studies of the inflammatory response in delirium is found in section 1.6.6-1.6.7.

In this exploratory study a multiplex assay approach was used to ask the following research questions: :

1) Are levels of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-7, IL-8, IL-12p70, Interferon (IFN)-γ, Granulocyte macrophage colony stimulating factor (GM-CSF) and Tumour Necrosis Factor (TNF)-α) elevated in CSF in delirium after hip fracture?

2) Are levels of anti-inflammatory cytokines (IL-4, IL-5, IL-10, IL-13) reduced in CSF in delirium after hip fracture?

Post hoc subgroup analyses also asked the following question:

1) Is there greater elevation in pro-inflammatory cytokines in those with delirium superimposed on dementia compared to delirium only, dementia only or neither?

5.2 Methods

5.2.1 Participant recruitment

Participants with acute hip fracture aged over 60 were recruited in an opportunistic manner by researchers in two related studies. The first was in the Orthopaedic Trauma Unit at the Royal Infirmary of Edinburgh. The second was in the Emergency Department of Oslo University Hospital and was part of a trial of orthogeriatric versus standard orthopaedic care following hip fracture (Oslo Orthogeriatric Trial, OOT) (Watne, Torbergsen et al. 2014). Details of subjects in each study are as follows:

Edinburgh study (CDHIP): Inclusion criteria were age over 60 and preoperative with acute hip fracture. Exclusion criteria were: nursing home resident, significant Parkinson's disease, major communication difficulties (aphasia, English not first language), those taking inhaled or oral steroids in the last 10 weeks, moribund on admission. I approached and recruited all participants.

Oslo study (OOT): Fractures as a result of high energy or multi-trauma were excluded. As with the Edinburgh study, those taking inhaled or oral steroids in the last 10 weeks (retrospective exclusion for the purposes of the present analysis), or who were moribund on admission were excluded. Participants were approached and recruited by members of the Orthopaedic trauma team.

On both sites, written informed consent was obtained from participants or their nominated next of kin if capacity to give informed consent was lacking. Ethical approval was obtained from Scotland A Research Ethics Committee (reference no. 09/MRE00/28) and the Regional Committee for Ethics in Medical and Health Research, Norway.

Seventy-six participants (N=46 from Edinburgh and N=30 from Oslo) were age-matched, 50% who ever developed delirium during the perioperative period and 50% who never developed delirium. For financial reasons, only one Luminex plate could be purchased at the time. Therefore only N=76 participants were included and were age-matched to remove one potential confounding factor.

5.2.2 Delirium assessment

In both sites, participants were assessed for delirium preoperatively, using a semi-structured interview with cognitive testing, review of casenotes and discussion with nursing or medical staff to complete the Confusion Assessment Method (Inouye, Vandyck et al. 1990) (CAM). The specific additional assessments in each site were as follows:

Edinburgh: Conscious level was assessed with the Observational Scale of Level of Alertness and Richmond Agitation Sedation Scale, there was additional assessment of attention (Edinburgh Delirium Test Box 1 (Brown, Fordyce et al. 2010)) and cognitive testing was with the Mini-Mental State Examination (Folstein, Folstein et al. 1975) with assessment of delirium severity using the Delirium Rating Scale Revised-98 (Trzepacz, Mittal et al. 2001). I performed all in-patient delirium assessments.

Oslo: Additional cognitive testing and assessment of delirium severity was with the Memorial Delirium Assessment Scale (Breitbart, Rosenfeld et al. 1997). Patients were classified as delirious if they scored positive on the CAM and fulfilled DSM IV criteria. Assessments were performed by a Geriatrician and small team of research nurses.

In Edinburgh, participants were again assessed for delirium daily from postoperative days 1-4 and on days 7 and once between days 10-14 or until transfer to a rehabilitation unit or discharge from hospital if these occurred sooner. In Oslo, participants were assessed daily until postoperative day 5 or until delirium-free.

5.2.2.1 Diagnosis of delirium

In both sites, patients were classified as delirious if they fulfilled DSM-IV criteria, informed by the CAM assessment process.

5.2.3 Assessment of prior dementia, function and co-morbidities

The Informant Questionnaire on Cognitive Decline in the Elderly (Jorm and Jacomb 1989) (IQCODE), a well-validated and widely used measure of pre-morbid cognition, was completed during a meeting with next of kin or other appropriate informant. I completed these in Edinburgh and Leiv Otto Watne in Oslo. A cut-off score of ≥3.44 (average score) was used to indicate evidence of probable prior dementia (Harwood, Hope et al. 1997).

Illness severity was estimated with the Age, Physiology and Chronic Health Evaluation (APACHE) II score (Knaus, Draper et al. 1985), which was modified in both centres in that: arterial PO_2 was not recorded in either centre because arterial blood gas sampling is not routine in patients with hip fracture. In addition in Oslo, haematocrit was not recorded as it is not routinely reported locally. Chronic disease burden was estimated with the Charlson Co-morbidity Index (Charlson, Pompei et al. 1987). Independence in personal activities of daily living was assessed with the Katz index of PADL (Katz, Ford et al. 1963) in Edinburgh, as discussed in section 2.6.3, and with the Barthel index (Mahoney and Barthel 1965) in Oslo. To harmonise these two scales, independence in PADL was defined as a Katz category A or B, or a Barthel score of ≥19. Information was also collected on type of fracture and the operation performed.

5.2.4 Sample collection

At the time of the operation to repair the hip fracture, if this was performed under spinal anaesthetic, the anaesthetist collected a sample of CSF after placement of the spinal needle and prior to the injection of anaesthetic agents. The sample was placed in a polypropylene universal container and put on ice. Samples were centrifuged as soon as possible at 1000 rpm, 4 ^{o}C for 10 minutes and stored in aliquots in polypropylene eppendorfs at -80 ^{o}C until assayed.

5.2.5 Cerebrospinal fluid assay

A high sensitivity Milliplex MAP human cytokine assay (HSCYTO 13-plex, Merck Millipore) was performed using a Luminex 200 reader (Biorad), as described in section 2.9.3. I completed the assay in Edinburgh. Samples from each participant were measured in singlicate due to sample availability and cost. The Luminex assay measured a panel of 13 cytokines, comprising IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, Interferon (IFN)-γ, Granulocyte macrophage colony stimulating factor (GM-CSF) and Tumour Necrosis Factor (TNF)-α. Assay characteristics are described in section 2.9.3.

5.2.6 Statistical analysis

Normality of distribution of the results was checked with histogram and Kolgomorov-Smirnov test. Any non-normally distributed cytokines were Log_{10} transformed. Group comparisons were performed with Student's *t* test for normally distributed variables, with Mann-Whitney U test for non-parametric continuous variables, and Pearson Chi-squared for

categorical variables. Results are expressed as mean (standard deviation) or median (interquartile range). Correlations were tested using Pearson correlation between raw cytokine values (if normally distributed) or log-transformed cytokine values (if non-normally distributed), to see whether members of the same cytokine groups changed together. In order to adjust for potential confounders, a logistic regression analysis model was built for CSF IL-5, using preoperative delirium as the dependent variable, using an Enter method. Co-variates were chosen if they were shown to be different between groups in the univariate analyses. The Statistical Package for the Social Sciences (SPSS) version 19.0 was used for data analysis. A value of $p<0.05$ for each analyses was considered significant, because the analyses were pre-specified.

5.3 Results

Seventy-six participants were age-matched (N=46 from Edinburgh, N=30 from Oslo), 24 men and 52 women. Table 5.1 shows the baseline characteristics of the combined sample. Median age was 83.5 years (IQR 77.8-87.3) in the delirium group and 83.5 years (IQR 78.0-88.0) in the no delirium group (p=0.831). Twenty-three participants developed delirium preoperatively, and 38 (50%) developed delirium at any stage in the perioperative period. Twenty participants had evidence of prior dementia (known diagnosis of dementia and/or IQCODE ≥3.44). The delirium group were more likely to have evidence of prior dementia, they were less functionally independent, and had higher acute illness severity (modified APACHE II) and greater chronic illness burden (CCI).

IL-1β was not detected by the assay, IL-2 was detected in 14/76 patients, IL-4 in 1/76 patients, IL-5, IL-6 and IL-8 in 76/76, IL-7 in 75/76, IL-10 in 71/76, IL-12p70 and IL-13 in 2/76, IFN-γ in 69/76, GM-CSF in 11/76 and TNF-α in 52/76. Further analysis was therefore not possible for IL-1β, IL-2, IL-4, IL-12p70, IL-13 or GM-CSF. Where the remaining cytokines were not detected, the manufacturer's reported lower limit of detection was used. Analyses were also performed with undetected cytokines set at 0pg/ml, and both approaches resulted in the same results in all cases.

No difference was found between those with and without preoperative delirium for the majority of the detected cytokines (Table 5.2). The only statistically significant difference was that IL-5 was lower in the group with preoperative delirium [mean 0.770 pg/ml (SD 0.342)] than in the group without [mean 1.013 pg/ml (SD 0.506)], p=0.045. There was no difference found in any of the cytokines between those who developed delirium at any stage in the perioperative period compared to those who did not (Table 5.2). IL-8 was higher in those with delirium preoperatively compared to those without delirium, but this was not significant. Levels of IL-6 were very similar between groups.

When groups with and without prior dementia were compared, regardless of delirium status, no difference was found for any of the detected cytokines between groups (Table 5.3).

A subgroup analysis of the groups with and without prior cognitive impairment was also performed. In those with prior dementia, no difference was found for any of the cytokines

between those with and without delirium (Table 5.4a). In those with no evidence of prior dementia, there was also no difference found for any of the cytokines between those with and without delirium (Table 5.4b).

Pearson's correlation between the cytokines revealed that many of the cytokines correlated positively with each other. The pro-inflammatory cytokines IL-6 and TNF-α correlated significantly, and also correlated with the pro-inflammatory chemokine IL-8. The T-helper 2 cytokines IL-5 and IL-10 correlated with each other. However, the T-helper 1 cytokines IL-7 and IFNγ did not correlate significantly with each other.

Table 5.1 A table showing the baseline characteristics of patients with and without delirium at any stage

	Delirium, N=38 Median (Interquartile range)	**No delirium, N=38** Median (Interquartile range)	**p value**	**Test statistic**
Age	83.5 years (IQR 77.8-87.3)	83.5 years (IQR 78.0-88.0)	0.831[a]	**702[c]**
Female	N=23 (61%)	N=29 (76%)	0.139[b]	**2.19[d]**
IQCODE score	Total 52.5 (IQR 48.8-61.0)	Total 48.0 (IQR 48.0-50.5)	0.001[a]	**395[c]**
	Average 3.28 (IQR 3.05-3.81)	Average 3.00 (IQR 3.00-3.16)	0.001[a]	**404[c]**
Alcohol Units/week	0 (IQR 0-4.25)	0 (IQR 0-2.50)	0.972[a]	**719[c]**
Type of accommodation	House or flat N=34	House or flat N=34	0.172[b]	**5.00[d]**
	Sheltered housing N=1	Sheltered housing N=3		
	Nursing home N=3	Nursing home N=0		
	Hospital N=0	Hospital N=1		
Independent in personal ADLs	N=22 (58%)	N=32 (84%)	0.011[b]	**6.40[d]**
Katz basic ADL score /6 (Edinburgh)	6 (IQR 5-6)	6 (IQR 6-6)	0.030[a]	**194[c]**
Barthel basic ADL score /20 (Oslo)	18 (IQR 15-19)	19 (IQR 18-20)	0.023[a]	**58.0[c]**
Modified APACHE II score	9.00 (IQR 7.75-11.00)	8.00 (IQR 6.00-11.00)	0.049[a]	**535[c]**
Charlson Co-morbidity Index	1.00 (IQR 0.75-2.25)	0.50 (IQR 0-1.25)	0.011[a]	**488[c]**
Type of fracture	Intracapsular N=28	Intracapsular N=23	0.292[b]	**2.46[d]**
	Extracapsular N=8	Extracapsular N=14		
	Periprosthetic N=2	Periprosthetic N=1		
Type of surgery	Hemiarthroplasty N=24	Hemiarthroplasty N=16	0.069[b]	**5.36[d]**
	Internal fixation N=14	Internal fixation N=19		
	Total hip replacement N=0	Total hip replacement N=3		

[a]Mann-Whitney-U test, [b]Pearson Chi-square

[c]Mann-Whitney-U test statistic, [d]Pearson Chi-square test statistic

Table 5.2 CSF levels of cytokines in patients with and without delirium preoperatively, and delirium at any stage. Results expressed as mean (standard deviation), Student's t test p value.

Cytokine	Preoperative delirium, mean (SD)			Delirium at any stage, mean (SD)		
	Yes N=23	No N=53	p value	Yes N=38	No N=38	p value
IL-5, pg/ml	0.77 (0.34)	1.01 (0.51)	**0.045**	0.86 (0.33)	1.02 (0.58)	0.150
IL-6, pg/ml	29.4 (24.0)	28.3 (51.3)	0.882	29.7 (26.2)	27.5 (32.1)	0.747
IL-7, pg/ml	2.19 (1.08)	2.68 (1.35)	0.129	2.42 (1.18)	2.65 (1.39)	0.444
IL-8, pg/ml	135.8 (117.0)	118.7 (72.8)	0.440	126.7 (96.8)	121.1 (79.5)	0.785
Log_{10} IL-10, pg/ml	0.74 (0.70)	0.66 (0.58)	0.602	0.75 (0.58)	0.63 (0.65)	0.416
IFN-γ, pg/ml	4.45 (8.36)	3.24 (2.32)	0.331	3.91 (6.60)	3.31 (2.46)	0.599
Log_{10} TNF-α, pg/ml	-0.55 (0.64)	-0.68 (0.50)	0.346	-0.58 (0.58)	-0.70 (0.52)	0.342

IL: Interleukin

IFN: Interferon

TNF: Tumour Necrosis Factor

Table 5.3 CSF levels of cytokines in patients with and without evidence of prior dementia according to IQCODE ≥3.44. Results expressed as mean (standard deviation), Student's t test p value.

Cytokine	Prior dementia (IQCODE ≥3.44), mean (SD)		
	Yes N=20	No N=55	p value
IL-5, pg/ml	0.91 (0.34)	0.95 (0.52)	0.755
IL-6, pg/ml	20.4 (15.5)	32.0 (32.3)	0.128
IL-7, pg/ml	2.28 (1.25)	2.63 (1.31)	0.303
IL-8, pg/ml	115.4 (55.8)	126.9 (98.2)	0.622
Log_{10} IL-10, pg/ml	0.73 (0.45)	0.68 (0.67)	0.773
IFN-γ, pg/ml	2.53 (2.11)	4.00 (5.65)	0.260
Log_{10} TNF-α, pg/ml	-0.673 (0.417)	-0.618 (0.589)	0.704

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly

IL: Interleukin

IFN: Interferon

TNF: Tumour Necrosis Factor

Table 5.4a Subgroup analysis of CSF levels of cytokines in patients with evidence of prior dementia (IQCODE ≥3.44), with and without delirium preoperatively, and with and without delirium at any stage. Results expressed as mean (standard deviation), Student's t test p value.

Cytokine	Group with prior dementia. Preoperative delirium, mean (SD)			Group with prior dementia. Delirium at any stage, mean (SD)		
	Yes N=11	No N=9	p value	Yes N=16	No N=4	p value
IL-5, pg/ml	0.81 (0.33)	1.03 (0.34)	0.153	0.88 (0.31)	1.02 (0.49)	0.512
IL-6, pg/ml	18.7 (16.9)	22.6 (14.3)	0.590	20.8 (16.3)	19.1 (13.8)	0.848
IL-7, pg/ml	1.80 (0.89)	2.86 (1.42)	0.058	2.22 (1.33)	2.50 (0.98)	0.701
IL-8, pg/ml	109.7 (59.3)	122.3 (53.8)	0.630	112.6 (52.4)	126.4 (75.9)	0.670
Log_{10} IL-10, pg/ml	0.69 (0.54)	0.78 (0.34)	0.658	0.66 (0.48)	1.00 (0.12)	0.193
IFN-γ, pg/ml	2.54 (2.30)	2.51 (1.99)	0.974	2.49 (2.23)	2.68 (1.80)	0.877
Log_{10} TNF-α, pg/ml	-0.74 (0.47)	-0.59 (0.34)	0.457	-0.70 (0.46)	-0.55 (0.11)	0.540

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly

IL: Interleukin

IFN: Interferon

TNF: Tumour Necrosis Factor

Table 5.4b Subgroup analysis of CSF levels of cytokines in patients without evidence of prior dementia (IQCODE <3.44), with and without delirium preoperatively, and with and without delirium at any stage. Results expressed as mean (standard deviation), Student's t test p value.

Cytokine	Group without prior dementia. Preoperative delirium, mean (SD)			Group without prior dementia. Delirium at any stage, mean (SD)		
	Yes N=12	No N=43	p value	Yes N=22	No N=33	p value
IL-5, pg/ml	0.75 (0.37)	1.01 (0.54)	0.126	0.85 (0.35)	1.02 (0.60)	0.237
IL-6, pg/ml	39.2 (25.9)	30.1 (33.9)	0.391	36.2 (30.2)	29.3 (33.8)	0.440
IL-7, pg/ml	2.54 (1.16)	2.65 (1.36)	0.805	2.56 (1.06)	2.67 (1.47)	0.753
IL-8, pg/ml	159.7 (151.2)	117.7 (77.5)	0.192	136.9 (119.5)	120.2 (82.2)	0.542
Log_{10} IL-10, pg/ml	0.80 (0.85)	0.65 (0.63)	0.505	0.81 (0.66)	0.60 (0.68)	0.263
IFN-γ, pg/ml	6.21 (11.3)	3.39 (2.40)	0.127	4.95 (8.40)	3.37 (2.57)	0.316
Log_{10} TNF-α, pg/ml	-0.38 (0.75)	-0.68 (0.53)	0.114	-0.49 (0.65)	-0.70 (0.54)	0.202

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly

IL: Interleukin

IFN: Interferon

TNF: Tumour Necrosis Factor

Table 5.5 Pearson's correlation between raw or Log_{10}-transformed cytokines measured in CSF, N=76

	IL-6	IL-7	IL-8	Log_{10} IL-10	IFNγ	Log_{10} TNF-α
IL-5	r=0.148 p=0.203	**r=0.412** **p<0.001**	**r=0.343** **p=0.002**	**r=0.339** **p=0.002**	r=0.066 p=0.573	**r=0.314** **p=0.006**
IL-6		**r=0.498** **p<0.001**	**r=0.519** **p<0.001**	**r=0.441** **p<0.001**	r= -0.001 p=0.965	**r=0.293** **p=0.010**
IL-7			**r=0.637** **p<0.001**	**r=0.433** **p<0.001**	r=0.117 p=0.312	**r=0.387** **p=0.001**
IL-8				**r=0.429** **p<0.001**	r=0.137 p=0.239	**r=0.341** **p=0.003**
Log_{10} IL-10					**r=0.353** **p=0.002**	**r=0.641** **p<0.001**
IFNγ						**r=0.554** **p<0.001**

r: Pearson's correlation statistic

IL: Interleukin

IFN: Interferon

TNF: Tumour Necrosis Factor

5.3.1 Logistic regression analysis

The demographic variables shown to be different between groups with and without delirium were IQCODE, modified APACHE II score, CCI and independence in activities of daily living (Table 5.1). A logistic regression model was built to adjust for the effect of these variables on the association between CSF IL-5 level and development of delirium preoperatively. The Wald statistic, odds ratios with 95% confidence intervals and p values for the logistic regression model are shown in Table 5.6, along with pseudo-R^2 values. There was only a trend towards significance for CSF IL-5 after adjusting for co-variates (odds ratio 4.00, p=0.095) (Table 5.6). There was no evidence of collinearity, and there were no concerns over goodness of fit (Hosmer-Lemeshow test p=0.277).

Table 5.6 Multivariate logistic regression analysis for CSF IL-5, built to adjust for the potential confounding effect of illness severity (modified APACHE II), co-morbidity (CCI), functional status (Katz or Barthel index) and prior dementia (IQCODE) on delirium status. The dependent variable was the development of delirium preoperatively.

Co-variate	CSF IL-5 model			
	Wald	OR[a]	95% CI[b] of OR[a]	p value
IQCODE total score	3.78	0.92	(0.84-1.00)	0.052
Modified APACHE II score	1.17	0.88	(0.70-1.11)	0.279
CCI	1.97	0.74	(0.48-1.13)	0.161
Independence in personal ADLs	4.32	3.94	(1.08-13.36)	0.038
CSF IL-5	2.79	4.00	(0.79-20.35)	0.095
Constant	2.77	0.10		

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly

APACHE: Acute Physiology, Age and Chronic Health Evaluation

CCI: Charlson Co-morbidity Index

ADL: Activities of Daily Living

IL: Interleukin

[a]OR: Odds ratio, [b]CI: Confidence interval. Pseudo-R^2: Cox and Snell 0.29, Nagelkerke 0.41

5.4 Discussion

The findings of this study generally did not support the hypotheses that there would be an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines in CSF in delirium after hip fracture. There was evidence of reduced IL-5 levels in the group with preoperative delirium, where delirium was active at the time of sample collection. This difference was consistent with the study hypothesis, however after adjusting for potential confounders, IL-5 was no longer associated with the development of preoperative delirium. The results of the logistic regression analysis suggested that in this cohort, loss of independence in activities of daily living was associated with preoperative delirium, suggesting more dependent, potentially frailer patients were more vulnerable to delirium with an odds ratio of 3.94 (p=0.038).

IL-5 is a primarily anti-inflammatory cytokine. It is part of the T-helper 2 cytokine response with IL-4, IL-10 and IL-13. It acts to stimulate B cell proliferation and immunoglobulin production to suppress inflammation (Ochi, Osoegawa et al. 2002). It also stimulates eosinophils and is a mediator of vascular permeability. The reduction in IL-5 in those with active delirium may represent a lack of a healthy anti-inflammatory response, perhaps relating to underlying dementia or frailty. Immunosenescence, a complex process in which immune function is attenuated by advancing age, includes reduced production of IL-5 and other cytokines from T-helper 2 lymphocytes (Gruver, Hudson et al. 2007). However, since both groups were age-matched, this should have limited the influence of ageing on results. There is also evidence of reduced immune-responsiveness in dementia; peripheral blood cells isolated from patients with Alzheimer's dementia have been found to produce less IL-5 (along with another T-helper 2 cytokine IL-13 and several pro-inflammatory cytokines) than age-matched controls (Richartz, Stransky et al. 2005).

This study did not replicate the results of MacLullich *et al* of higher CSF IL-8 in delirium, although the direction of change in those with active delirium was towards higher IL-8 in delirium. Previous studies of CSF IL-6 in delirium are at odds with the present findings, with higher IL-6 in delirium associated with a flare of Systemic Lupus Erythematosus (Katsumata, Harigai et al. 2007), and lower IL-6 in those with acute hip fracture who are about to develop

delirium postoperatively (Westhoff, Witlox et al. 2013). However, the participants involved in those two studies were different, and it may be that different pathophysiological processes are involved in delirium in each setting. In particular, neuropsychiatric SLE involves active CNS inflammatory processes, particularly during flares (Kasama, Odai et al. 2008). Given that our cohort is more comparable to the Westhoff study, one might expect that this study would find lower IL-6, however the direction of change in the present study was to higher IL-6 levels in delirium, but this difference was slight and non-significant.

There are several potential explanations for the present negative results. The results may reflect a genuine lack of changes in the measured cytokines in the CNS in delirium in the population of hip fracture patients that can be detected in CSF. This could either be because there is no alteration in the central inflammatory response or because changes in cytokine signalling are at a brain cellular level and not detectable in CSF. Another possibility is that the magnitudes of any changes were too small to be detected in a study of this size, and with the relatively insensitive multiplex assay method used. In particular the present study may be too small to robustly examine differences in cytokines between those with delirium superimposed on dementia, delirium only, dementia only and those with neither delirium nor dementia, where the microglial priming hypothesis would suggest that those with delirium superimposed on dementia should have the greatest CNS pro-inflammatory response to peripheral insults such as hip fracture. However, this study is larger than many of the previous studies of CSF biomarkers in delirium, and of a comparable size to other CSF studies in other neurological conditions. Another explanation is that the severity of delirium was not sufficient, and that changes in CSF cytokines are only measurable in severe delirium or after prolonged delirium. Finally, it is possible that there is change in the inflammatory profile in CSF in only some patients. Delirium has highly heterogeneous and overlapping aetiologies (Laurila, Laakkonen et al. 2008). In the present study, there are several potential aetiologies in each patient, including: trauma, concurrent illnesses on admission to hospital (infection, etc.), pain, analgesic drugs, psychological stress, dehydration, etc. It is possible that inflammatory pathways were only implicated in some patients, and this study was not sufficiently large to detect this. The fact that many of the cytokines correlated with each other and with other family members suggests that the assay was biologically valid. T-helper 2 cytokines did not correlate with each other, which may be because different pathways were active in these patients.

Future studies should involve larger cohorts, and different settings. Control groups for comparison could include matched elective surgical patients, since peripheral trauma may be associated with elevated CSF IL-8 (hip fracture patients versus elective lower limb surgery) (Chuang, Power et al. 2005), and out-patients with dementia, since many patients with dementia develop delirium following hip fracture and other acute insults. Since this study did not detect all of the cytokines measured at sufficient levels to analyse, future studies could employ more sensitive assays and a wider range of cytokines and chemokines. One could also attempt to take account of the likely aetiology of delirium. A standardised method measuring all the major precipitating factors of delirium and attributing it to the most likely cause might also be a way of identifying whether there is a different inflammatory profile in delirium due to certain aetiologies. The profile in different motor subtypes could also be examined. Patients with sepsis from a peripheral source, with and without associated delirium, could also be compared; a search of the literature found little research on CSF cytokines in sepsis except in the setting of meningitis, where CSF cytokines including IL-8, Il-1β and TNF-α are markedly elevated (Ostergaard, Benfield et al. 1996, Bociaga-Jasik, Garlicki et al. 2012).

In summary, this exploratory study tested the hypothesis that CSF levels of pro-inflammatory cytokines would be higher and anti-inflammatory cytokines would be lower in a group of age-matched patients with delirium after hip fracture. The results of the study largely did not support this hypothesis. The T-helper 2 anti-inflammatory cytokine IL-5 was found to be lower in those who developed delirium preoperatively, but this difference was no longer significant after adjusting for important confounders in a multivariate analysis. The fact that the cytokines largely correlated with each other and within immunological groups lends confidence to this being a meaningful result. Future studies could use more sensitive assays and measure a wider range of cytokines, and examine the inflammatory profile in delirium of different subtypes and different aetiological groups.

6 Chapter 6: Levels of cerebrospinal fluid S100B and tau in hip fracture patients with delirium versus controls

6.1 Introduction

Delirium is a syndrome of acute central nervous system (CNS) dysfunction which may be precipitated by diverse acute peripheral and CNS disorders, and drugs. Delirium is associated both with immediate adverse effects, and increased risk of future cognitive impairment and acceleration of dementia (MacLullich, Beaglehole et al. 2009). Understanding the CNS disturbances underlying delirium as well as potential mechanisms linking delirium with new or accelerated neurodegeneration is a priority. However, the pathophysiology of delirium remains largely obscure (MacLullich, Ferguson et al. 2008). Increases in cerebrospinal fluid (CSF) cortisol, inflammatory markers and other potential biomarkers have been reported (Hall, Shenkin et al. 2011), and neuroimaging findings in delirium include ventricular enlargement and white matter lesions, suggesting possible neuronal and glial changes (Soiza, Sharma et al. 2008), but biological evidence of acute CNS dysfunction in delirium remains lacking.

S100B is a calcium-binding protein found primarily in astrocytes, but also in oligodendrocytes, neurons, lymphocytes, ependymal cells and epithelial cells of the choroid plexus (Michetti, Corvino et al. 2012), and in some non-CNS sites. S100B is secreted by astrocytes under metabolic stress, and has cytokine-like properties. S100B is a biomarker of CNS damage: increased CSF and serum S100B are linked with adverse CNS outcomes, including complications in neurointensive care patients, lesion volume in traumatic brain injury, and poorer outcomes in stroke (Michetti, Corvino et al. 2012).

Elevated serum S100B has been found in delirium in hip fracture patients (van Munster, Bisschop et al. 2010) and in elderly general medical inpatients (van Munster, Korevaar et al. 2010). In another study no differences in CSF S100B levels were found in a group of elderly general medical inpatients with persistent delirium compared with outpatients with Alzheimer's dementia (Caplan, Kvelde et al. 2010) though there was no control group of acutely unwell people without delirium.

There are no published studies which have compared levels of CSF S100B in acutely unwell patients with and without delirium. This is important because CSF S100B levels largely reflect astroglial activation and are likely more representative of a CNS origin of S100B.

Tau is a microtubule associated protein which is primarily located in neuronal axons. It may undergo several post-translational changes including phosphorylation, and hyperphosphorylated tau aggregates are more neurotoxic (Clavaguera, Grueninger et al. 2014). Several neurodegenerative diseases involve pathological inclusions of tau aggregates, most notably Alzheimer's dementia, but also progressive supranuclear palsy, corticobasal degeneration and frontotemporal dementia (Clavaguera, Grueninger et al. 2014). In a mouse model, anaesthesia-induced hypothermia induced massive tau hyperphosphorylation, suggested as a possible cause of postoperative cognitive dysfunction and acceleration of dementia after surgery (Planel, Richter et al. 2007).Tau is widely used as a CSF biomarker for Alzheimer's dementia; a recent meta-review reported that the majority of studies demonstrate increased levels of the order of 300% in patients with Alzheimer's dementia vs. healthy controls (Ferrerira, Perestelo-Perez et al. 2014). It is not specific for dementia, however, more a reflection of neuronal loss. Tau level correlates with brain atrophy and age (Randall, Mosconi et al. 2014).

In delirium, a borderline significant elevation in serum tau levels has been found in critically ill patients with "non-inflamed" delirium (van den Boogaard, Kox et al. 2011). In CSF, Witlox *et al* found no association between tau or phosphorylated-tau and the risk of postoperative delirium in patients with hip fracture (Witlox, Kalisvaart et al. 2011). Xie *et al* measured the ratios of β-amyloid-40/tau and β-amyloid-42/tau in CSF in patients undergoing elective arthroplasty, and found that those in the lowest quartile of both ratios (suggesting lower β-amyloid and/or higher tau, although neither marker is reported alone) had higher incidence and greater delirium symptom severity (Xie, Swain et al. 2014).

This study asked the following research questions:

1) Are CSF S100B and tau levels higher in elderly patients with delirium following acute hip fracture compared to those without delirium?

2) Are higher baseline salivary and CSF cortisol levels associated with higher CSF S100B levels?

6.2 Methods

6.2.1 Participant recruitment and cognitive assessment

Participants with acute hip fracture aged over 60 years were recruited prospectively but not consecutively by a geriatrician (RJH) in an acute orthopaedics ward in Edinburgh, Scotland, between 2009 and 2011, as described in chapter 2. Exclusion criteria were: nursing home resident, co-morbid disease with prognosis of less than one year, Parkinson's disease, corticosteroid prescription or inability to communicate in English. Informed consent was obtained from participants or legal proxies. Ethical approval was obtained from Scotland A Research Ethics Committee. Delirium was assessed with a semi-structured interview supplemented with assessments of level of consciousness (Richmond Agitation Sedation Scale), attention (Edinburgh Delirium Test Box), and cognition (Mini-Mental State Examination). Delirium was diagnosed with the Confusion Assessment Method and the Delirium Rating Scale Revised-98 (DRS-R98); considered present with a positive CAM score or a total DRS-R98 score of >17.75. Assessments took place preoperatively, on the day of surgery, and then daily from postoperative days 1-4 and on days 7 and once between days 10-14 or until transfer to a rehabilitation unit or discharge from hospital. The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) was used to ascertain prior cognitive impairment. The APACHE (Age, Physiology and Chronic Health Evaluation) II score was used to estimate acute illness severity preoperatively; the score was modified by removing arterial PO_2 because arterial blood gas sampling is not routine in patients with hip fracture.

6.2.2 Sample collection

Saliva was collected preoperatively at two timepoints, 0800-1000 and 1500-1700, if this was feasible in the time before hip fracture repair. CSF was collected using the spinal needle prior to the injection of anaesthetic agents. A paired serum sample was collected at the time of spinal anaesthetic. CSF and serum samples were placed in polypropylene containers and put on ice, then centrifuged at 1000 rpm for CSF and 3000 rpm for serum at 4^{o}C for 10 minutes and stored at -80 ^{o}C.

6.2.3 Assays

CSF S100B was measured using a validated in-house Enzyme-Linked Immunosorbent Assay (ELISA), and tau using a commercially-available ELISA at the National CJD Surveillance Unit, Edinburgh, UK (Green, Keir et al. 1997), by Alison Green and Mary Andrews. The original intention had been to also measure CSF β-amyloid and phosphorylated-tau, however due to budgetary restrictions, only total tau and S100B were measured. Cortisol was measured in CSF, serum and saliva using ELISA (CSF and saliva: Salimetrics, serum: MP Biomedicals) by RJH.

6.2.4 Statistical analysis

S100B and tau results were log-transformed due to non-parametric distribution, and following log-transformation, normality of distribution was checked with Kolgomorov-Smirnov test. Group comparisons between those with and without delirium during the preoperative period, and at any stage in the perioperative period, were then performed with Student's *t* test. Correlations between raw CSF S100B and tau levels and baseline measures of delirium severity (DRS-R98 severity score), illness severity (modified APACHE II score) and prior dementia (total IQCODE, total MMSE) were tested with Spearman's Rho. This was to test for any acute relationship between severity of delirium and illness, and active CNS damage, and to see whether higher levels of the dementia biomarker tau were associated with more severe delirium and with worse tests of global cognition with MMSE and of prior dementia with IQCODE. Correlations were also tested between raw CSF S100B levels and baseline measures of cortisol in CSF, serum and saliva with Spearman's Rho, to determine whether there is a relationship between CNS damage and astrogliosis as measured with S100B and activation of the HPA axis.

6.3 Results

Forty-five patients were recruited (Figure 6.1). Table 6.1 provides details of the participants. Eight (17.8%) participants developed delirium preoperatively, and 19 (42.2%) during the two week perioperative period. Age was not significantly different between those with and without delirium. Six participants had prior cognitive impairment, with a diagnosis of dementia or average IQCODE score ≥ 3.44; all six were in the delirium group, but only two had developed delirium preoperatively. Five of the six patients in the "prior dementia" group had a known diagnosis of dementia, of whom three had a diagnosis of Alzheimer's dementia, and two had no aetiology listed in their medical records.

6.3.1 CSF S100B and tau

Mean Log_{10} CSF S100B concentrations were significantly elevated in those with preoperative delirium compared to those without delirium (-0.156 (SD 0.238) vs -0.306 (SD 0.162), respectively, Student's t-test t=2.18, df=43, p=0.035) (Figure 6.2). There was no difference in Log_{10} CSF S100B concentrations in those participants who became delirious during the two week perioperative period compared to participants who did not become delirious (mean -0.239 (SD 0.182) vs. -0.308 (SD 0.184), respectively, Student's t-test t=1.25, df=43, p=0.218). Log_{10} CSF S100B concentrations in those with and without evidence of prior cognitive impairment were not different (mean -0.276 (SD 0.192) vs. -0.230 (SD 0.135), respectively, Student's t-test t=-0.559, df=40, p=0.579).

Mean Log_{10} CSF tau concentrations were not significantly different in those with preoperative delirium compared to those without delirium (2.57 (SD 0.23) vs 2.55 (SD 0.24), respectively, Student's t-test t=0.227, df=43, p=0.821). There was also no difference in Log_{10} CSF tau concentrations in those participants who became delirious during the two week perioperative period compared to participants who did not become delirious (mean 2.54 (SD 0.22) vs. 2.57 (SD 0.25), respectively, Student's t-test t=-0.353, df=43, p=0.726) (Figure 6.3). Log_{10} CSF tau concentrations in those with and without evidence of prior cognitive impairment were not different (mean 2.68 (SD 0.33) vs. 2.56 (SD 0.21), respectively, Student's t-test t=1.19, df=40, p=0.243).

6.3.2 Correlations

Neither CSF S100B (rho=0.095, p=0.536) nor tau (rho=-0.222, p=0.143) correlated with preoperative DRS-R98 severity score. CSF S100B did not correlate with modified APACHE II score (rho=0.009, p=0.952). CSF tau level did not correlate with baseline total MMSE score (rho=0.013, p=0.936) nor total IQCODE score (rho=0.045, p=0.778). There was a trend towards positive correlation of CSF tau with age (rho=0.264, p=0.079).

CSF S100B level did not correlate with any of the baseline cortisol measures (CSF cortisol rho=0.108, p=0.480, baseline serum cortisol rho=0.205, p=0.181, baseline AM salivary cortisol rho=0.200, p=0.606, baseline PM salivary cortisol rho=0.317, p=0.406).

CSF S100B and tau did not correlate with each other (rho=0.081, p=0.595).

Figure 6.1 Diagram showing recruitment numbers and patients excluded for S100B substudy

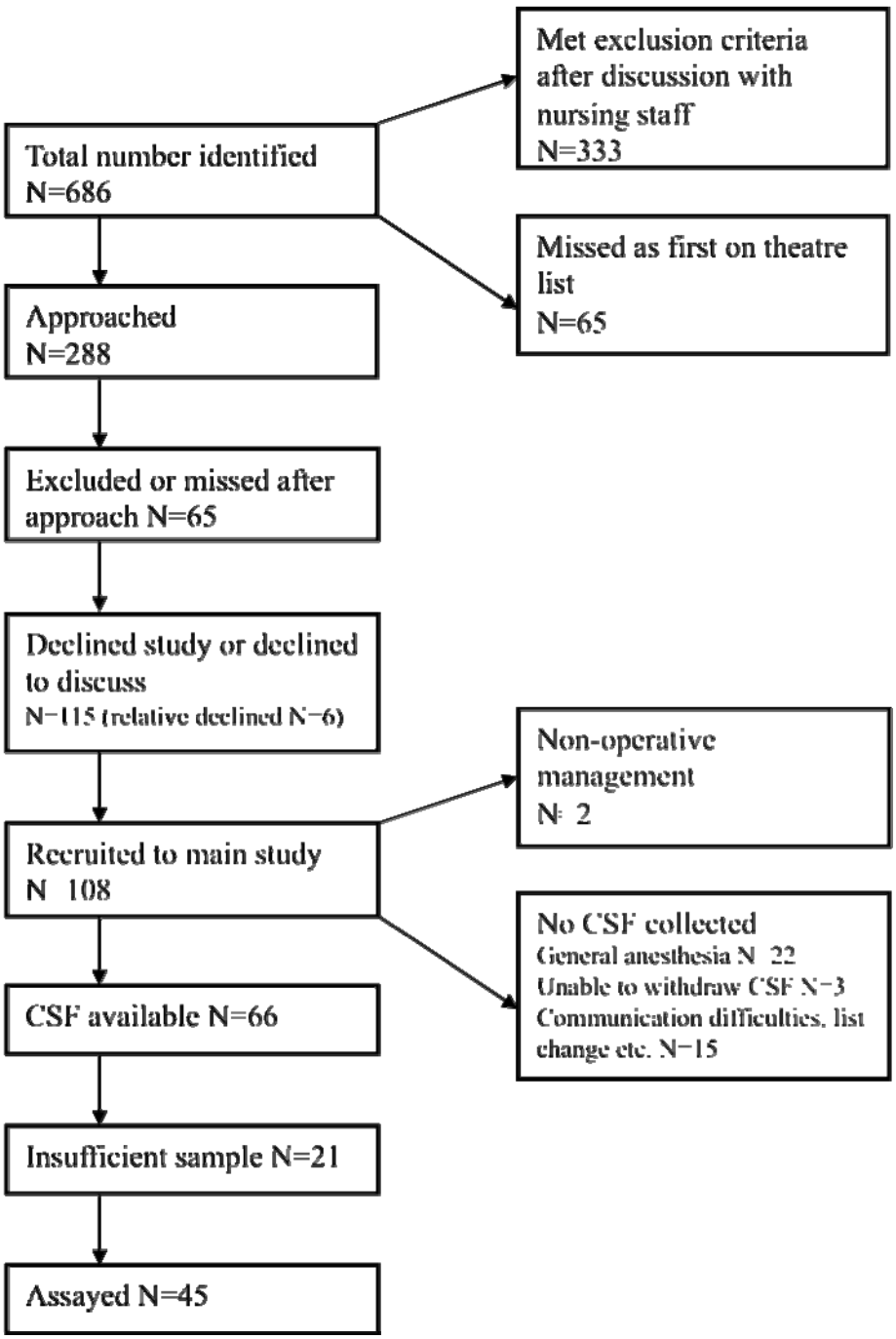


Table 6.1 showing baseline medical, surgical and functional demographics of the cohort

	Delirium, N=19	**No delirium, N=26**	**p value**	**df**	**Test score**
Age	81.3 years (SD 6.7)	78.9 years (SD 9.9)	0.377[#]	43	**0.892**
Female	N=12 (63%)	N=19 (73%)	0.297[£]	1	**1.089**
IQCODE score	Total 54.79 (SD 8.57) Average 3.42 (SD 0.54)	Total 49.35 (SD 1.99) Average 3.08 (SD 0.12)	0.005[#] 0.005[#]	40 40	**2.956** **2.954**
Living alone	N=8 (42%)	N=19 (73%)	0.091[£]	2	**4.794**
Package of care	N=6 (32%)	N=8 (31%)	0.954[£]	1	**0.003**
Mobility at baseline	Independent, no aids N=5 With a stick N=5 With 2 sticks N=2 With a mobilator N=7	Independent, no aids N=16 With a stick N=7 With 2 sticks N=2 With a mobilator N=1	0.021[£]	3	**9.742**
Katz basic ADL score /6	6 (IQR 4-6)	6 (IQR 6-6)	0.057[$]		**MWU 181.0**
Lawton instrumental ADL score /8	4 (IQR 3-8)	7 (IQR 6-8)	0.019[$]		**MWU 142.0**
Modified APACHE II score*	8.63 (SD 2.11)	8.08 (SD 2.80)	0.472[#]	43	**0.725**
Charlson Co-morbidity Index	1 (IQR 1-2)	1 (IQR 0-2)	0.383[$]		**MWU 210.5**
Type of fracture	Intracapsular N=16 Extracapsular N=2 Periprosthetic N=1	Intracapsular N=14 Extracapsular N=12 Periprosthetic N=0	0.098[£]	4	**7.834**
Type of surgery	Hemiarthroplasty N=14 Internal fixation N=5 Total hip replacement N= 0	Hemiarthroplasty N=10 Internal fixation N=15 Total hip replacement N=1	0.057[£]	2	**5.716**
Type of anesthesia	**Spinal anesthetic N=19**	**Spinal anesthetic N=26**			

[#]Student's t-test, [£]Pearson Chi-square, [$]Mann-Whitney U test. Mean (Standard Deviation) or Median (Interquartile Range). IQCODE – Informant Questionnaire of Cognitive Decline in the Elderly. ADL – Activities of Daily Living. *APACHE II (Acute Physiology And Chronic Health Evaluation II) score modified by removing Arterial Blood Gas result, since this is not routinely performed

Figure 6.2 Log_{10} CSF S100B levels in patients with and without preoperative delirium.

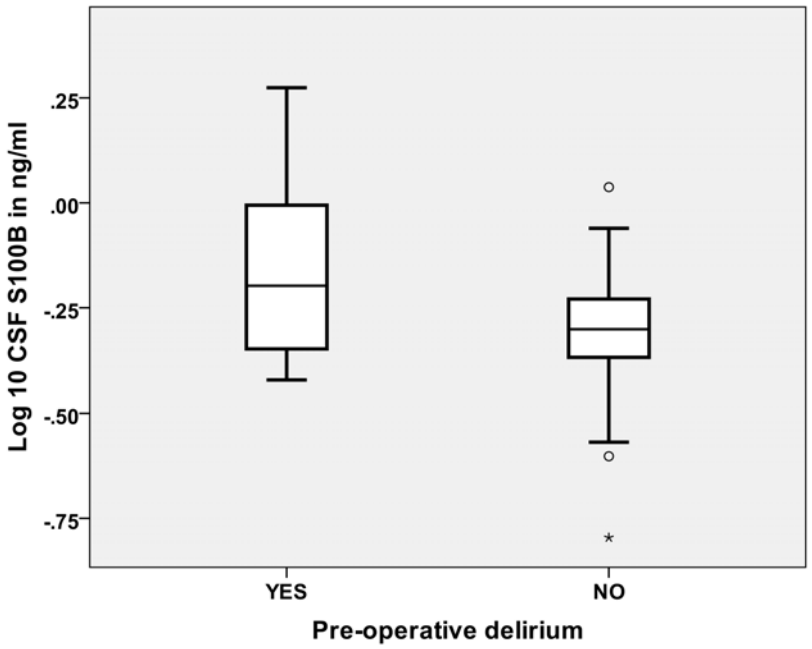


Figure 6.3 Log10 CSF S100B levels in patients with and without delirium at any stage

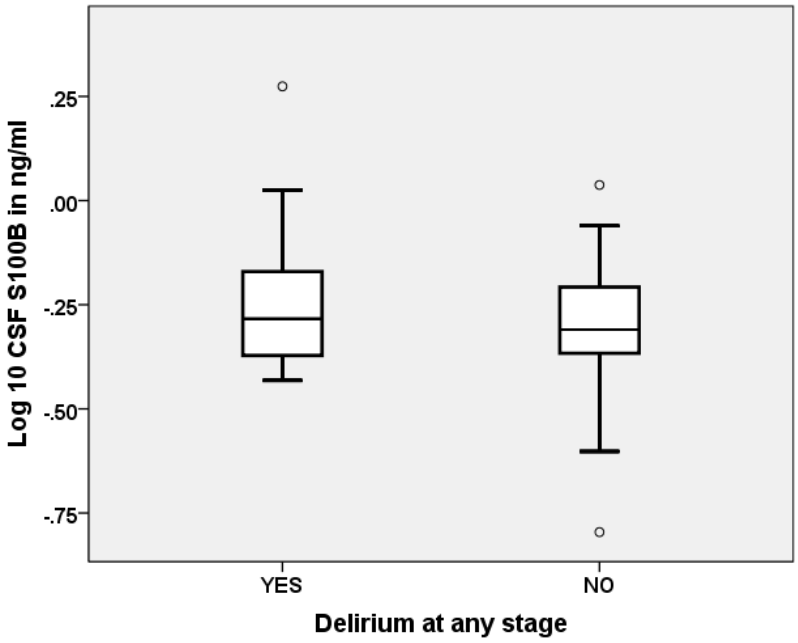


Figure 6.4 Log10 CSF tau levels in patients with and without preoperative delirium

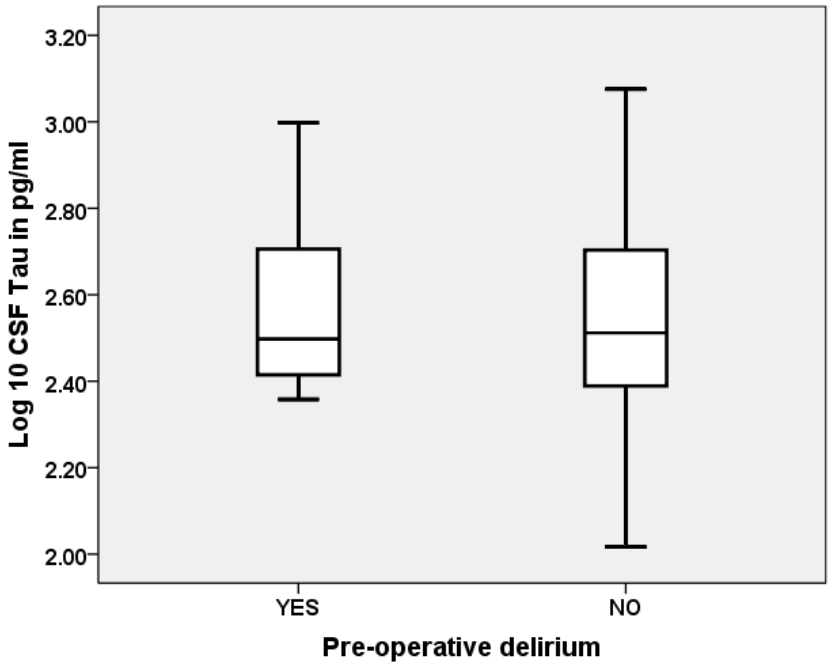
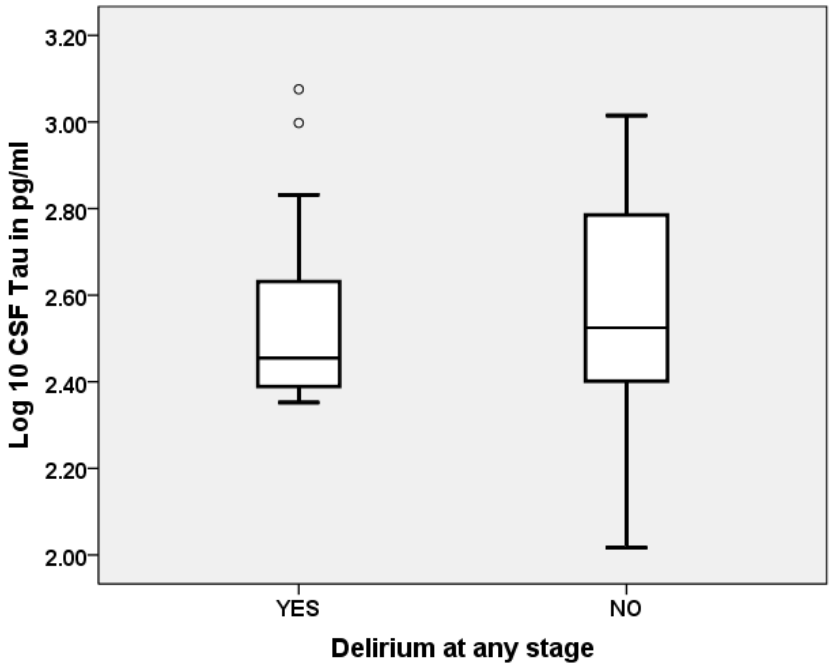


Figure 6.5 Log_{10} CSF tau levels in patients with and without delirium at any stage



6.4 Discussion

These results provide novel evidence that S100B, a marker of astrocyte activation and potential CNS dysfunction or damage, is elevated in the CSF in patients with current delirium. These findings are consistent with two prior studies reporting elevated serum S100B in patients with delirium both following hip fracture and in elderly acute medical patients (van Munster, Bisschop et al. 2010, van Munster, Korevaar et al. 2010). In those studies, serum S100B levels were highest during or after delirium. Consistent with this I found the highest levels in CSF samples collected during delirium, although there was no correlation between CSF S100B and severity of delirium, suggesting no linear relationship between delirium severity and activation of S100B in the CNS. The origin of S100B in the serum is uncertain, however, due to non-CNS sources such as adipose tissue. The present study suggests a CNS origin for elevated S100B levels in delirium, because the CNS has by far the highest concentration of S100B, and peripheral levels are generally lower than the levels I found here (Petzold, Keir et al. 2003). Another study comparing acutely unwell delirious patients with stable dementia outpatients did not find higher CSF S100B in delirium (Caplan, Kvelde et al. 2010) but that finding is not directly comparable with the present study because of the lack of an acute control group.

This study also replicates the findings of Witlox *et al* that there was no difference in CSF tau levels in delirium, nor any relationship with delirium severity. I therefore did not find evidence of greater tau release from neurons in active delirium. Dementia is undoubtedly a risk factor for delirium (Juliebo, Bjoro et al. 2009), and all six participants with dementia in this study also developed delirium during the perioperative period, but I did not find evidence of greater tau pathology when those with dementia or those with delirium were compared to those without. There was also no relationship between tau and measures of global cognition or prior dementia. This may be because of the relatively small numbers of patients in this study, or because of different dementia aetiologies in this study, or because factors other than tau pathology are responsible for the increased delirium risk in dementia.

Elevated CSF S100B in delirium may be due to leakage from damaged or dying astrocytes and neurons, in which S100B is normally found. However, S100B may also be actively

secreted from astrocytes in response to stimuli which may not induce astrocyte damage or even dysfunction. Such stimuli include metabolic stress (oxygen and glucose deprivation), lipopolysaccharide, pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor-(TNF)-α, β-amyloid, and glutamate; expression may be down-regulated by Interferon-(IFN)-γ and EGF (Epidermal Growth Factor) (Guerra, Tortorelli et al. 2011, Michetti, Corvino et al. 2012). This study did not find any relationship between CSF S100B and CSF, serum or salivary levels of cortisol, suggesting that the exaggerated stress response seen in delirium as discussed in chapters 4 and 7 may not be involved in the astroglial activation suggested by elevation of S100B in active delirium. This concurs with previous research in rodents suggesting stress-induced serum S100B release is independent of glucocorticoids (Scaccionoce, Del Bianco et al. 2004).

Following release from astrocytes, S100B may have autocrine effects, triggering the release of TNF-α and IL-6. Additional cytokine-like activities of S100B are largely transduced via RAGE (Receptor for Advanced Glycation Endproducts). S100B may also have direct effects on neurons. At low (nanomolar) concentrations, S100B has been found to be largely neurotrophic and neuroprotective. However, at higher (micromolar) concentrations, S100B exerts neurotoxic effects, by activating RAGE pathways and through the generation of reactive oxygen species (Michetti, Corvino et al. 2012). RAGE activation has been implicated in multiple disease pathologies, particularly those involving inflammation (Guerra, Tortorelli et al. 2011). After stimulation by TNF-α and acting via RAGE, S100B activates microglia, and increases IL-1β, TNF-α, and cyclooxygenase-2 expression, thus stimulating CNS inflammation. Aberrant CNS inflammatory responses may be important in the pathophysiology of delirium (MacLullich, Ferguson et al. 2008).

If there are acutely elevated S100B levels in delirium, the resultant neurotoxicity could plausibly lead to disruption of neurotransmission in critical cognitive attentional pathways. Additionally, the acute elevation demonstrated here in delirium may provide a means by which delirium and dementia interact, for example through accelerating Alzheimer's dementia. This may be through an acute neurotoxic effect of S100B during the acute phase of delirium leading to irreversible damage, the propagation of a CNS inflammatory response as described above, or through the increased generation of β-amyloid plaques and hyperphosphorylated tau neurofibrillary tangles (Michetti, Corvino et al. 2012).

Some limitations of this study must be acknowledged. The sample size was small. The patients were old and had all recently suffered hip fracture. I did not assess participants every day in the postoperative period, and may have missed brief episodes of delirium. I only found a significantly higher level of S100B in those who were already delirious at the time the sample was collected, and not in the whole group who became delirious perioperatively. This may be because delirium needs to be established, potentially with acute astrocyte activation and ongoing CNS dysfunction and perhaps damage, to see a rise in S100B. Also, postoperative delirium in emergency patients is more common than delirium in medical patients and has a greater range of aetiologies. I did not measure S100B in the serum due to the uncertain origin of peripheral measurement, although the comparison of CSF and serum levels would be valuable in interpreting serum S100B levels in future studies.

Although there was no difference in tau levels at baseline and no relationship with delirium, these markers were only measured at one timepoint, and tau or phosphorylated tau may have risen with prolonged delirium or following the insult of anaesthetic. It would be very difficult in practical and ethical terms to measure these CSF markers repeatedly in patients with delirium. Although serum tau and phosphorylated tau measurement may have additional sources, it may be a more feasible way to look at their relationship with delirium, postoperative cognitive change and anaesthetic insult in future studies, ideally in an elective cohort with measurement of premorbid cognition.

This study suggests that delirium is associated with acute CNS dysfunction as measured by increased CSF S100B levels. These findings parallel multiple other studies in other neurological conditions demonstrating that CSF S100B elevation is a marker of acute CNS dysfunction. Through promoting inflammation and other effects, elevated S100B levels may be relevant to both acute delirium and its association with longer-term cognitive decline. Future work with larger samples and more diverse precipitants will help to further shed light on this promising line of enquiry.

7 Chapter 7: The relationship between perioperative delirium and serum, salivary and CSF cortisol

7.1 Introduction

Several lines of evidence suggest that there is an exaggerated and prolonged cortisol response to stressors in delirium, and there may be flattening of cortisol diurnal rhythm. This has been discussed in more detail in section 1.5. It is well recognised that steroids given in clinical practice may precipitate delirium and psychosis (Wolkowitz, Reus et al. 1997). Multiple stressors relevant to patients with hip fracture, including trauma, surgery, anaesthesia, dehydration, infection, pain and psychological stress, may precipitate delirium and activate the hypothalamic-pituitary-adrenal (HPA) axis. Of course, activation of the HPA axis is necessary in such a physically and psychologically stressful situation as a frail elderly person who has fractured their hip. However, an exaggerated activation of the HPA axis with high cortisol levels, potentially due to loss of normal negative feedback of the HPA axis as may be seen in aging and dementia, or the effect of the serial insults as listed above, may plausibly lead to delirium. Elevation of cortisol may be sustained for at least eight weeks after hip fracture (Barton, Horan et al. 2001), where up to 53% of patients develop delirium (Bruce, Ritchie et al. 2007), however little is known about the relationship between cortisol and delirium past the very acute phase, as detailed below.

Studies examining cortisol levels in delirium have often measured it in serum at one baseline timepoint, but it has been shown to be elevated in postoperative delirium following cardiac surgery (Mu, Wang et al. 2010) and non-cardiac surgery (Shi, Wang et al. 2010), and in patients with critical illness (van den Boogaard, Kox et al. 2011) and acute coronary syndrome (Colkesen, Giray et al. 2013). Studies examining serial cortisol levels have found a greater rise between pre- and postoperative serum cortisol levels in those with postoperative delirium following elective arthroplasty (Cerejeira, Batista et al. 2013), and higher levels over several days encompassing a delirium episode following hip fracture (Bisschop, de Rooij et al. 2011).

Cortisol diurnal rhythm may also be disrupted in delirium, with elevation of evening cortisol levels. Loss of normal diurnal rhythm may be seen in dementia, depression and Cushing's disease (Herbert, Goodyer et al. 2006). This is often accompanied by disruption of sleep-wake cycle, which is almost universally affected in delirium (Meagher, Leonard et al. 2010). Flattening of cortisol diurnal rhythm has been demonstrated in postoperative cognitive dysfunction following non-cardiac surgery (Rasmussen, O'Brien et al. 2005). Only one previous very small study has examined cortisol diurnal rhythm in delirium, where loss of diurnal rhythm was found in 3/7 male patients who developed delirium following non-cardiac surgery (McIntosh, Bush et al. 1985). No previous study of the HPA axis in delirium has taken the detailed measurements of baseline CSF, serial morning serum cortisol and serial cortisol diurnal rhythm in saliva, encompassing a delirium episode, that have been measured in the present study.

This study asked the following research questions:

1) Is delirium after hip fracture associated with high cortisol levels in serum?
2) Is delirium associated with loss of cortisol diurnal rhythm measured in saliva, with elevation in afternoon salivary cortisol levels?
3) Are the derangements in cortisol levels and cortisol diurnal rhythm more exaggerated in those with delirium superimposed on dementia?

7.2 Methods

Participants were recruited and assessed as described in detail in Chapter 2. Assessment for delirium was performed preoperatively, and on postoperative days 1, 2, 3, 4, 7 and once between days 10-14, unless participants were discharged or transferred to an orthogeriatric rehabilitation ward prior to this point. Delirium assessment comprised a semi-structured interview with additional testing of cognition, arousal and attention. The Delirium Rating Scale Revised '98 (DRS-R98) was used to assess severity, the Observational Scale of Level of Arousal (OSLA) and the Richmond Agitation Sedation Scale (RASS) to assess level of consciousness, the Edinburgh Delirium Test Box-1 (EDTB-1) to assess attention and the Mini-Mental State Examination (MMSE) to assess global cognition. Diagnosis of delirium was by DSM-IV criteria informed by the Confusion Assessment Method (CAM) protocol.

The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) was used to assess for prior dementia, and previous diagnoses of dementia were also documented. An IQCODE average score cut-off of ≥3.44 was used to suggest prior dementia, and of ≥3.19 to suggest prior mild cognitive impairment (MCI). For subgroup analyses, those with probable prior MCI and dementia were collapsed into one group. Other variables considered in this study were illness severity with the Acute Physiology, Age and Chronic Health Evaluation (APACHE) II score, which was modified by omitting the PO2 score, co-morbid illness burden using the Charlson Co-morbidity Index (CCI), frailty using a simple frailty score, the Rockwood Frailty Index, and function using the Katz scale for personal activities of daily living and the Lawton scale of instrumental activities of daily living.

CSF was collected at the time of spinal anaesthetic as described in section 2.4.2 and chapter 4. Serum samples were collected at the time of spinal anaesthetic and between 8-10am on day 4 and once between days 10-14 postoperatively in patients who were still in the orthopaedic trauma ward. Diurnal saliva samples were collected at baseline, and on postoperative days 4, 7 and between days 10-14 postoperatively in patients who were still in the orthopaedic trauma ward. Samples were processed and stored as described in section 2.5. Cortisol was measured in duplicate (CSF in singlicate) in batches with ELISA (CSF and Saliva: Salimetrics, Serum: MP Biomedicals) as described in section 2.9.1, 2.10.1 and 2.11.

7.2.1 Univariate analyses

Normality of distribution was tested using histogram plots and Kolgomorov-Smirnov tests. Group comparisons examined cortisol levels in serum and saliva at each timepoint, comparing those who did and did not develop delirium during the perioperative period, with the Mann Whitney U test. Group comparisons also examined cortisol levels in those who did or did not fulfil criteria for delirium on the day of assessment and sample collection, with Mann Whitney U test. Further subgroup analyses compared cortisol levels between subgroups with delirium superimposed on MCI/dementia, delirium only, MCI/dementia only and neither delirium nor MCI/dementia, with the Kruskal Wallis test. A p value of <0.05 was considered significant.

7.2.2 Logistic regression modelling statistical methods

This analysis was performed by Dan Davis. The analysis considered four of blocks time where cortisol exposures could be linked to delirium outcomes: block 1 (days 0 and 1); block 2 (days 2, 3 and 4); block 3 (day 7); block 4 (single observation between day 10 and 14). The outcome was defined as delirium (yes / no) within any of these time blocks. The exposures were absolute cortisol (measured by serum) and cortisol AM:PM ratio in saliva. These variables were positively skewed, necessitating Log_e transformation. Important covariates selected *a priori* were: age, sex, IQCODE-defined dementia (cut-off >3.44) and Charlson Co-morbidity Index (to index some co-morbidity related predisposing factors) and APACHE II score (to assess acute illness-related precipitating factors). The analysis was therefore designed to estimate the effect of cortisol levels on delirium, independent of any associations with chronic illness burden and acute illness severity.

Logistic regression models were estimated, considering separately each participant and block. These observations are not truly independent as observations within participants are correlated. To account for this, robust standard errors were estimated, clustering the estimates at the participant level. Complete case analysis was used. Missing data were considered missing-at-random and correlation matrices were unstructured. Post-estimation testing for violation of assumptions included the Hosmer-Lemeshow test for goodness-of-fit.

7.2.3 Correlations

Correlations were also tested between measures of delirium severity (DRS-R98 severity score), inattention (EDTB-1 score), level of arousal (OSLA total score) and global cognition (MMSE score) and cortisol levels at each time point during the perioperative period, using Spearman's Rho, to determine whether greater HPA axis activation is associated with worse scores on the components of the delirium assessment and of global cognition. Correlations were also tested between cortisol measures and baseline predisposing and precipitating factors including age, number of hours between triage in Accident and Emergency and operation, Charlson Co-morbidity Index, number of prescribed medications, APACHE II score, IQCODE average score, Katz scale of personal activities of daily living and Lawton scale of instrumental activities of daily living, using Spearman's Rho. This was to investigate whether any of these risk factors for delirium may be acting through activation of the HPA axis.

7.3 Results

One hundred and eight participants were recruited to the study. During the perioperative period, two participants were excluded because their fracture was managed conservatively, and one participant was excluded after day 4 because of a significant and unpredicted complication which required ICU admission including steroid therapy. One participant died and seven participants withdrew consent to continue participating in the study during the perioperative period (up to day 10-14). Data and samples were included for participants until the point at which they were excluded, withdrawn or died. No serum, CSF or saliva samples were available from an additional two participants due to logistical problems or refusal. Participant numbers at the day 10-14 timepoint were smaller due to a large proportion of patients either having been discharged or moved to an orthogeriatric rehabilitation ward prior to this timepoint.

Table 7.1 shows baseline characteristics of the final included cohort of N=104 participants in whom at least one biological sample was available. Delirium was diagnosed in N=42 participants during the perioperative period, N=13 of whom developed delirium prior to their operation, and an additional N=29 who developed delirium postoperatively. The delirium group were more likely to have dementia, to be more functionally impaired, to be frailer and to be taking a higher number of drugs. They had higher illness severity according to APACHE II. The delirium group also waited longer for their operation; this may be because the delirium group were more likely to have had preoperative medical complications which necessitated delays to surgery, or that waiting longer for surgery increases the risk of delirium. By examining waiting times in groups with prevalent (developing preoperatively) and incident (postoperative only) versus never delirium, the wait was higher in both delirium subgroups [prevalent delirium 43 (30-77) hours, incident delirium 40 (31-48) hours, never delirium 28 (19-43) hours, Kruskal-Wallis p=0.004, pairwise comparisons adjusted p=0.042 and p=0.016 respectively].

Although patients who were intended to have spinal anaesthesia were included in the study, for various reasons, some went on to have general anaesthetic. Of the N=104 included participants, N=23 had general anaesthetic, N=77 had spinal anaesthetic, and N=4 had both. There were no differences in rates of delirium between groups either pre-operatively (which

may have influenced anaesthetic choice; Pearson Chi-square 0.624, p=0.732), on post-operative day one (Pearson Chi-square 1.95, p=0.745) or at any point during the perioperative period (Pearson Chi-square 0.452, p=0.798). Participants were not excluded from the study on the basis that they had a general anaesthetic, to limit attrition.

Table 7.1 Baseline characteristics of the included cohort

	Delirium, N=42 Median (Interquartile range)	**No delirium, N=62** Median (Interquartile range)	**p value**	**Test statistic**
Age	83.0 years (77.0-88.3 IQR)	80.5 years (70.0-87.0 IQR)	0.147[a]	**1084[c]**
Female	N=24 (57%)	N=41 (66%)	0.353[b]	**0.863[d]**
IQCODE score	Total 50 (IQR 48-54)	Total 48 (IQR 48-54)	0.001[a]	**683[c]**
	Average 3.13 (IQR 3.00-3.38)	Average 3.00 (IQR 3.00-3.13)	<0.001[a]	**664[c]**
Dementia status	Normal N=20	Normal N=44	0.008[b]	**9.561[d]**
(MCI cut-off IQCODE >3.19, Dementia >3.44)	MCI N=11	MCI N=11		
	Dementia N=8	Dementia N=2		
Alcohol Units/week	1.5 (IQR 0-6.25)	0.5 (IQR 0-4.00)	0.523[a]	**1212[c]**
Type of accommodation	House or flat N=38	House or flat N=54	0.597[b]	**0.280[d]**
	Sheltered housing N=4	Sheltered housing N=8		
Katz basic ADL score /6	6 (IQR 5-6)	6 (IQR 6-6)	0.043[a]	**1101[c]**
Lawton instrumental ADL score /8	6 (IQR 4-8)	7 (IQR 6-8)	0.053[a]	**979[c]**
Rockwood brief frailty index	Independent N=23	Independent N=47	0.003[b]	**14.14[d]**
	Bladder incontinence N=4	Bladder incontinence N=10		
	≥1 other ADL needs N=10	≥1 other ADL needs N=5		
	Two or three ADL needs N=4	Two or three ADL needs N=0		
Number of medications	6 (IQR 4-8.25)	4 (IQR 3-7)	0.022[a]	**959[c]**
Modified APACHE II total score	9 (IQR 7-11.25)	8 (IQR 6-10)	0.004[a]	**866[c]**
Charlson Co-morbidity Index	1 (IQR 0-3)	1 (IQR 0-2)	0.095[a]	**1061[c]**
Type of fracture	Intracapsular N=26	Intracapsular N=34	0.653[b]	**0.852[d]**
	Extracapsular N=14	Extracapsular N=26		
	Periprosthetic N=2	Periprosthetic N=2		
Type of surgery	Hemiarthroplasty N=21	Hemiarthroplasty N=22	0.012[b]	**11.00[d]**
	Internal fixation N=0	Internal fixation N=7		
	Total hip replacement N=18	Total hip replacement N=33		
	Open reduction and internal fixation N=3	Open reduction and internal fixation N=0		
Waiting time for operation[e]	41 (30-49) hours (IQR)	28 (19-43) hours (IQR)	0.001[a]	**721[c]**
Type of anaesthesia	Spinal N=32	Spinal N=45	0.798[b]	**0.452[d]**
	General N=9	General N=14		
	Both N=1	Both N=3		

[a]Mann-Whitney-U test, [b]Pearson Chi-square, [c]Mann-Whitney U test statistic, [d]Pearson Chi square test statistic

[e]Time from triage in Accident and Emergency to operation start time

7.3.1 Cortisol levels during the perioperative period

Cortisol was not normally distributed in serum, saliva or CSF, so non-parametric statistics were used. The relationship between delirium and cortisol in CSF is discussed in full in chapter 4. Serum cortisol levels rose postoperatively in those with and without delirium (Figure 7.1). This rise may be attributable in part to the fact that the baseline serum sample was collected at the time of spinal anaesthetic, which could be between 8am and 5pm, whereas the subsequent samples were collected between 8am and 10am, closer to the circadian peak of cortisol. Median serum cortisol levels were higher in the delirium group at each timepoint during the perioperative period, but this was not significant. Serum cortisol levels were generally slightly higher in the groups with delirium only and delirium superimposed on MCI/dementia during the perioperative period, but this was not significant (Table 7.2). When those who did or did not fulfil criteria for delirium on the day of sample collection were compared, morning serum cortisol levels were significantly higher in those who fulfilled criteria for delirium on postoperative day 4 (Table 7.4).

Morning salivary cortisol was significantly higher in the delirium group preoperatively, but the numbers in both groups were small. Postoperatively, morning salivary cortisol levels rose in both groups (Figure 7.2), but there was no significant difference between those with and without delirium. There was evidence of flattening of cortisol diurnal rhythm in the delirium group with a rise in afternoon salivary cortisol; this difference was significant at days 4 and 7 postoperatively in the whole delirium group, and on postoperative day 4 in those fulfilling criteria for delirium on the day of sampling. The AM:PM salivary cortisol ratio was lower in the whole delirium group throughout the postoperative period, which was significant on postoperative day 4 (Table 7.3), and there was a trend towards significance in those with delirium on the day of sampling on postoperative day 4 (Table 7.4). This suggests loss of diurnal rhythm, which here persisted past the immediate perioperative period (see chapter 9). Subgroup comparisons based on the combination of delirium and MCI/dementia status revealed significant differences between salivary cortisol levels measured preoperatively in the morning and on day 4 in the afternoon, and in the AM:PM cortisol ratio on day 4. The highest cortisol levels were in those with delirium superimposed on MCI/dementia at both timepoints, with pairwise comparisons finding a significant difference between subgroups with delirium superimposed on MCI/dementia and neither delirium nor MCI/dementia (preoperative AM cortisol adjusted p=0.047, day 4 PM cortisol adjusted p=0.020). The lowest and therefore most abnormal AM:PM salivary cortisol ratio on day 4 was in the group

with delirium only (median ratio 1.4), with similar ratios in the group with delirium superimposed on MCI/dementia (median ratio 1.7) and the group with MCI/dementia only (median ratio 1.8). Pairwise comparisons did not demonstrate a significant difference between subgroups, although there was a trend towards significance between the groups with delirium only and neither delirium nor dementia, adjusted p=0.064.

Table 7.2 Morning serum cortisol levels during the perioperative period in those with and without delirium, and subgroups with delirium superimposed on MCI/dementia, delirium only, MCI/dementia only and neither delirium nor MCI/dementia. Group comparisons are with Mann-Whitney U or Kruskal-Wallis at each timepoint.

Timepoint	Median serum cortisol in nmol/L (interquartile range)							
	Delirium at any stage			Delirium and dementia subgroups				
	Yes	No	MWU P	Delirium and MCI/dementia	Delirium only	MCI/Dementia only	Neither	K-W P
Preoperative	480.2 (392.2-625.3) N=34	458.7 (327.7-601.0) N=54	777 0.227	476.8 (374.1-690.3) N=17	479.9 (406.7-531.4) N=14	370.1 (269.4-611.4) N=10	463.0 (319.3-596.0) N=39	1.69 0.640
Day 4	598.8 (484.0-706.2) N=32	523.1 (457.2-661.3) N=52	669 0.133	603.4 (444.6-735.5) N=16	556.0 (501.3-687.3) N=15	463.9 (429.2-549.9) N=13	539.9 (461.1-665.9) N=39	4.38 0.224
Day 10-14	546.4 (472.6-643.5) N=16	475.1 (389.6-508.6) N=18	90 0.062	545.7 (451.6-653.0) N=9	547.1 (470.5-640.6) N=7	515.6 (373.5-694.5) N=5	474.4 (405.3-499.4) N=13	4.29 0.231

MWU: Mann-Whitney U test

K-W: Kruskal-Wallis test

Table 7.3 Diurnal salivary cortisol levels during the perioperative period in those with and without delirium, and subgroups based on delirium and dementia status. Group comparisons are with Mann-Whitney U or Kruskal-Wallis.

Timepoint	Median salivary cortisol in nmol/L (interquartile range)							
	Delirium at any stage			Delirium and dementia subgroups				
	Yes	No	MWU p	Delirium and MCI/dementia	Delirium only	MCI/Dementia only	Neither	K-W p
Preoperative AM	11.2 (8.2-14.8) N=11	6.9 (5.6-9.4) N=16	**39 0.016**	11.8 (9.7-16.8) N=5	9.9 (6.2-13.6) N=6	5.8 (5.3-8.2) N=4	7.0 (6.1-10.2) N=11	**8.32 0.040**
Preoperative PM	5.5 (4.0-10.2) N=9	6.9 (3.7-12.2) N=13	56 0.867	4.8, 9.6 N=2	5.7 (3.9-9.4) N=6	N=1	8.0 (3.6-12.9) N=12	1.08 0.782
Preoperative AM:PM ratio	2.6 (1.1-3.2) N=5	1.4 (0.7-1.9) N=7	10 0.268	N=0	2.6 (1.1-3.2) N=5	N=1	1.4 (0.7-2.0) N=6	1.51 0.469
Day 4 AM	16.8 (11.9-29.6) N=32	17.4 (10.6-26.7) N=49	758 0.802	19.3 (9.4-29.6) N=16	14.3 (11.7-34.1) N=15	10.9 (7.7-24.2) N=13	18.1 (13.8-27.5) N=35	4.05 0.256
Day 4 PM	12.6 (8.5-17.7) N=28	7.9 (5.7-11.2) N=45	**332 0.001**	13.6 (9.9-19.0) N=14	12.3 (7.3-23.3) N=13	7.2 (5.0-12.3) N=10	7.9 (5.7-10.9) N=35	**12.12 0.007**
Day 4 AM:PM ratio	1.6 (1.0-2.5) N=27	2.1 (1.5-3.2) N=43	**363 0.009**	1.7 (0.9-2.6) N=14	1.4 (1.0-2.5) N=12	1.8 (1.4-2.2) N=10	2.3 (1.5-3.6) N=33	**8.61 0.035**
Day 7 AM	14.1 (11.6-20.5) N=29	14.2 (10.9-24.5) N=34	488 0.945	14.8 (11.1-18.8) N=14	14.0 (12.4-24.0) N=14	12.2 (6.0-25.1) N=8	14.3 (11.4-24.6) N=26	1.41 0.704
Day 7 PM	9.8 (6.2-14.7) N=22	7.2 (4.1-9.6) N=36	**261 0.031**	7.3 (6.0-14.6) N=11	11.3 (6.7-18.1) N=10	6.6 (4.0-8.3) N=7	7.4 (4.4-9.8) N=29	5.48 0.140
Day 7 AM:PM ratio	1.6 (1.1-2.7) N=21	1.9 (1.1-3.6) N=30	272 0.411	2.1 (1.2-2.8) N=10	1.6 (1.4-2.3) N=10	1.9 (0.6-3.2) N=6	1.9 (1.1-4.1) N=24	0.77 0.856
Day 10-14 AM	12.4 (8.8-19.2) N=16	13.1 (9.7-16.1) N=23	179 0.886	11.2 (8.7-17.5) N=9	18.5 (8.6-19.5) N=7	10.6 (7.8-15.2) N=6	13.6 (10.6-17.0) N=17	2.01 0.570
Day 10-14 PM	8.7 (4.8-14.8) N=16	5.7 (3.7-9.5) N=18	102 0.147	7.8 (5.2-12.2) N=8	10.1 (4.7-18.8) N=8	6.8 (5.2-9.4) N=4	5.2 (3.6-9.8) N=14	2.65 0.449
Day 10-14 AM:PM ratio	1.7 (1.0-2.5) N=15	2.1 (1.3-3.1) N=18	112 0.421	1.9 (1.0-2.5) N=8	1.6 (1.0-3.4) N=7	1.3 (1.1-3.0) N=4	2.4 (1.4-3.1) N=14	1.51 0.678

MWU: Mann-Whitney U test

K-W: Kruskal-Wallis test

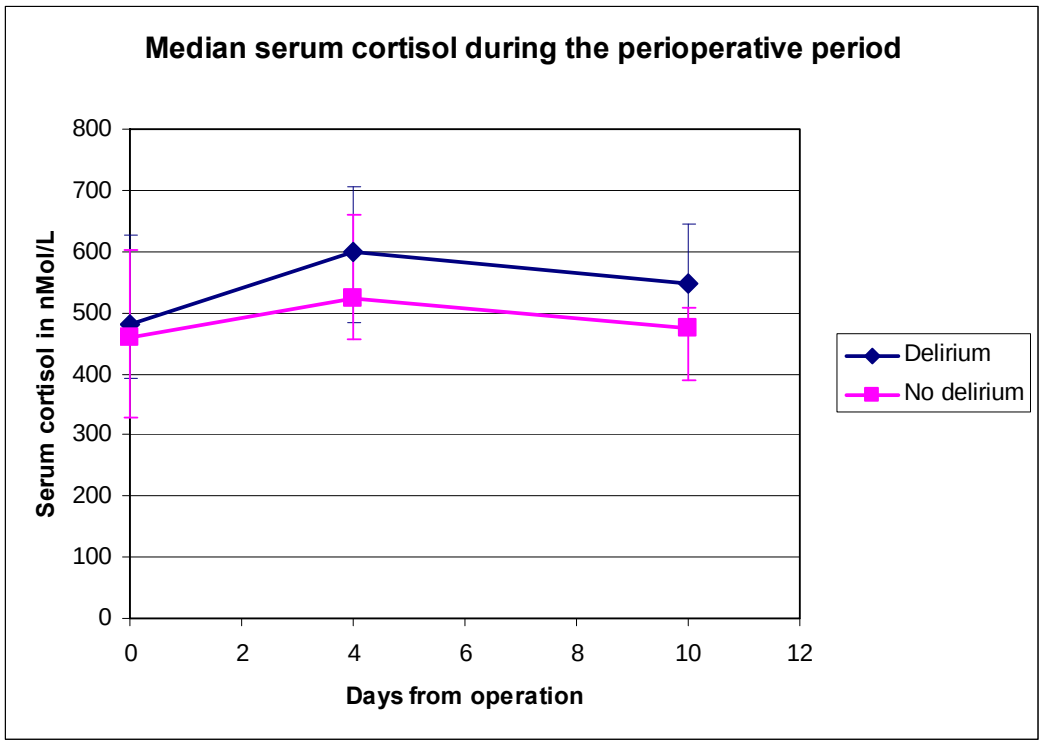
Table 7.4 Cortisol measures during the perioperative period, grouped by those who did or did not fulfil criteria for delirium on the day of sampling

Cortisol measure	Delirium on the day, Median cortisol in nmol/L (IQR)		
	Yes	No	MWU, p
Preoperative AM saliva	8.2, 9.7 N=2	8.2 (6.2-12.4) N=25	20, p=0.689
Preoperative PM saliva	N=0	5.7 (3.7-11.4) N=22	
Preoperative saliva AM:PM ratio	N=0	1.42 (1.00-2.48) N=12	
Preoperative serum	490.7 (389.3-603.8) N=11	463.0 (358.2-606.0) N=77	371, p=0.508
Day 4 AM saliva	20.1 (12.9-35.4) N=17	15.4 (10.4-26.7) N=63	433, p=0.228
Day 4 PM saliva	13.0 (10.7-21.9) N=18	8.2 (5.7-11.5) N=54	**234, p=0.001**
Day 4 saliva AM:PM ratio	1.6 (1.0-2.6) N=17	2.0 (1.3-2.9) N=52	**315, p=0.077**
Day 4 serum	679.4 (517.1-736.5) N=17	524.1 (455.1-654.8) N=66	**365, p=0.027**
Day 7 AM saliva	11.6 (10.1-20.0) N=5	14.2 (11.5-23.1) N=57	111, p=0.434
Day 7 PM saliva	8.0 (4.1-15.8) N=5	7.7 (4.7-12.3) N=53	131, p=0.979
Day 7 saliva AM:PM ratio	2.1 (0.8-2.7) N=4	1.7 (1.1-2.8) N=47	84, p=0.748
Day 10-14 AM saliva	9.5, 14.8 N=2	13.0 (9.2-18.2) N=37	33, p=0.826
Day 10-14 PM saliva	6.8, 8.3 N=2	6.6 (4.2-11.5) N=32	26, p=0.699
Day 10-14 saliva AM:PM ratio	1.0, 2.0 N=2	1.7 (1.2-2.5) N=31	26, p=0.706
Day 10-14 serum	478.8, 561.7 N=2	493.3 (419.8-561.5) N=32	24, p=0.602

MWU: Mann Whitney U test statistic

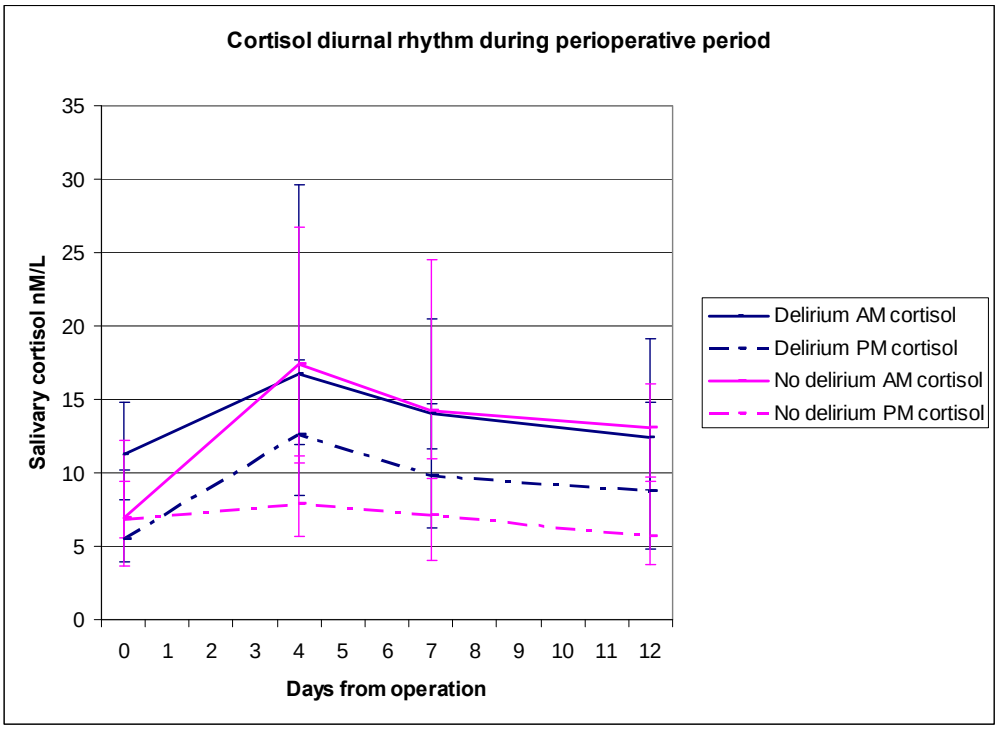
IQR: Interquartile range

Figure 7.1 Serum cortisol levels during the perioperative period, delirium at any stage



Preoperative serum cortisol ever vs. never delirium MWU=777, p=0.227, postoperative day 4 serum cortisol ever vs. never delirium MWU=669, p=0.133, postoperative day 10-14 serum cortisol ever vs. never delirium MWU=90, p=0.062.

Figure 7.2 Cortisol diurnal rhythm (saliva) during the perioperative period, delirium at any stage



Preoperative AM salivary cortisol ever vs. never delirium MWU=39, p=0.016, PM salivary cortisol ever vs. never delirium MWU=56, p=0.867. Postoperative day 4 AM salivary cortisol ever vs. never delirium MUW=758, p=0.802, PM salivary cortisol ever vs. never delirium MWU=332, p=0.001. Postoperative day 7 AM salivary cortisol ever vs. never delirium MWU=488, p=0.945, PM salivary cortisol ever vs. never delirium MWU=261, p=0.031. Postoperative day 10-14 AM salivary cortisol ever vs. never delirium MWU=179, p=0.886, PM salivary cortisol MWU=102, p=0.147.

7.3.2 Logistic regression analysis

Complete data for logistic regression were available in 73 participants, contributing to 99 blocks of analysis. Log_e-transformation normalised the cohort at the majority of timepoints. Serum cortisol was associated with delirium, even after adjustment for predisposing and precipitating factors (for each standard deviation increase in Log_e-cortisol in serum, odds increased 10.9, 95% CI 1.56 to 75.7, p=0.02). The AM:PM cortisol ratio was inversely associated with delirium (that is, the lower and more abnormal the AM:PM ratio, the greater the risk of delirium), but this was not statistically significant (for each SD increase in cortisol AM:PM ratio, OR 0.42, 95% CI 0.15 to 1.23, p=0.11). The presence of dementia (known or as ascertained by IQCODE) was associated with an 8.7 fold increase in rate of delirium (p<0.01). There were no concerns about goodness-of-fit (p=0.30).

Table 7.5 Logistic regression analysis with delirium as the outcome variable

Variable	Odds ratio	Robust SE	Z	P	95% CI
Log_e of salivary cortisol AM:PM ratio	0.42	0.23	-1.58	0.11	0.15 – 1.23
Log_e of serum cortisol	10.87	10.77	2.41	0.02	1.56 – 75.71
Dementia (IQCODE)	8.72	6.52	2.90	<0.01	2.02 – 37.75
CCI	1.04	0.17	0.24	0.81	0.76 – 1.43
Age	1.02	0.05	0.44	0.66	0.92 – 1.13
Sex	0.84	0.55	-0.26	0.80	0.24 – 3.01
APACHE II score	1.09	0.19	0.52	0.60	0.78 – 1.52
Constant	$3.48e^{-09}$	$2.11e^{-08}$	-3.21	<0.01	2.39e-14 – 0.0005

SE – standard error

CI – confidence interval

IQCODE – Informant Questionnaire of Cognitive Decline in the Elderly

CCI – Charlson co-morbidity index

APACHE II – Age, Physiology and Chronic Health Evaluation II

Number of observations – 99

Pseudo-R^2 – 0.204

7.3.3 Correlations between the HPA axis and delirium features

Tables 7.6 and 7.7 show correlations between measures of the HPA axis during the perioperative period and features of delirium (delirium severity, level of arousal, inattention and global cognition) at each time-point. At baseline, morning salivary cortisol correlated

negatively with MMSE score (higher cortisol with worse global cognition). Serum cortisol at time of anaesthetic correlated positively with delirium severity (DRS-R98) and level of arousal (OSLA) (higher cortisol with worse delirium and more abnormal level of arousal) measured at the baseline assessment. Cortisol measured in CSF at the same timepoint also correlated positively with DRS-R98 and OSLA. At day 4, DRS-R98 correlated positively with afternoon salivary cortisol, and OSLA correlated positively with morning serum cortisol and morning and afternoon salivary cortisol. EDTB-1 score correlated with AM:PM salivary cortisol ratio (more inattention with a lower and more abnormal ratio). MMSE correlated negatively with afternoon salivary cortisol. At day 7, no cortisol measures correlated with any of the measures of delirium, arousal, attention or cognition. At day 10-14, DRS-R98 and OSLA correlated positively with afternoon salivary cortisol, suggesting less of the normal afternoon dip in cortisol with more severe delirium and abnormal arousal.

Table 7.6 Correlations between baseline and day 4 measures of cortisol in serum, saliva and CSF, and measures of delirium severity, level of arousal, inattention and global cognition

Cortisol measure	Baseline				Day 4			
	DRS-R98	OSLA	EDTB-1	MMSE	DRS-R98	OSLA	EDTB-1	MMSE
Morning serum (baseline at time of anaesthetic)	**R=0.234 p=0.028 N=88**	**R=0.307 p=0.004 N=88**	R= -0.143 p=0.297 N=55	R=0.048 p=0.662 N=86	R=0.086 p=0.441 N=83	**R=0.240 p=0.030 N=82**	R= -0.043 p=0.742 N=74	R= -0.169 p=0.130 N=81
AM saliva	R=0.349 p=0.075 N=27	R= -0.019 p=0.928 N=26	R= -0.353 p=0.098 N=23	**R= -0.414 p=0.032 N=27**	R=0.042 p=0.714 N=80	**R=0.254 p=0.024 N=79**	R=0.064 p=0.631 N=58	R= -0.131 p=0.249 N=79
PM saliva	R= -0.118 p=0.600 N=22	R=0.089 p=0.703 N=21	R=0.160 p=0.489 N=21	R= -0.198 p=0.377 N=22	**R=0.284 p=0.016 N=72**	**R=0.310 p=0.008 N=71**	R= -0.268 p=0.057 N=51	**R= -0.337 p=0.004 N=71**
AM:PM saliva ratio	R=0.555 p=0.061 N=12	R= -0.196 p=0.564 N=11	R= -0.491 p=0.125 N=11	R= -0.402 p=0.196 N=12	R= -0.209 p=0.085 N=69	R= -0.041 p=0.737 N=68	**R=0.381 p=0.008 N=48**	R=0.181 p=0.140 N=68
CSF	**R=0.453 p<0.001 N=66**	**R=0.301 p=0.014 N=66**	R= -0.229 p=0.155 N=55	R= -0.065 p=0.612 N=64				

R = Spearman's Rho

DRS-R98 = Delirium Rating Scale Revised '98; OSLA = Observational Scale of Level of Alertness

EDTB-1 = Edinburgh Delirium Test Box-1; MMSE = Mini-Mental State Examination

Table 7.7 Correlations between postoperative day 7 and day 10-14 measures of cortisol in serum and saliva, and measures of delirium severity, level of arousal, inattention and global cognition

Cortisol measure	Day 7				Day 10-14			
	DRS-R98	OSLA	EDTB-1	MMSE	DRS-R98	OSLA	EDTB-1	MMSE
Morning serum					R=0.277 p=0.113 N=34	R=0.179 p=0.319 N=33	R=0.087 p=0.661 N=28	R= -0.271 p=0.127 N=33
AM saliva	R= -0.027 p=0.834 N=62	R=0.108 p=0.407 N=61	R= -0.105 p=0.489 N=46	R=0.007 p=0.959 N=59	R=0.145 p=0.377 N=39	R=0.219 p=0.187 N=38	R= -0.133 p=0.475 N=31	R= -0.043 p=0.797 N=38
PM saliva	R=0.202 p=0.128 N=58	R=0.178 p=0.186 N=57	R= -0.057 p=0.712 N=45	R= -0.166 p=0.229 N=54	**R=0.467** **p=0.005** N=34	**R=0.390** **p=0.023** N=34	R= -0.039 p=0.841 N=29	R= -0.306 p=0.083 N=33
AM:PM saliva ratio	R= -0.138 p=0.335 N=51	R= -0.142 p=0.324 N=50	R= -0.057 p=0.729 N=39	R=0.048 p=0.744 N=48	R= -0.188 p=0.294 N=33	R= -0.200 p=0.266 N=33	R= -0.120 p=0.544 N=28	R=0.134 p=0.464 N=32

R = Spearman's Rho

DRS-R98 = Delirium Rating Scale Revised '98

OSLA = Observational Scale of Level of Alertness

EDTB-1 = Edinburgh Delirium Test Box-1

MMSE = Mini-Mental State Examination

7.3.4 Correlations between cortisol measures and delirium predisposing and precipitating factors

There was a positive correlation between age and CSF cortisol (rho=0.289, p=0.019), and serum cortisol at baseline (rho=0.347, p=0.001), and also with PM salivary cortisol on day 7 (rho=0.334, p=0.010) and AM salivary cortisol on day 14 (rho=0.371, p=0.020). Number of hours from triage in Accident and Emergency to operation correlated with preoperative cortisol AM:PM ratio (rho=0.662, p=0.019) and with preoperative serum cortisol (rho=0.234, p=0.031). APACHE II score at baseline correlated positively with afternoon salivary cortisol on postoperative days 4 (rho=0.297, p=0.011) and 7 (rho=0.347, p=0.008). IQCODE average score correlated inversely with AM:PM cortisol ratio on day 4 (rho= -0.258, p=0.033). Katz personal ADL score correlated inversely with preoperative serum cortisol (rho= -0.272, p=0.010) and negatively with postoperative day 4 afternoon salivary cortisol (rho= -0.231, p=0.049). Lawton instrumental ADL score correlated inversely with CSF cortisol (rho= -0.301, p=0.016) and with postoperative day 10-14 afternoon salivary cortisol (rho= -0.463, p=0.006) and AM:PM ratio (rho=0.459, p=0.007). Charlson co-morbidity index did not correlate with any of the cortisol measures, and the number of prescribed medications on admission did not correlate with baseline cortisol measures.

7.4 Discussion

This study found several significant relationships between cortisol and delirium. These findings will now be summarised, followed by a discussion of their implications.

Morning serum cortisol was elevated in the delirium group throughout the perioperative period, peaking on day 4 postoperatively. This difference was statistically significant only for those with active delirium on postoperative day 4. When longitudinal serum cortisol measures were examined in the multivariate logistic regression analysis, it was found to be associated with delirium after adjusting for age, gender, illness severity, co-morbidity and dementia status. However, due to the number of participants, although several important co-variates were included, there may have been other factors influencing cortisol profile such as waiting time for operation, type of anaesthetic or functional level which could not be accounted for.

Morning salivary cortisol was higher in the delirium group preoperatively, and rose in both groups postoperatively. Afternoon salivary cortisol levels were significantly higher in the delirium group on postoperative days 4 and 7, and in those with active delirium on postoperative day 4. There was consequent flattening of the cortisol diurnal rhythm (as indicated by a lower AM:PM ratio), with an attenuation of the diurnal nadir, with a lower AM:PM ratio in the delirium group throughout the perioperative period, which was significant on postoperative day 4. The highest afternoon cortisol levels were in the group with delirium superimposed on dementia/MCI and then the delirium only group, and the lowest AM:PM ratio was in the delirium only group, followed by the delirium on dementia/MCI group. The multivariate logistic regression analysis found a trend towards significance for a lower cortisol AM:PM ratio in the delirium group after adjusting for co-variates. Baseline APACHE II score was shown to correlate with afternoon salivary cortisol level on postoperative days 4 and 7, and it may be that illness severity has an influence on the loss of cortisol diurnal rhythm in delirium.

Cortisol in CSF and serum at baseline correlated with delirium severity and level of arousal. Postoperatively, on days 4 and 10-14, afternoon salivary cortisol correlated positively with delirium severity and level of arousal, suggesting more severe delirium and more abnormal arousal with higher and therefore abnormal afternoon cortisol levels There were more

consistent relationships between cortisol measures and measures of arousal and delirium severity than with inattention or global cognition, suggesting HPA axis activation may be more involved in some features of delirium than others.

The results of the correlations tested between predisposing and precipitating factors of delirium and cortisol measures suggest that the longer a patient waits from arrival in hospital to operation, the greater the activation of the HPA axis, potentially due to more opportunity for exposure to stress, pain and other activators of the HPA axis, which would likely impact on delirium risk. Higher illness severity scores, worse IQCODE (prior dementia) scores and worse functional ADL scores were all associated with greater failure of cortisol diurnal rhythm to reach its nadir, and may be factors influencing this loss of diurnal rhythm in delirium or in acute illness. Co-morbidity and polypharmacy do not seem to have a particular impact on the HPA axis in this cohort.

In summary, these findings provide support for the hypothesis that there is an exaggerated and sustained hypothalamic-pituitary-adrenal axis cortisol response to stress in delirium. This elevation in cortisol release was both central in CSF (see chapter 4) and peripheral as measured in serum and saliva. This study is also the first I am aware of to examine cortisol diurnal rhythm in delirium longitudinally, and suggests that there is maintained circadian drive in the AM, with a failure to shut down cortisol release in the PM. There are several possible reasons for this change which are not mutually exclusive, including the effects of stress or acute illness, impaired negative feedback on the HPA axis or impaired metabolism or clearance of cortisol.

In previous studies where cortisol has been measured at a single timepoint, usually preoperatively or on admission, it has been shown to be associated with postoperative delirium after cardiac surgery (Mu, Wang et al. 2010) and non-cardiac surgery (Shi, Wang et al. 2010), and to be elevated in those with delirium associated with acute coronary syndrome (Colkesen, Giray et al. 2013), elective cardiac surgery (Plaschke, Fichtenkamm et al. 2010) and critical illness (Pfister, Siegemund et al. 2008, van den Boogaard, Kox et al. 2011). Longitudinal studies have shown that high preoperative levels of serum cortisol before elective cardiac surgery predict risk of postoperative delirium (Kazmierski, Banys et al. 2013). Those who develop delirium post-abdominal surgery have higher cortisol levels (Kudoh, Takese et al. 2005), and in patients undergoing elective arthroplasty, a greater rise in

serum cortisol from pre-op to post-op was associated with a greater risk of developing delirium (Cerejeira, Batista et al. 2013). In hip fracture, CSF cortisol taken at induction of anaesthetic has been shown to be elevated in those who develop delirium perioperatively (Pearson, de Vries et al. 2010), and in serial serum samples encompassing a delirium episode, to be higher in those with delirium but not after adjusting for prior dementia (Bisschop, de Rooij et al. 2011).

There are several potential consequences of sustained elevation in cortisol levels. Peripherally, this elevated cortisol promotes a pro-catabolic state which may hamper physical and functional recovery from hip fracture and increase vulnerability to infection. These catabolic effects could be expected to be worse in those who had delirium. In the CNS, sustained elevation of cortisol levels over weeks to months may be associated with hippocampal atrophy, and on a cellular level cortisol increases neuronal vulnerability to cell damage from other insults, and reduces neuronal cell dendritic length and cell volume (Tata and Anderson 2010). Increased HPA axis activity (morning plasma cortisol) has also been shown to be associated with more rapid deterioration in cognitive test scores in patients with Alzheimer's dementia (Csernansky, Dong et al. 2006).

Failure of cortisol levels to fall in the PM could potentially disrupt sleep-wake cycle, and such disruption is extremely common in delirium (Meagher, Moran et al. 2007). It would also mean that the potentially harmful effects of exposure of cells to elevated cortisol levels for sustained periods would be more prolonged, without the normally observed reduction of levels in the evening. Indeed, higher evening cortisol levels in a population-based study have been shown to be associated with cognitive impairment (Johar, Emeny et al. 2015). Cortisol diurnal rhythm has not been examined in detail before in delirium. However in an early study McIntosh *et al* demonstrated loss of circadian rhythm of cortisol and β-endorphin in three of seven male patients who developed delirium in surgical intensive care after non-cardiac surgery (McIntosh, Bush et al. 1985). Dysregulation of cortisol and control of the HPA axis in delirium is further suggested by the demonstration of abnormal dexamethasone suppression tests in delirium in patients with lower respiratory tract infection (O'Keeffe and Devlin 1994) and delirium superimposed on dementia (Robertsson, Blennow et al. 2001), with failure of dexamethasone to suppress cortisol levels. In elderly patients with delirium associated with a lower respiratory tract infection, this loss of negative feedback of the HPA

axis persisted up to eight weeks later, even when the acute illness and delirium episode had resolved (O'Keeffe and Devlin 1994).

In postoperative cognitive dysfunction, Rasmussen *et al* found a similar pattern of change in cortisol diurnal rhythm with elevation in both morning and afternoon levels and significant flattening of the circadian rhythm at one week after non-cardiac surgery in patients who developed postoperative cognitive dysfunction (Rasmussen, O'Brien et al. 2005). Although the Rasmussen study did not assess specifically for postoperative delirium, it is possible that some of these patients had delirium as well as POCD. Comparable perioperative timepoints in this study and the Rasmussen study are preoperative and postoperative day 7; given the small numbers of preoperative samples in this study, these values are difficult to compare. At day 7, median morning salivary cortisol values are very similar in those with delirium, POCD and no delirium/POCD. Median day 7 afternoon salivary cortisol, which was elevated in POCD in the Rasmussen study, is higher still in delirium as measured in this study.

There are some limitations of this study which must be acknowledged. Although participants were assessed regularly for delirium, with timings aimed to balance accurate and sensitive delirium diagnosis with the burden to participants, due to the fluctuating nature of delirium, some mild or short episodes may have been missed. Although the multivariate logistic regression analysis aimed to adjust for known important predisposing and precipitating factors for delirium, due to the numbers of participants and datapoints it was not possible to include all co-variates, and the influence of waiting time for operation, type of anaesthetic and functional level in particular could not be accounted for. There are also missing serum and saliva samples at all timepoints, but in particular the preoperative saliva samples and day 10-14 samples. Preoperatively, because patients were often recruited on the morning of surgery, and were fasting, it was often only possible to attempt one saliva sample collection (if any, due to requisite AM and PM sample time windows), and these were sometimes dry or declined. By day 10-14, participants were often either discharged or transferred to an orthogeriatric rehabilitation facility. To address this, an ethical amendment was submitted early in the study to widen the time window of this assessment from day 14 only to days 10-14. This increased the number of participants assessed at this time-point, but numbers were still relatively small. Numbers in subgroups with dementia only and delirium superimposed on dementia are also small, and the subgroup analyses must be viewed as exploratory. The majority of participants with probable dementia in this study developed delirium. Although

patients intended for spinal anaesthesia were included in the study, for various reasons some went on to have general anaesthesia and a small number had both. There was no difference in rates of delirium based on anaesthetic type, in keeping with a recent systematic review and meta-analysis which did not find any difference in the odds ratio of developing post-operative delirium between patients who had regional or general anaesthesia (Mason, Noel-Storr et al. 2010). There is some evidence that regional anaesthesia reduces surgical stress intraoperatively and during the immediate postoperative period. Patients undergoing total hip replacement under combined spinal and epidural anaesthesia had lower intraoperative cortisol than those under general anaesthesia (Lattermann, Belohlavek et al. 2005), a small trial of spinal versus general anaesthesia in laparoscopic cholecystectomy also found lower intraoperative cortisol levels (Das, Bhattacharya et al. 2015), and high spinal blockade for cardiac surgery has been shown to be associated with lower cortisol levels until 4h post-operatively (Lee, Grocott et al. 2003). However, epidural versus general anaesthesia was not found to influence urinary corticol excretion during the intraoperative period or over the first 24h postoperatively in patients undergoing lower limb revascularisation (Breslow, Parker et al. 1993), and no difference was found in cortisol levels 24h after haemorroidectomy in those who had spinal or general anaesthesia (Buyukkocak, Caglayan et al. 2006). Kudoh *et al* examined HPA axis response to epidural versus general anaesthetic in patients with schizophrenia undergoing abdominal surgery. They found lower cortisol levels in the epidural group 15 minutes after skin incision and at the end of surgery, but by post-operative days 1 and 2 there was no longer any difference (Kudoh, Takese et al. 2003). There is therefore some evidence that the immediate cortisol stress response to surgery is lower with regional anaesthesia, however less is known about the effects after 24-48h postoperatively. At the timepoints tested in this study, anaesthetic type may be less likely to have had a signficiant impact. With greater participant numbers, it would be ideal to account for anaesthetic type in multivariate analysis, and further research is needed into the influence of anaesthetic type on stress reponse to surgery beyond the immediate perioperative period.

This is an observational study, and causation cannot be firmly established. This is important because delirium itself is stressful, and could therefore plausibly lead to increased cortisol release. The results of the logistic regression analysis suggest that cortisol level is predictive of delirium status at the next time it was assessed. The fact that this relationship was still significant after adjusting for important covariates including acute illness severity and chronic illness burden also suggest that the elevation of cortisol seen in delirium is not

necessarily just a marker of how "sick" someone is. Due to the relatively small sample size, I was unable to adjust for other covariates which have been shown to particularly affect salivary cortisol measurement, such as season, smoking and sociodemographic status (Vreeburg, Kruijtzer et al. 2009, Arsenault-Lapierre, Chertkow et al. 2010).

In conclusion, this study lends further support to the hypothesis that there is greater activation of the HPA axis in delirium. Serum cortisol was elevated in those with delirium on the day of sampling postoperatively, and serum cortisol level predicted delirium development after adjusting for important predisposing and precipitating factors. Cortisol levels in serum correlate with measures of delirium severity and arousal. This study is unusual in that it has assessed cortisol levels longitudinally in delirium, and in multiple modalities. It concurs with previous longitudinal studies of serum cortisol in delirium following hip fracture (Bisschop, de Rooij et al. 2011) and elective arthroplasty (Cerejeira, Batista et al. 2013), but I found that the relationship was independent of the co-variates measured including prior cognition. This is also the most detailed study to date of longitudinal cortisol diurnal rhythm in delirium. There is evidence of flattening of cortisol diurnal rhythm in delirium, with elevated afternoon levels, and lower AM:PM cortisol ratio. There was a trend to significance for the relationship between delirium and AM:PM cortisol ratio after adjusting for covariates. Subgroup analyses suggest that this loss of diurnal rhythm is in those with delirium alone and delirium superimposed on dementia. Future studies could compare these cortisol levels with patients with and without dementia undergoing elective surgery, to assess the impact of the hip fracture trauma on the HPA axis, and also with out-patients with dementia. It would also be possible to make a more detailed assessment of cortisol diurnal rhythm with more salivary cortisol measurements through the 24hour period, with additional samples collected on wakening and at bedtime. This could perhaps be done in a more focussed study of cortisol diurnal rhythm to minimise any additional burden on participants, either during elective surgery with accurate baseline assesement or in medical in-patients, patients with stroke or patients with hip fracture during a delirium episode.

8 Chapter 8: The relationship between delirium and peripheral inflammatory markers

8.1 Introduction

This study explores the peripheral (serum) inflammatory response in patients with delirium after hip fracture. Infection and trauma, including surgery, are common precipitants of delirium and induce an inflammatory response in the host. Features of sickness behaviour have much in common with delirium in the elderly, and delirium has been proposed as a maladaptive sickness behaviour response (Cunningham and MacLullich 2013). Animal modelling has shown delirium-like behavioural changes in older mice and mice with neurodegenerative disease in response to a peripheral inflammatory stimulus (Godbout, Chen et al. 2005, Cunningham, Campion et al. 2009). In the central nervous system in these mice, the peripheral inflammatory stimulus leads to an exaggerated central inflammatory response, due to the activation of "primed" microglia.

Studies examining serum cytokines and chemokines in delirium have found mixed results. In hip fracture specifically, the pro-inflammatory cytokine interleukin (IL)-6 and pro-inflammatory chemokine IL-8 have been shown to be elevated in serum in those with delirium (Beloosesky, Hendel et al. 2007, van Munster, Bisschop et al. 2010, MacLullich, Edelshain et al. 2011), but not in all studies (Lemstra, Kalisvaart et al. 2008). Delirium after cardiac surgery is associated with higher levels of chemokines (Rudolph, Ramlawi et al. 2008), and delirium after elective arthroplasty with a higher pro/anti-inflammatory ratio (Cerejeira, Nogueira et al. 2012).

Some of these studies only measured inflammatory markers at a single timepoint, and few have measured inflammatory profile after the immediate perioperative period; CRP and IL-8 have been measured up to 30 days post-hip fracture in one study (Beloosesky, Hendel et al. 2007). By measuring several serum cytokines and chemokines at three timepoints during the perioperative period, with frequent and detailed assessments for delirium, this study aims to capture any change in inflammatory profile during delirium. Also, by examining inflammatory profile up to one year post-hip fracture, this study will be able to explore

whether there might be evidence of a persistent inflammatory response after delirium, and explore the relationship with persistent symptoms of delirium.

This study therefore asked the following research questions:

1) Is delirium after hip fracture associated with derangements of inflammatory markers in serum, specifically:

   1a) High levels of pro-inflammatory cytokines?

   1b) Low levels of anti-inflammatory cytokines?

   1c) High levels of chemokines?

2) Is there an imbalance in the ratio of pro to anti-inflammatory cytokines, in favour of a pro-inflammatory state?

3) Do any differences in inflammatory profile persist in the delirium group during the year post-hip fracture?

4) What is the relationship between inflammatory markers and morning serum cortisol and cortisol AM:PM ratio measured at the same timepoints?

8.2 Methods

Patients were recruited and assessed for delirium as described in Chapter 2. Serum samples were collected at the time of anaesthetic induction (baseline) and between 8-10am on day 4 and once between days 10-14 postoperatively, and between 8-10am at the follow-up visits at 3, 6 and 12 months after hip fracture. Saliva samples for cortisol were collected between 0800-1000 (AM) and between 1500-1700 (PM) at baseline, postoperative days 4, 7, and 10-14, and at 3, 6 and 12 months. Samples were processed and stored as described in section 2.5.2. Ten cytokines and chemokines were measured by Luminex assay (R&D) as described in section 2.10.2. The manufacturers' (R&D) recommendations on sample collection and storage for measurement of cytokines in serum are that samples are allowed to clot for 30 minutes at room temperature, centrifuged and stored immediately; samples were processed and stored as soon as possible. Cortisol was also measured with ELISA in serum (MP Biomedicals) and saliva (Salimetrics) as described in sections 2.10.1 and 2.11 respectively.

The cytokines and chemokines measured in the Luminex assay were as follows:

- IL-1β (interleukin-1β), a pro-inflammatory cytokine
- IL-1ra (interleukin-1 receptor antagonist), the endogenous antagonist of IL-1β
- IL-5, a T-helper 2 anti-inflammatory cytokine that stimulates B cell growth and immunoglobulin production
- IL-6, a pro-inflammatory cytokine
- IL-8 (also known as CXCL8), a chemokine produced by macrophages that induces chemotaxis of neutrophils and other granulocytes towards infection
- IL-10, a T-helper 2 anti-inflammatory cytokine
- MIP-1α (macrophage inflammatory protein 1α, also known as CCL3), produced by macrophages after stimulation with bacterial endotoxin
- MIP-1β (macrophage inflammatory protein 1β, also known as CCL4), produced by macrophages after stimulation with bacterial endotoxin
- MCP-1 (monocyte chemoattractant protein 1, also known as CCL2), which recruits monocytes, memory T cells and dendritic cells to the site of inflammation
- TNF-α (tumour necrosis factor α), a pro-inflammatory cytokine, a key player in the acute phase response

The minimum detectable dose (MDD) for the respective inflammatory markers were as follows: IL-1β: 0.27pg/ml, IL-1ra: 4.05pg/ml, IL-5: 0.33pg/ml, IL-6: 0.36pg/ml, IL-8: 0.39pg/ml, IL-10: 0.13pg/ml, MCP-1: 0.16pg/ml, MIP-1α: 8.11pg/ml, MIP-1β: 0.44pg/ml, TNF-α: 0.60pg/ml.

8.2.1 Group comparisons

Normality of distribution was tested using histogram plots and Kolgomorov-Smirnov tests. Group comparisons first examined individual raw cytokine or chemokine levels in serum at each timepoint, comparing those who did and did not develop delirium at any stage during the perioperative period, with Mann Whitney U test. The pro/anti-inflammatory ratio of cytokines was also calculated as previously described by Cerejeira *et al,* with the following formula, using raw values: (IL-1β+TNF-α+IL-6+IL-8)/IL-10 (Cerejeira, Nogueira et al. 2012). The difference between pro/anti-inflammatory ratio during the perioperative period was then tested with Mann-Whitney U test. In addition to group comparisons between the whole group who developed delirium during the perioperative period versus the group who did not, group comparisons were also performed at each sampling timepoint between those who did or did not have active delirium on that day.

Group comparisons were also performed for individual cytokines and chemokines during the follow-up period, and this grouping took into account whether or not the patient had developed delirium during the preceding time period, i.e. up until 3, 6 or 12 months. Subgroup comparisons were also tested between patients with delirium superimposed on dementia/mild cognitive impairment (MCI), delirium only, dementia/MCI only, and neither delirium nor dementia/MCI, with Kruskal-Wallis test.

Further exploratory analyses were also performed where a difference was found in levels of inflammatory markers, in order to adjust for the effects of potentially important confounding variables including important predisposing and precipitating risk factors for delirium. Analysis of covariance was used with the log-normalised cytokine or chemokine value as the dependent variable, delirium status as the fixed factor, and age, gender, prior dementia (known diagnosis or IQCODE ≥3.44), Charlson co-morbidity index and modified-APACHE II score as covariates. A full factorial model was used with Type IV sum of squares to take account of missing values. Individual models were constructed for each significant result. The models used for cytokines and chemokines measured during the follow-up period did not

include baseline APACHE II score as a covariate, since this was an acute measure of illness severity at baseline.

8.2.2 Inflammatory marker subgrouping

Following the precedent of a study by Rudolph *et al* which investigated whether there was a difference in the postoperative inflammatory response of patients with and without delirium after cardiac surgery, cytokines and chemokines were divided into subgroups and analysed further (Rudolph, Ramlawi et al. 2008). The three subgroups, chosen *a priori* and based on the known roles of the cytokines and chemokines within the inflammatory response were:

1. Pro-inflammatory cytokines: IL-1β, IL-6, TNF-α
2. Anti-inflammatory cytokines: IL-1ra, IL-5, IL-10
3. Chemokines: IL-8, MIP-1α, MIP-1β, MCP-1

Due to skewed data, all cytokines and chemokines were first log-normalised. At each timepoint, the log-normalised cytokines were standardised to the mean of the no-delirium group at the same timepoint, to produce a marker Z score, with the following equation:

Marker Z score = (Log-cytokine value – Mean of no delirium group at same timepoint) / standard deviation of no delirium group at same timepoint

A class Z score was then calculated for each subgroup of cytokines or chemokines, at each timepoint, by calculating the mean marker Z score for each subgroup. Student's t-test was then applied, comparing the class Z scores between those with and without delirium at any stage.

To investigate the inflammatory response to the hip fracture surgery, the postoperative change in cytokine or chemokine level was calculated, by subtracting the log-normalised cytokine value at baseline from the log-normalised cytokine value at postoperative day 4. This postoperative change was then standardised to the mean change in the no delirium group to produce the Change Z score, using the following equation:

Change Z = (postoperative change in log-normalised cytokine – no delirium group mean postoperative change) / no delirium group standard deviation of postoperative change

Class Change Z scores were then calculated for the three subgroups, and Student's t test applied to compare the standardised postoperative change in cytokine and chemokine classes between those with and without delirium at any stage.

8.2.3 Correlations

Finally, correlations were tested between measures of delirium severity (DRS-R98 severity score), inattention (EDTB-1 score), level of arousal (OSLA total score) and global cognition (MMSE score) and the individual cytokines and chemokines measured contemporaneously at baseline, day 4 and days 10-14 postoperatively, and at 3, 6 and 12 months follow-up, using Spearman's Rho. This was to determine whether worse scores on components of the delirium assessment were associated with more disruption in the inflammatory profile, i.e. higher pro-inflammatory cytokines and chemokines and lower anti-inflammatory cytokines. Correlations were also tested with Spearman's Rho among the cytokines and chemokines themselves at each timepoint to assess whether markers with a biological relationship change together, which would increase the likelihood that any findings were biologically plausible. Correlations were also tested with serum cortisol, collected at the same time, and AM:PM cortisol ratio measured in saliva on the same day, with Spearman's Rho, to assess the relationship between HPA axis activation and loss of diurnal rhythm with activation of inflammation. Finally, correlations were tested between CSF (as measured in a different Luminex assay as described in chapter 5) and baseline serum cytokines with Spearman's Rho, to examine whether the relationship between the central and peripheral release of cytokines is maintained, or lost, the latter suggesting different central and peripheral processes, in keeping with the neuroinflammatory hypothesis This is limited by the use of different assays, however.

8.3 Results

One-hundred and eight participants were enrolled into the study. Two participants were excluded due to non-operative treatment as they would not be comparable to the rest of the cohort, and a further participant was excluded after day 4 due to an unpredictable and significant complication which required ICU admission and steroid therapy. During the perioperative period, one participant died and N=7 participants withdrew consent to continue participating. As discussed in Chapter 7, although patients intended for spinal anaesthesia were included, some went on to have general anaesthesia or both; these participants were not excluded from further follow-up on this basis. Data and all available samples were used for participants up until the point of withdrawal, exclusion or death. Serum samples were available for N=84 participants at baseline, N=83 at day 4 and N=34 between days 10-14. Delirium was diagnosed in N=42 participants, N=13 preoperatively (prevalent delirium) and N=29 postoperatively (incident delirium). Table 7.1 in chapter 7 shows demographic characteristics at baseline of the included cohort of N=104 participants who had at least one biological sample. As discussed in chapter 7, the delirium group was more likely to have dementia, to be more functionally impaired, frailer and be on more medication. They had higher illness severity according to APACHE II. The delirium group also waited longer for their operation, which may have been both a cause (longer wait increasing risk of incident delirium) and effect of delirium (longer wait due to medical complications delaying surgery in those with prevalent delirium). During the follow-up period, two participants who had not developed delirium during the perioperative period were found to have developed new delirium at one of the follow-up assessments, one participant at 3 months and one at 6 months.

8.3.1 Detection of inflammatory markers

During the perioperative period, the majority of cytokines were detected in most or all samples. IL-5 and MIP-1α were detected in around a half to two thirds of samples. During the follow-up period, levels of most cytokines and chemokines were lower, and in particular levels of IL-5, IL-10 and MIP-1α were very low or undetected. All original values were used in the analysis.

8.3.2 Inflammatory profile during the perioperative period

All the cytokines and chemokines were not normally distributed so non-parametric statistics were used for raw data group comparisons. Table 8.2 shows the results of group comparisons for individual cytokines and chemokines and the pro/anti-inflammatory ratio between those who did or did not develop delirium during the perioperative period. Figures 8.1 to 8.10 show individual serum cytokine and chemokine levels during the perioperative period, comparing the groups who did or did not develop delirium at any stage.

8.3.2.1 Univariate analyses

IL-1ra and IL-6 levels were significantly higher in the delirium group at baseline and at day 10-14. IL-8 levels were significantly higher in the delirium group throughout the perioperative period (day 0 – day 10-14). MIP-1β levels were higher in the delirium group at day 10-14. The pro/anti-inflammatory ratio rose slightly in both groups postoperatively, and it was higher in the delirium group throughout the perioperative period, suggesting a shift to a pro-inflammatory state. There was no difference in IL-1β, TNF-α, IL-5, IL-10, MCP-1 and MIP-1α between those who did or did not develop delirium at any stage in the perioperative period.

Levels of serum inflammatory markers at each timepoint were also compared between those who did or did not have active delirium on the day of sampling, as shown in Table 8.3. At baseline, those with active delirium had lower levels of MIP-1β than those who did not. On postoperative day 4, those with active delirium had higher levels of IL-6, IL-8 and TNF-α, and a higher pro/anti-inflammatory ratio than those without delirium. On postoperative day 10-14, numbers with active delirium were very small (N=2), however levels of IL-1β were still significantly higher in delirium than in those without.

8.3.2.2 Multivariate analyses

As an exploratory analysis, cytokines where there was a group difference in the initial univariate analysis were chosen for Analysis of Covariance to adjust for potential confounders. The log-normalised cytokine or chemokine value was the dependent variable, delirium status the fixed factor, and age, gender, prior dementia, Charlson co-morbidity index and modified-APACHE II score were covariates. The results of the ANCOVA tests are shown in Table 8.4 for group comparisons between those with delirium at any stage from day

0 to day 10-14 postoperatively, and Table 8.5 for group comparisons between those with and without active delirium on the day of assessment.

After adjusting for covariates, delirium at any stage from day 0 – day 10-14 remained significantly associated with LogIL-6 and LogMIP-1β at postoperative day 10-14.

After adjusting for covariates, LogMIP-1β was still associated with active delirium at baseline, there was a trend towards significance between LogIL-6 and active delirium on day 4, and LogIL-1β was still associated with active delirium on day 10-14.

Table 8.2 Serum cytokine or chemokine levels (raw values) during the perioperative period in those with and without delirium at any stage during the perioperative period*

Cytokine or chemokine	Baseline, Median (IQR), pg/ml			Postoperative day 4, Median (IQR), pg/ml			Postoperative day 10-14, Median (IQR), pg/ml		
	Delirium N=31	No delirium N=53	MWU p	Delirium N=32	No delirium N=51	MWU p	Delirium N=16	No delirium N=18	MWU p
IL-1β	1.8 (1.2-2.6)	1.5 (0.7-2.3)	702 0.268	1.3 (0.5-2.5)	1.2 (0.7-2.6)	783 0.753	1.6 (1.1-2.2)	1.2 (0.8-3.0)	137 0.795
IL-1ra	3457 (1829-6504)	2199 (1269-3624)	**539 0.009**	4015 (2422-5983)	4190 (2440-6962)	755 0.565	2930 (2106-7983)	1848 (1595-3571)	**82 0.032**
IL-5	0.8 (0.4-1.7)	0.7 (0-2.1)	773 0.647	0.4 (0-0.9)	0.7 (0-0.9)	713 0.326	0.5 (0-1.0)	0 (0-0.7)	101 0.116
IL-6	21.5 (16.9-65.8)	18.4 (8.5-34.6)	**587 0.030**	15.5 (10.5-41.5)	14.6 (8.8-29.8)	738 0.466	9.6 (7.2-17.0)	6.5 (5.1-7.7)	**64 0.005**
IL-8	22.1 (18.3-34.4)	17.1 (11.0-26.1)	**573 0.021**	30.9 (24.3-41.3)	21.9 (13.7-34.7)	**581 0.028**	31.9 (21.9-43.1)	18.4 (8.4-26.0)	**70 0.011**
IL-10	2.1 (1.6-2.9)	2.0 (1.8-3.1)	806 0.882	1.8 (1.6-2.6)	2.1 (1.7-2.7)	704 0.294	2.1 (1.5-2.4)	1.8 (1.4-2.3)	124 0.489
MCP-1	270.0 (202.4-310.9)	247.5 (163.6-342.8)	755 0.538	269.9 (211.6-357.2)	248.9 (171.8-347.3)	779 0.729	347.5 (224.9-424.9)	261.8 (137.9-352.1)	90 0.062
MIP-1α	0 (0-41.3)	24.0 (0-85.4)	659 0.109	24.0 (0-70.3)	0 (0-75.3)	797 0.849	32.6 (0-67.8)	56.7 (0-109.5)	107 0.185
MIP-1β	97.0 (48.3-187.2)	97.8 (58.9-143.0)	777 0.680	83.2 (44.9-164.3)	110.0 (64.6-135.4)	743 0.495	113.2 (72.0-151.8)	39.9 (25.3-105.0)	**77 0.021**
TNF-α	9.4 (5.2-12.9)	8.4 (5.1-10.4)	696 0.245	11.1 (6.9-12.8)	9.4 (6.1-11.8)	713 0.333	10.4 (8.6-12.2)	8.2 (4.2-11.6)	113 0.284
Pro/anti-inflammatory ratio	28.0 (23.0-38.1)	20.5 (14.1-29.7)	**474 0.004**	35.1 (25.9-41.3)	23.6 (16.3-34.9)	**528 0.010**	23.4 (20.9-48.7)	17.6 (12.4-26.5)	**73 0.025**

MWU: MannWhitney U test statistic

*Perioperative period from baseline (time of operation) to day 10-14 postoperatively

Table 8.3 Serum cytokine or chemokine levels (raw values) during the perioperative period in those with and without active delirium on the day of sampling during the perioperative period

Cytokine or chemokine	Baseline, Median (IQR), pg/ml			Postoperative day 4, Median (IQR), pg/ml			Postoperative day 10-14, Median (IQR), pg/ml		
	Delirium N=10	No delirium N=74	MWU p	Delirium N=17	No delirium N=65	MWU p	Delirium N=2	No delirium N=32	MWU p
IL-1β	1.8 (0.9-3.1)	1.6 (0.9-2.3)	342 0.699	1.6 (0.6-2.7)	1.2 (0.6-2.6)	520 0.710	2.6, 8.4	1.3 (0.9-2.0)	**2.5 0.014**
IL-1ra	2922 (1807-6351)	2408 (1404-4785)	305 0.369	5518 (3242-6398)	4091 (2407-6629)	480 0.407	2159, 26490	2287 (1657-3827)	17 0.321
IL-5	0.2 (0-0.8)	0.7 (0.3-2.1)	238 0.065	0.4 (0-1.3)	0.7 (0-0.9)	540 0.880	0, 0.5	0.3 (0-0.9)	29 0.856
IL-6	21.1 (14.6-69.3)	10.7 (19.7-42.1)	324 0.521	27.6 (12.8-53.0)	13.8 (8.9-25.3)	**348 0.019**	9.8, 11.2	7.3 (5.5-9.5)	12 0.175
IL-8	21.9 (17.2-32.9)	19.0 (11.9-30.2)	311 0.411	32.4 (24.8-43.9)	23.2 (15.8-34.1)	**375 0.042**	17.0, 24.1	22.4 (13.8-35.1)	31 0.970
IL-10	1.9 (1.5-2.4)	2.1 (1.7-3.1)	289 0.260	2.4 (1.6-3.0)	2.0 (1.6-2.5)	480 0.403	0, 1.1	1.9 (1.5-2.4)	21 0.471
MCP-1	212.8 (149.4-301.6)	272.2 (191.7-342.1)	283 0.229	262.1 (158.3-391.4)	252.2 (182.3-326.3)	545 0.932	203.4, 318.6	296.4 (159.5-381.3)	25 0.649
MIP-1α	0 (0-39.3)	24.0 (0-73.2)	273 0.155	49.6 (0-68.1)	0 (0-75.3)	545 0.922	0, 26.8	49.6 (0-94.4)	25 0.649
MIP-1β	47.6 (31.9-93.5)	101.9 (68.3-150.7)	**195 0.015**	93.6 (51.5-184.5)	93.6 (54.7-134.2)	519 0.702	50.2, 82.5	91.0 (33.7-124.3)	31 0.970
TNF-α	7.7 (6.2-12.4)	8.7 (5.1-10.9)	368 0.978	12.5 (7.7-14.5)	9.4 (6.1-11.6)	**379 0.046**	12.5, 13.4	9.8 (4.7-11.4)	10 0.128
Pro/anti-inflammatory ratio	25.5 (22.6-44.2)	24.5 (15.3-33.4)	238 0.179	36.7 (27.7-45.3)	25.8 (16.6-36.1)	**331 0.014**	N=1	22.1 (15.7-29.8)	N/A

MWU: MannWhitney U test statistic

Table 8.4 Exploratory Analysis of Covariance to adjust for confounders for those cytokines and chemokines where there was a significant difference between groups in the univariate analysis; delirium at any stage from baseline to day 10-14

Timepoint	Inflammatory marker	Delirium at any stage Y/N (1, N-1) F	Delirium at any stage Y/N P
Baseline	LogIL-1ra	(1,75) 1.38	0.244
	LogIL-6	(1,75) 2.78	0.100
	LogIL-8	(1,75) 1.26	0.266
	LogPro/Anti Ratio	(1,72) 0.17	0.680
Day 4	LogIL-8	(1,81) 2.05	0.157
	LogPro/Anti Ratio	(1,74) 3.70	0.059
Day 10-14	LogIL-1ra	(1,33) 0.45	0.511
	LogIL-6	(1,33) 5.07	**0.033**
	LogIL-8	(1,33) 1.55	0.224
	LogMIP-1β	(1,30) 4.76	**0.039**
	LogPro/Anti Ratio	(1,30) 0.99	0.329

Analysis of Covariance, with log-normalised cytokine or chemokine as the dependent variable, delirium at any stage during the perioperative period as the fixed factor, and age, gender, prior dementia (known or IQCODE ≥3.44), modified APACHE II score and Charlson comorbidity index as covariates.

Table 8.5 Exploratory Analysis of Covariance to adjust for confounders for those cytokines and chemokines where there was a significant difference between groups in the univariate analysis; delirium active on the day the serum sample was collected

Timepoint	Inflammatory marker	Delirium on the day Y/N (1, N-1) F	Delirium on the day Y/N P
Baseline	LogMIP-1β	(1,72) 4.63	**0.035**
Day 4	LogIL-6	(1,80) 3.65	0.060
	LogIL-8	(1,80) 0.42	0.519
	LogTNF-α	(1,78) 0.42	0.518
	LogPro/Anti Ratio	(1,73) 0	0.995
Day 10-14	LogIL-1β	(1,31) 7.34	**0.012**

Analysis of Covariance, with log-normalised cytokine or chemokine as the dependent variable, delirium active on the day of sampling as the fixed factor, and age, gender, prior dementia (known or IQCODE ≥3.44), modified APACHE II score and Charlson comorbidity index as covariates

Figure 8.1 Median serum IL-1β during the perioperative period in those with and without delirium at any stage

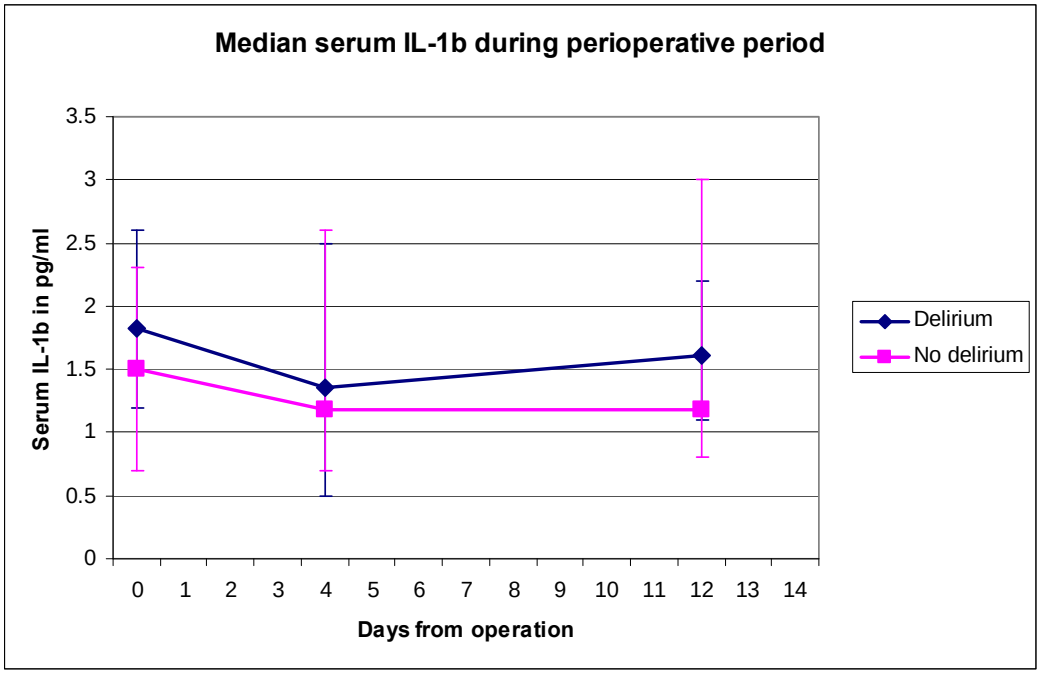


Figure 8.2 Median serum IL-1ra during the perioperative period in those with and without delirium at any stage

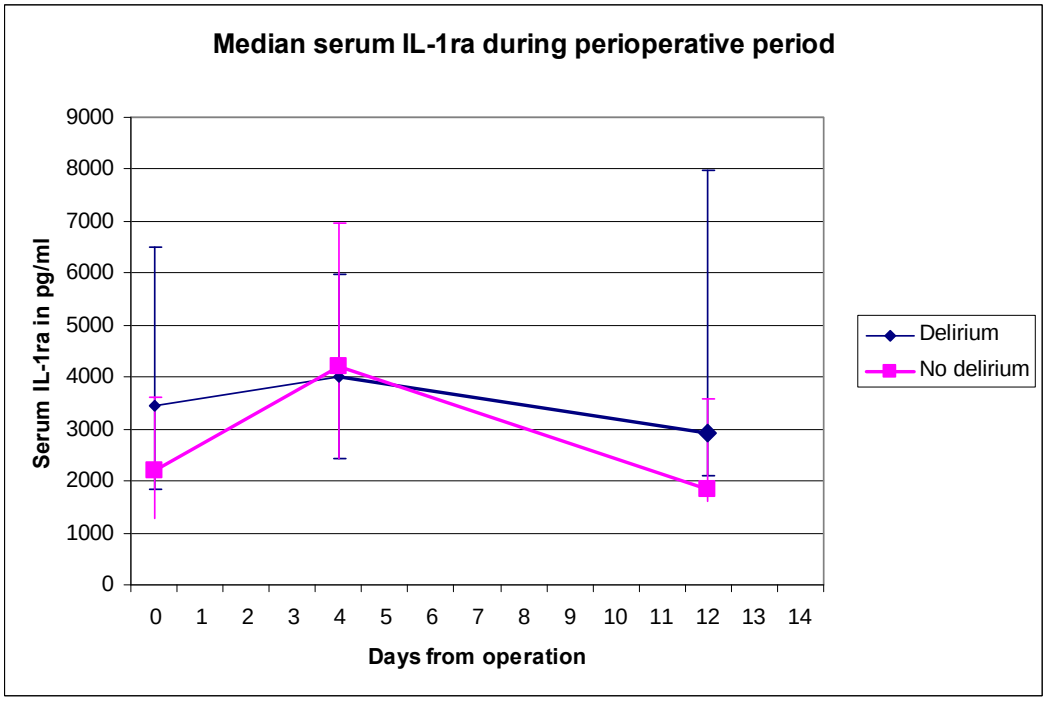


Figure 8.3 Median serum IL-5 during the perioperative period in those with and without delirium at any stage

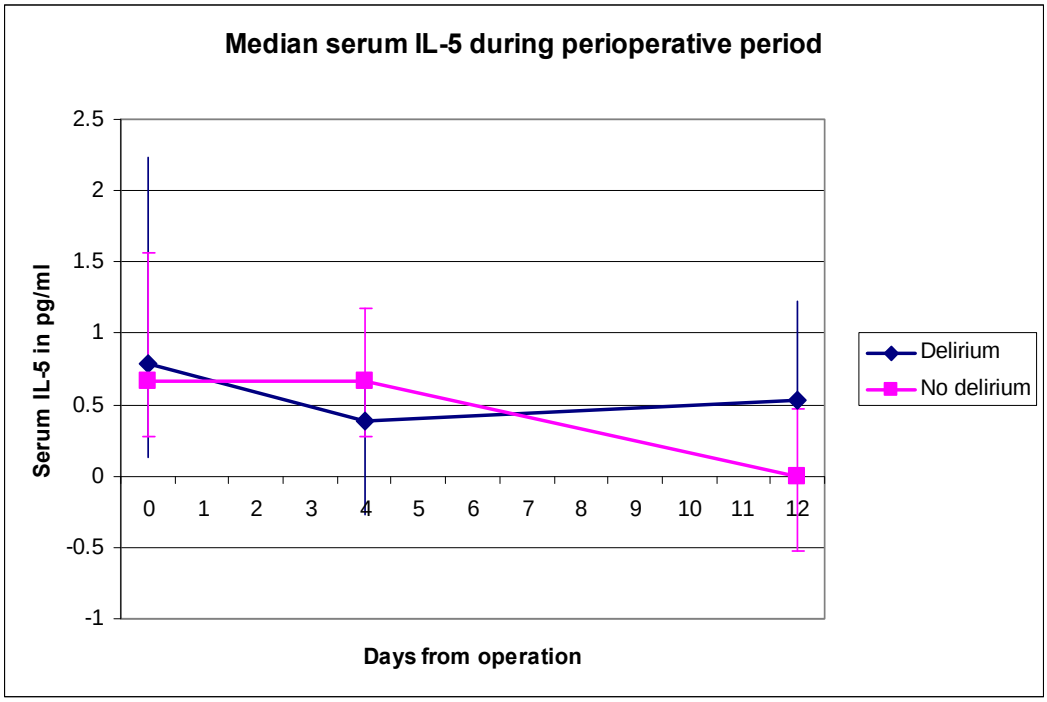


Figure 8.4 Median serum IL-6 during the perioperative period in those with and without delirium at any stage

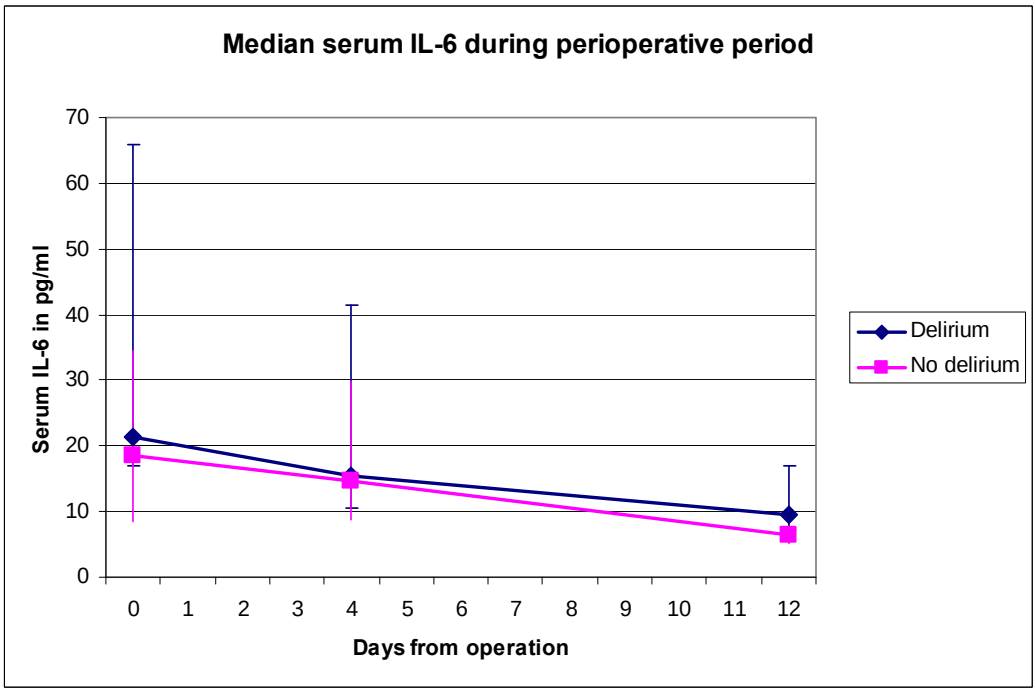


Figure 8.5 Median serum IL-8 during the perioperative period in those with and without delirium at any stage

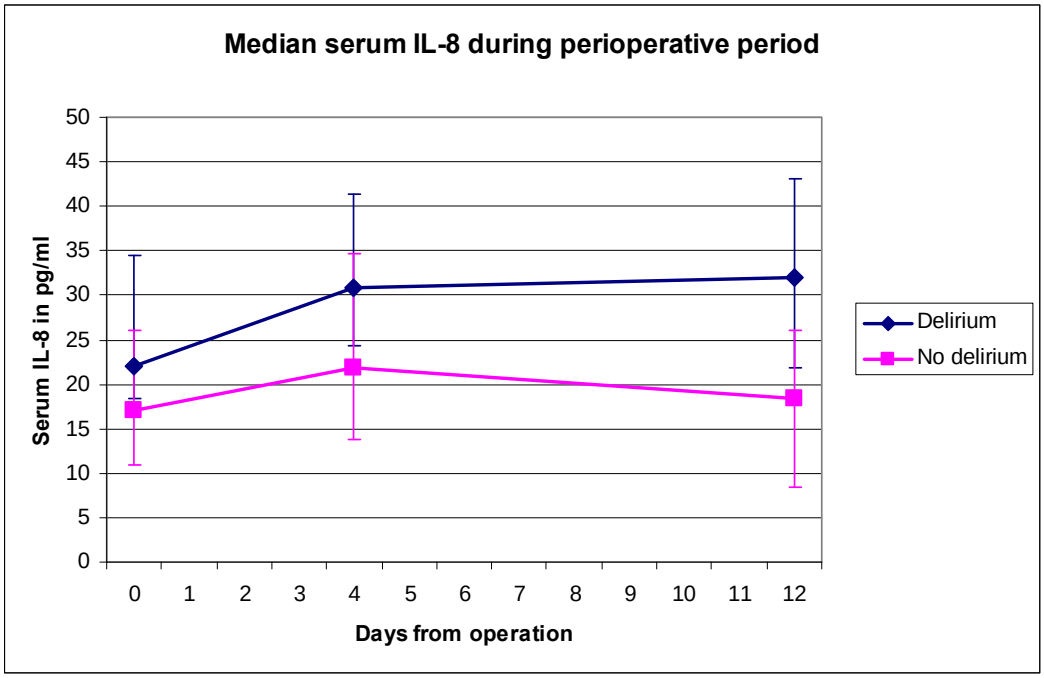


Figure 8.6 Median serum IL-10 during the perioperative period in those with and without delirium at any stage

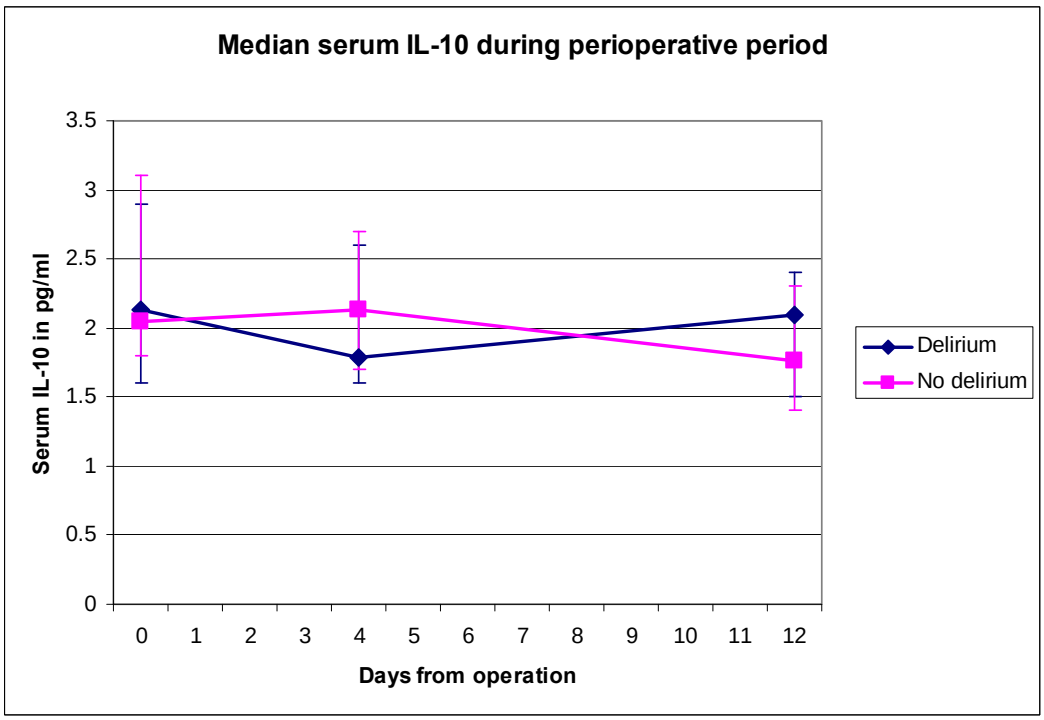


Figure 8.7 Median serum MCP-1 during the perioperative period in those with and without delirium at any stage

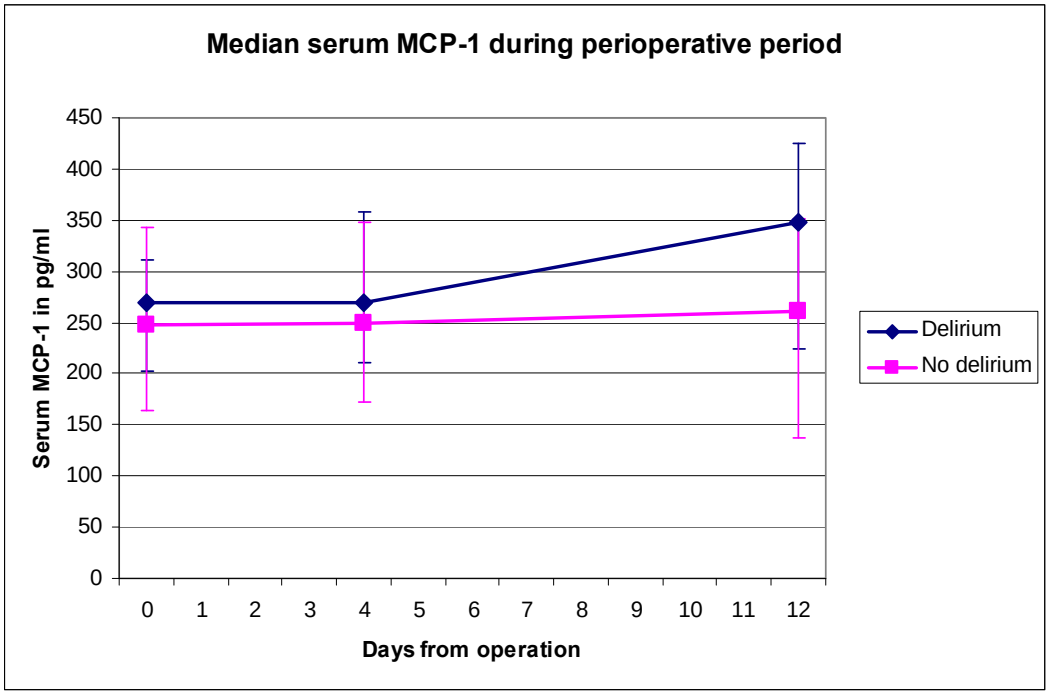


Figure 8.8 Median serum MIP-1α during the perioperative period in those with and without delirium at any stage

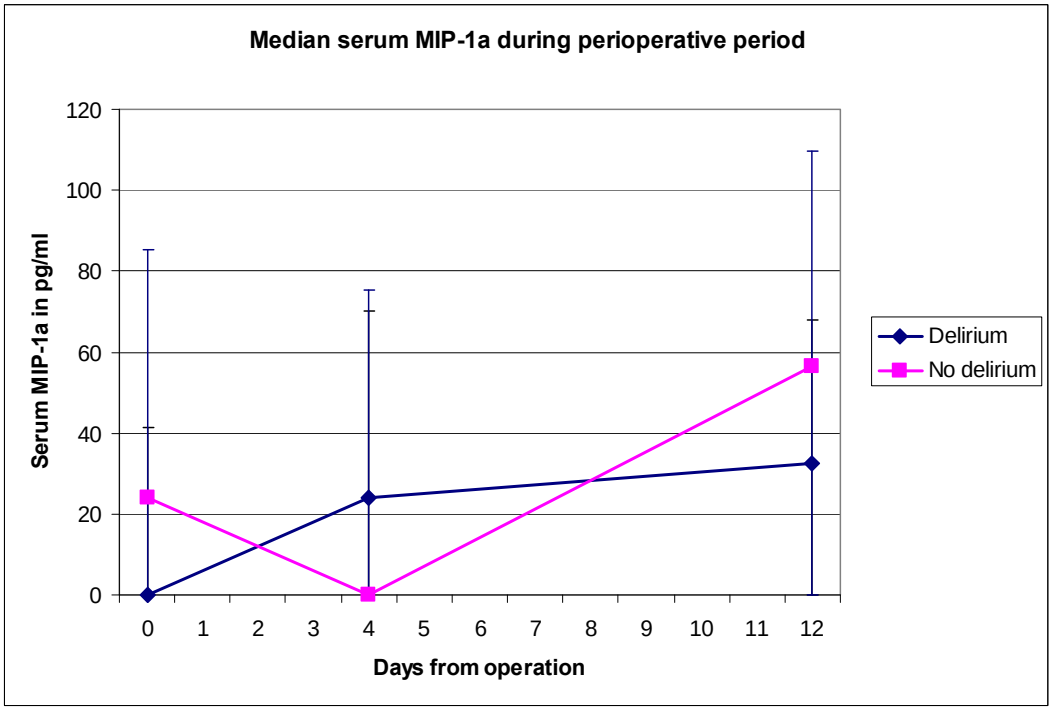


Figure 8.9 Median serum MIP-1β during the perioperative period in those with and without delirium at any stage

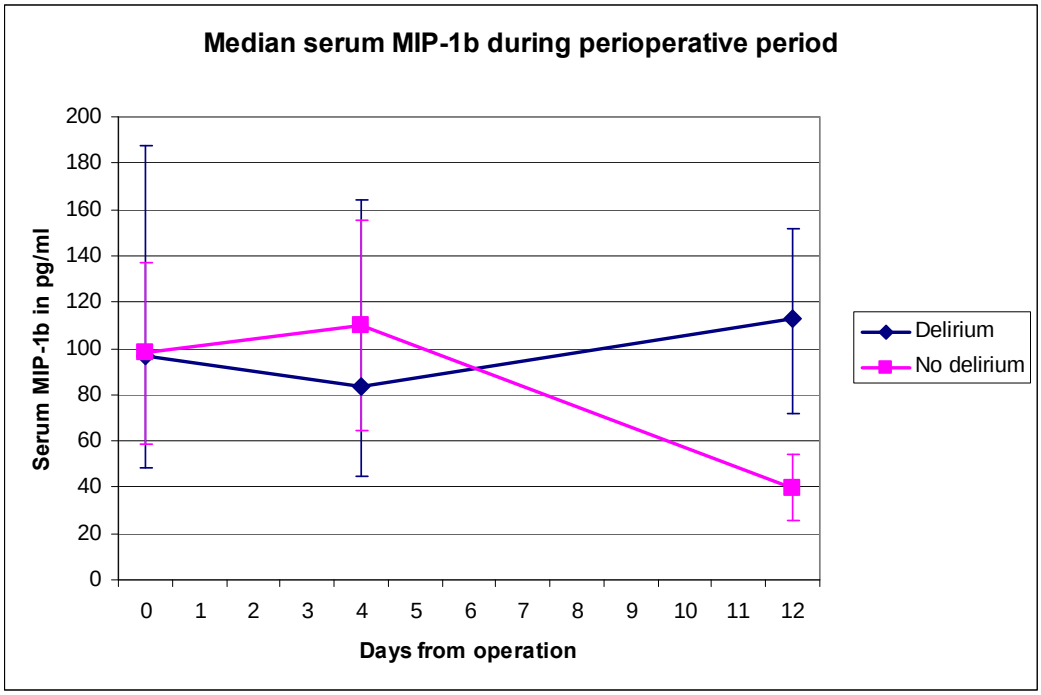
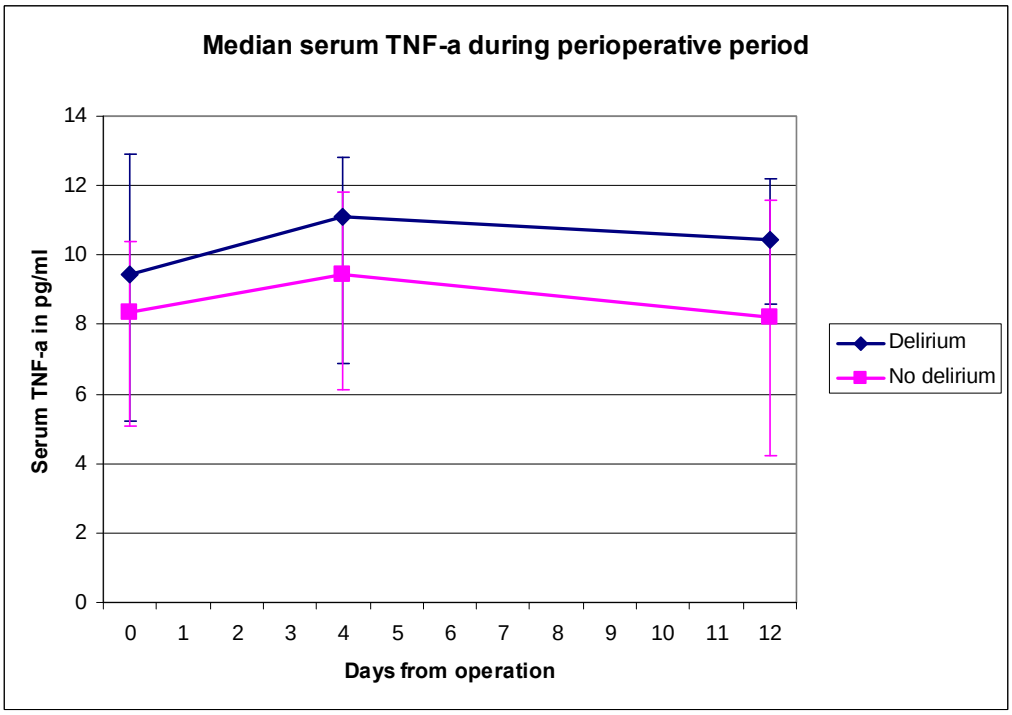


Figure 8.10 Median serum TNF-α during the perioperative period in those with and without delirium at any stage



8.3.3 Inflammatory marker subgroup profile during the perioperative period

Table 8.6 shows the Class Z scores for the delirium and no delirium groups for the three inflammatory marker subgroups. There was a trend towards a higher Class Z score in delirium in the pro-inflammatory subgroup at baseline, and this was significant at day 10-14. There was also a trend towards a higher Class Z score in delirium in the anti-inflammatory subgroup at day 10-14. There was no significant difference in the chemokine subgroup at any timepoint. Table 8.7 shows the Change Z scores (baseline to postoperative day 4) in those with and without delirium for the three inflammatory marker subgroups. There was greater change between baseline and day 4 in all three subgroups of cytokines and chemokines in the delirium group, which was significant in the pro-inflammatory and chemokine subgroups.

Analysis of covariance, with Class Z score or Change Z score as the dependent variable, delirium at any stage during the perioperative period as the fixed factor and age, gender, prior dementia (known or IQCODE ≥3.44), Charlson comorbidity index and modified APACHE II score as covariates was performed to account for the effect of potentially confounding precipitating and predisposing risk factors for delirium. There was no significant relationship between delirium and pro-inflammatory Class Z score at day 10-14 after adjusting for covariates ($F(1,33)$ 2.22, $p=0.148$). There was a trend towards significance for proinflammatory Change Z score ($F(1,64)$ 3.33, $p=0.073$). There was no significant relationship between delirium and chemokine Change Z score after adjusting for covariates ($F(1,64)$ 2.55, $p=0.116$).

Table 8.6 The difference by Student's t test between class Z scores for the three cytokine and chemokine subgroups, between those with and without delirium during the perioperative period.

Inflammatory marker subgroup	Timepoint	Delirium Class Z score mean (SD)	No delirium Class Z score mean (SD)	test statistic	p value
Pro-inflammatory IL-1β, IL-6, TNF-α	Baseline	0.305 (0.615)	0.008 (0.734)	1.90	0.061
	Day 4	-0.025 (0.904)	-0.020 (0.746)	-0.03	0.977
	Day 10-14	0.642 (0.759)	0.000 (0.798)	2.40	**0.023**
Anti-inflammatory IL-1ra, IL-5, IL-10	Baseline	0.241 (0.721)	-0.048 (0.806)	1.65	0.104
	Day 4	-0.328 (0.802)	-0.069 (0.731)	-1.51	0.134
	Day 10-14	0.765 (1.436)	-0.054 (0.882)	2.03	0.051
Chemokine IL-8, MIP-1α, MIP-1β, MCP-1	Baseline	0.080 (0.452)	-0.004 (0.096)	0.59	0.554
	Day 4	-0.012 (0.693)	0.001 (0.683)	-0.09	0.932
	Day 10-14	0.359 (0.645)	-0.024 (0.817)	1.50	0.143

Table 8.7 The difference by Student's t test between Change Z scores from baseline to day 4 for the three cytokine and chemokine subgroups, between those with and without delirium during the perioperative period.

Inflammatory marker subgroup	Delirium N=23 Change Z score mean (SD)	No delirium N=43 Change Z score mean (SD)	test statistic	p value
Pro-inflammatory IL-1β, IL-6, TNF-α	-0.420 (0.842)	-0.002 (0.652)	-2.24	**0.029**
Anti-inflammatory IL-1ra, IL-5, IL-10	-0.371 (0.675)	0.000 (0.806)	-1.88	0.065
Chemokine IL-8, MIP-1α, MIP-1β, MCP-1	-0.405 (0.734)	0.037 (0.890)	-2.03	**0.046**

8.3.4 Inflammatory profile during the follow-up period

8.3.4.1 Univariate analyses

Table 8.8 shows the results of group comparisons for individual cytokines and chemokines measured during the follow-up period, between those who did or did not develop delirium during the perioperative period or were found to be delirious at follow-up at assessment at 3, 6 or 12 months. Figures 8.11 to 8.20 show individual serum cytokine and chemokine levels during the follow-up period for the delirium and no delirium groups. As would be expected, levels of most of the cytokines and chemokines were lower during the follow-up period than in the acute phase post-hip fracture and operation, and some were virtually undetected during the follow-up period. Since IL-10 was often undetected during the follow-up period, the pro/anti-inflammatory ratio could not be calculated. There was no significant difference between groups for most of the inflammatory proteins. Serum IL-6 was significantly elevated in the delirium group at 3 months post-hip fracture, and there was a trend towards significant elevation at 6 months, but levels were much lower by 12 months. Serum IL-8 remained elevated in the delirium group throughout the follow-up period, which was significant at 3 and 6 months, and a trend towards significance at 12 months. TNF-α also remained elevated in the delirium group during the follow-up period, a difference which was significant at 3, 6 and 12 months.

8.3.4.2 Multivariate analyses

Table 8.9 shows the results of the exploratory analysis of covariance tests. After adjusting for potential confounders, delirium remained significantly associated with LogIL-8 at 3 months.

Table 8.8 Serum cytokine levels during the follow-up period in those who did or did not develop delirium during the perioperative period, or during the intervening 3, 6 or 12 months

Cytokine or chemokine	3 months, Median (IQR), pg/ml			6 months, Median (IQR), pg/ml			12 months, Median (IQR), pg/ml		
	Delirium N=35	No delirium N=45	MWU p	Delirium N=28	No delirium N=45	MWU p	Delirium N=24	No delirium N=44	MWU p
IL-1β	1.4 (0.7-1.9)	1.2 (0.3-1.5)	675 0.272	1.4 (1.1-1.6)	1.2 (1.0-1.7)	581 0.574	0.2 (0-0.7)	0 (0-0.4)	420 0.135
IL-1ra	1769 (1306-2461)	1505 (1199-2110)	642 0.158	1584 (966-2101)	1213 (1022-1604)	527 0.243	1357 (1050-1782)	1306 (1056-1895)	511 0.827
IL-5	0.7 (0-0.9)	0.5 (0-0.8)	711 0.438	0.7 (0.3-0.9)	0.7 (0.5-0.9)	622 0.927	0 (0-0)	0 (0-0)	528 1.000
IL-6	6.6 (4.1-12.0)	4.2 (3.4-6.3)	**562 0.028**	6.2 (3.0-10.2)	4.3 (2.8-5.4)	472 0.072	0 (0-4.6)	0 (0-0)	467 0.277
IL-8	19.3 (14.4-25.3)	12.6 (10.5-19.2)	**478 0.003**	17.6 (13.3-23.5)	11.8 (9.9-17.1)	**421 0.018**	19.2 (14.5-30.2)	16.6 (10.1-24.3)	384 0.065
IL-10	1.0 (0-1.4)	0.4 (0-1.3)	698 0.371	0 (0-0.2)	0 (0-0)	577 0.402	0 (0-0)	0 (0-0)	504 0.293
MCP-1	306.3 (248.1-418.5)	314.9 (244.8-377.5)	718 0.500	300.5 (240.1-346.7)	299.4 (223.6-367.0)	623 0.937	315.0 (257.2-387.4)	302.2 (223.7-381.4)	480 0.538
MIP-1α	0 (0-37.8)	0 (0-29.8)	719 0.419	0 (0-57.1)	0 (0-0)	529 0.134	0 (0-0)	0 (0-0)	519 0.677
MIP-1β	92.1 (62.3-113.8)	83.0 (54.3-105.8)	750 0.712	77.3 (52.0-105.4)	82.8 (54.0-100.4)	599 0.721	76.4 (41.4-96.4)	78.9 (49.1-99.2)	502 0.734
TNF-α	8.7 (6.3-10.6)	6.9 (4.8-8.9)	**554 0.023**	8.7 (5.7-11.2)	7.2 (5.7-8.4)	**458 0.050**	4.2 (1.6-6.0)	2.5 (0.8-4.4)	**372 0.044**

Table 8.9 Exploratory ANCOVA results for follow-up period cytokines and chemokines where there was a group difference in the univariate analysis, delirium at any stage from day 0 until the timepoint at which the serum sample was collected

Timepoint	Inflammatory marker	Delirium up until timepoint Y/N (1, N-1) F	Delirium up until timepoint Y/N P
3 months	LogIL-6	(1,78) 1.16	0.286
	LogIL-8	(1,78) 5.29	**0.024**
	LogTNF-α	(1,77) 0.30	0.589
6 months	LogIL-8	(1,71) 0.30	0.589
	LogTNF-α	(1,69) 0.13	0.719
12 months	LogTNF-α	(1,54) 2.18	0.147

Analysis of Covariance, with log-normalised cytokine or chemokine as the dependent variable, delirium at any point until the time assessed (i.e. 3, 6 or 12 months) as the fixed factor, and age, gender, prior dementia (known or IQCODE ≥3.44) and Charlson comorbidity index as covariates.

Figure 8.11 Median serum IL-1β during the study period in those with and without delirium at any stage

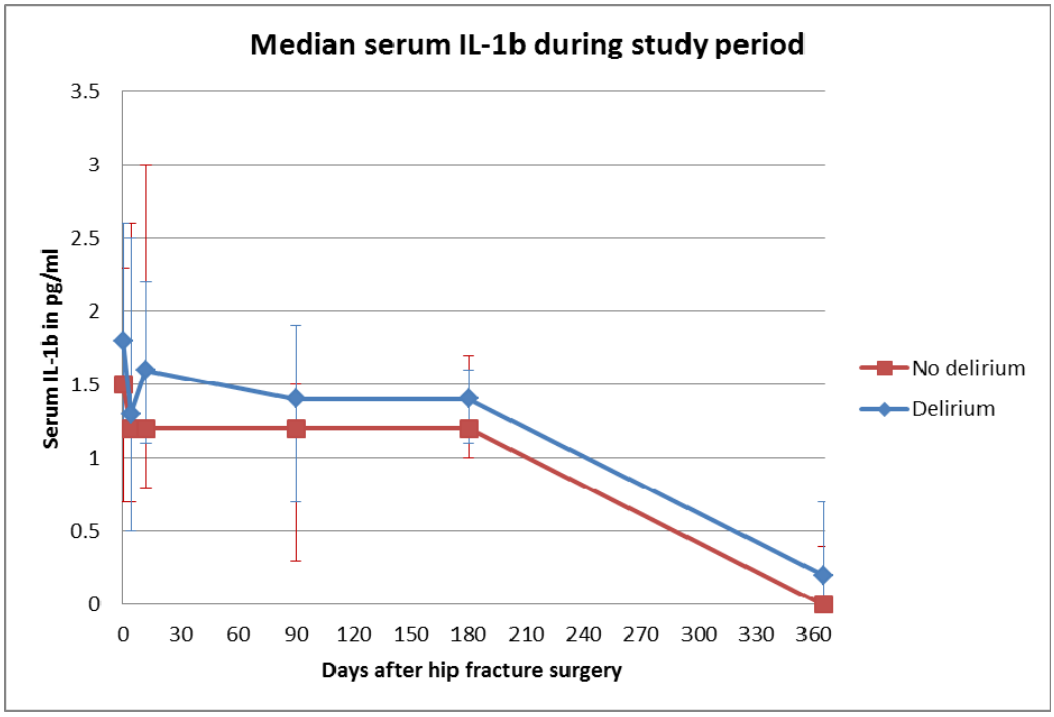


Figure 8.12 Median serum IL-1ra during the study period in those with and without delirium at any stage

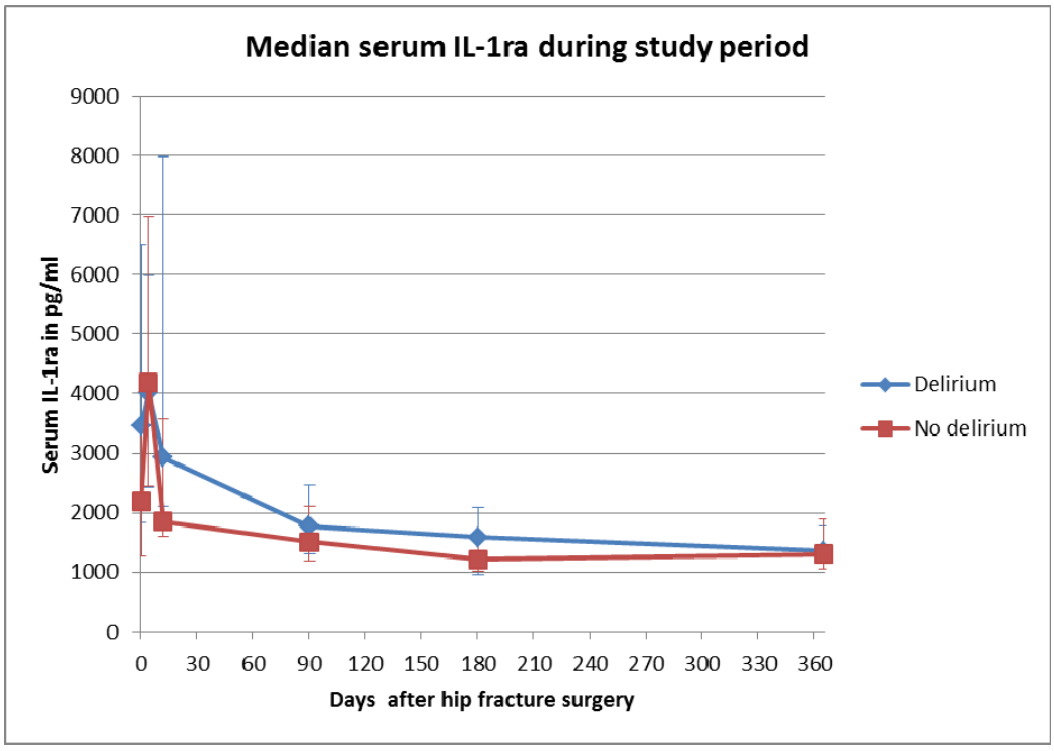


Figure 8.13 Median serum IL-5 during the study period in those with and without delirium at any stage

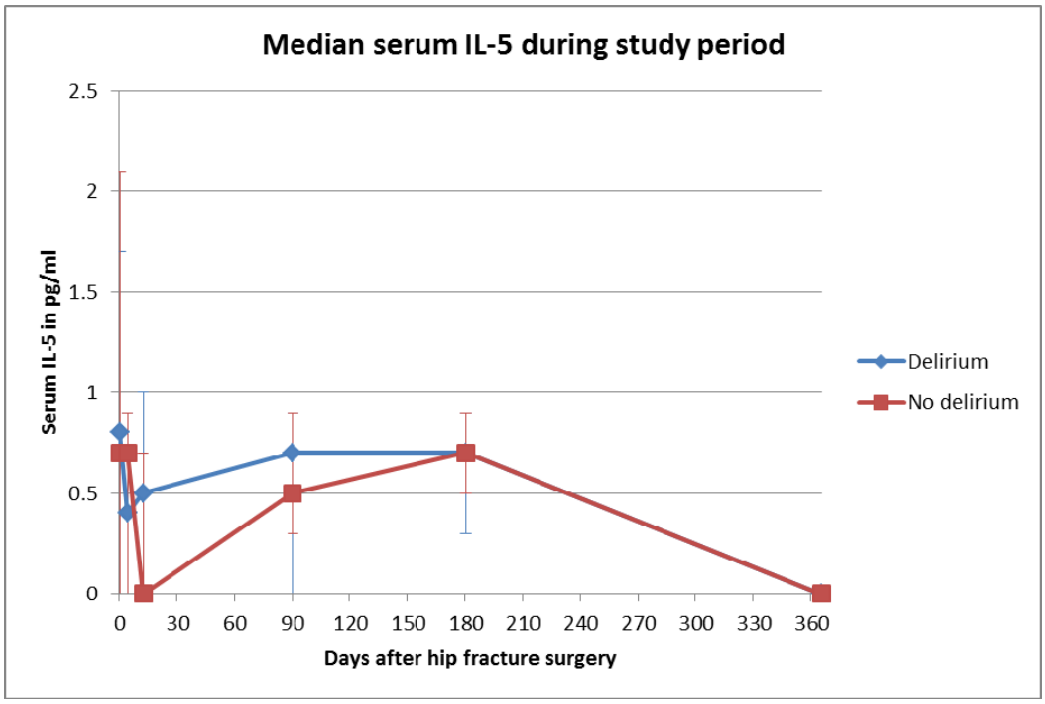


Figure 8.14 Median serum IL-6 during the study period in those with and without delirium at any stage

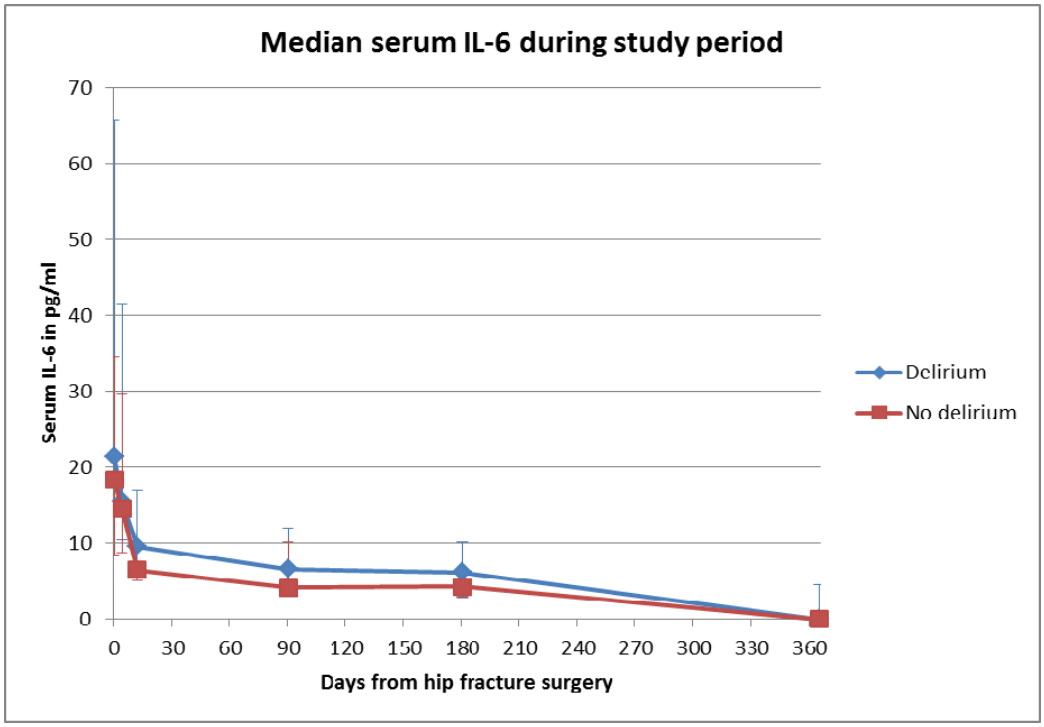


Figure 8.15 Median serum IL-8 during the study period in those with and without delirium at any stage

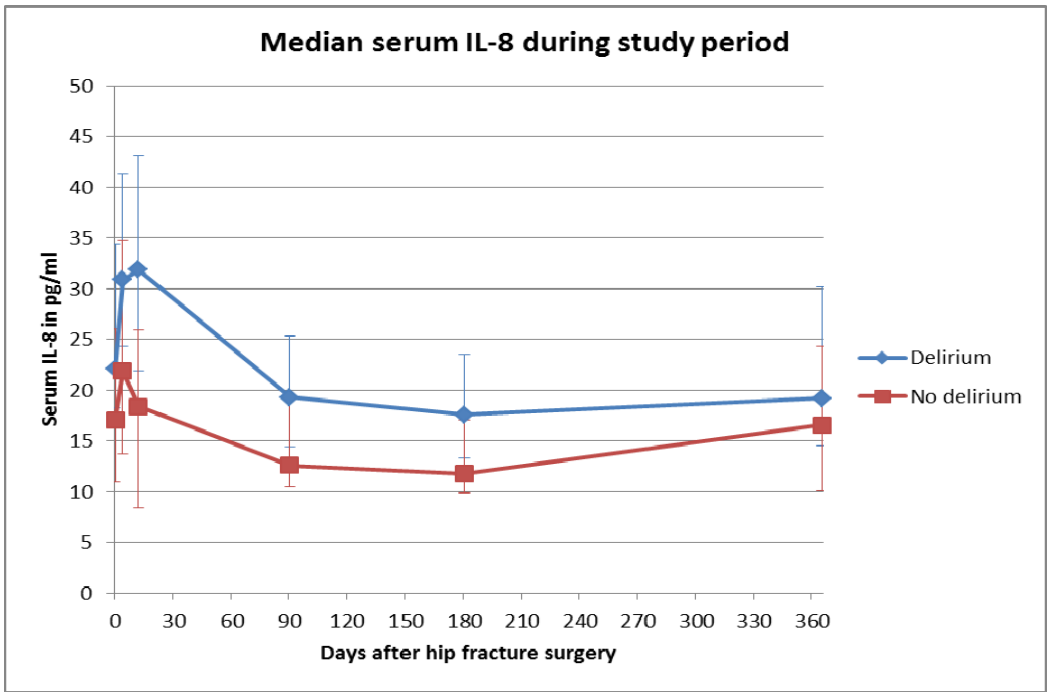


Figure 8.16 Median serum IL-10 during the study period in those with and without delirium at any stage

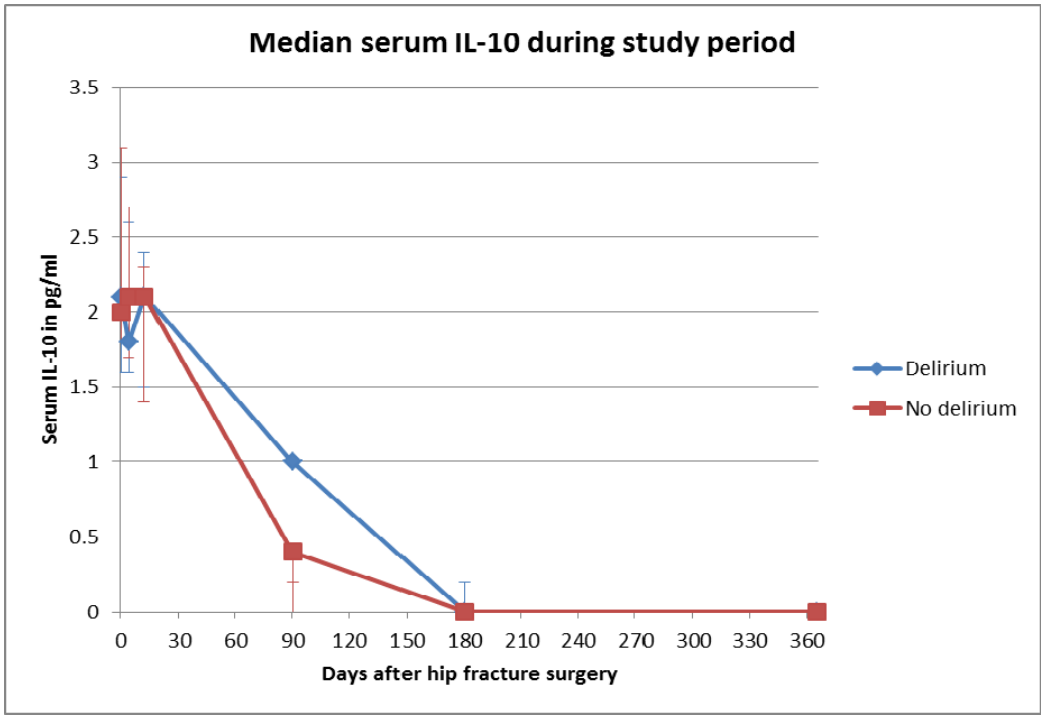


Figure 8.17 Median serum MCP-1 during the study period in those with and without delirium at any stage

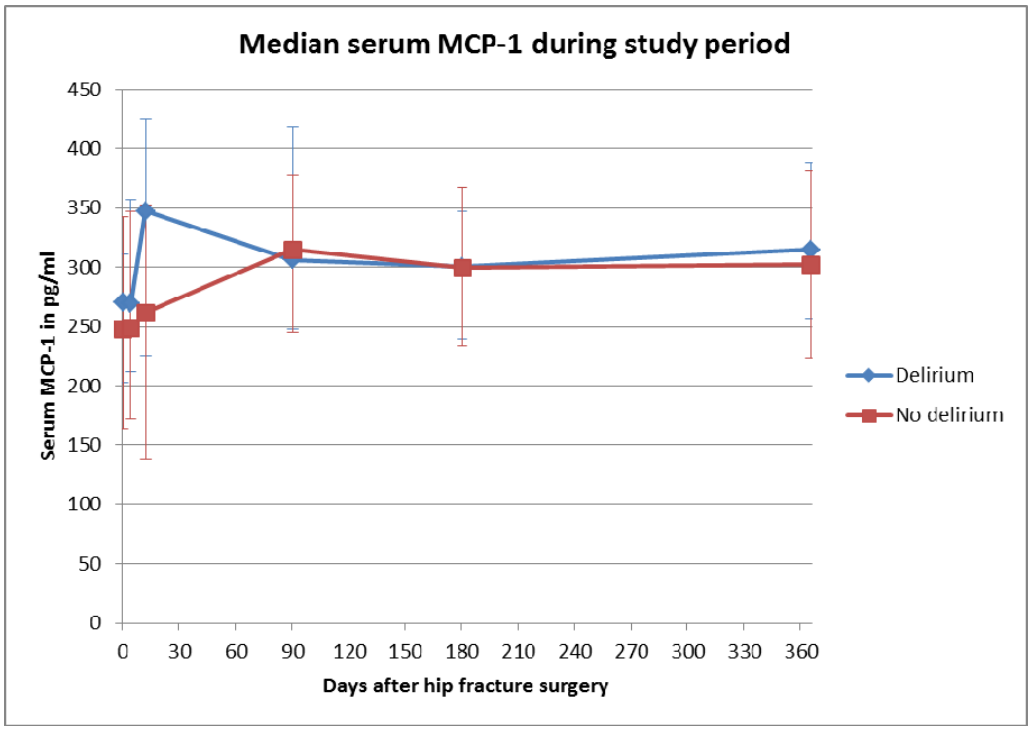


Figure 8.18 Median serum MIP-1α during the study period in those with and without delirium at any stage

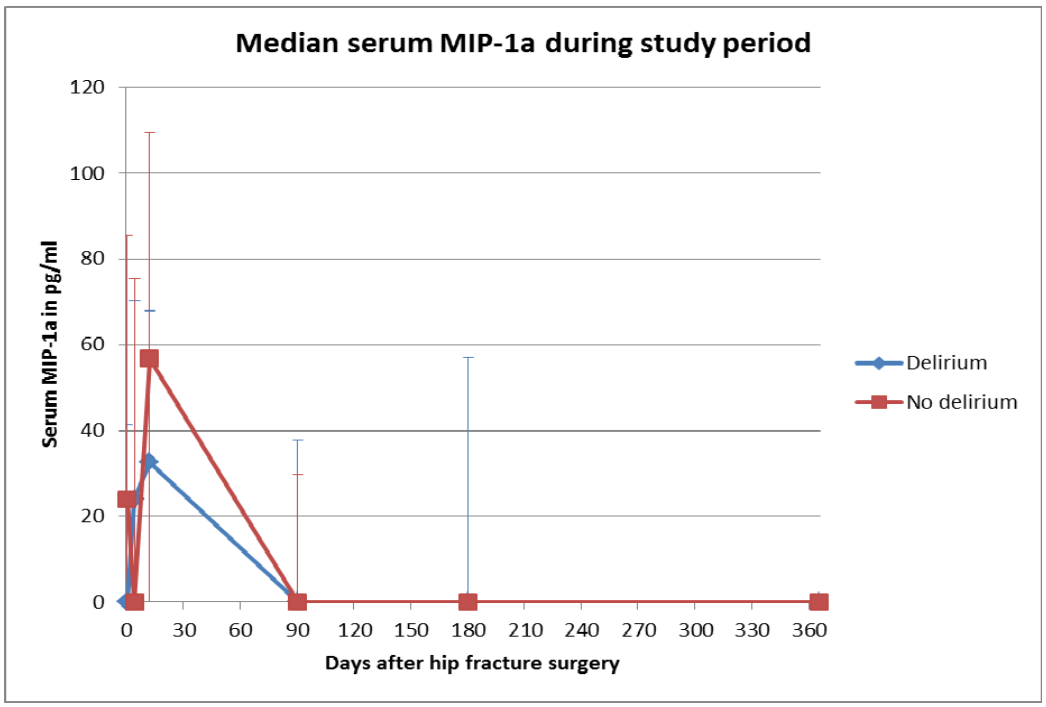


Figure 8.19 Median serum MIP-1β during the study period in those with and without delirium at any stage

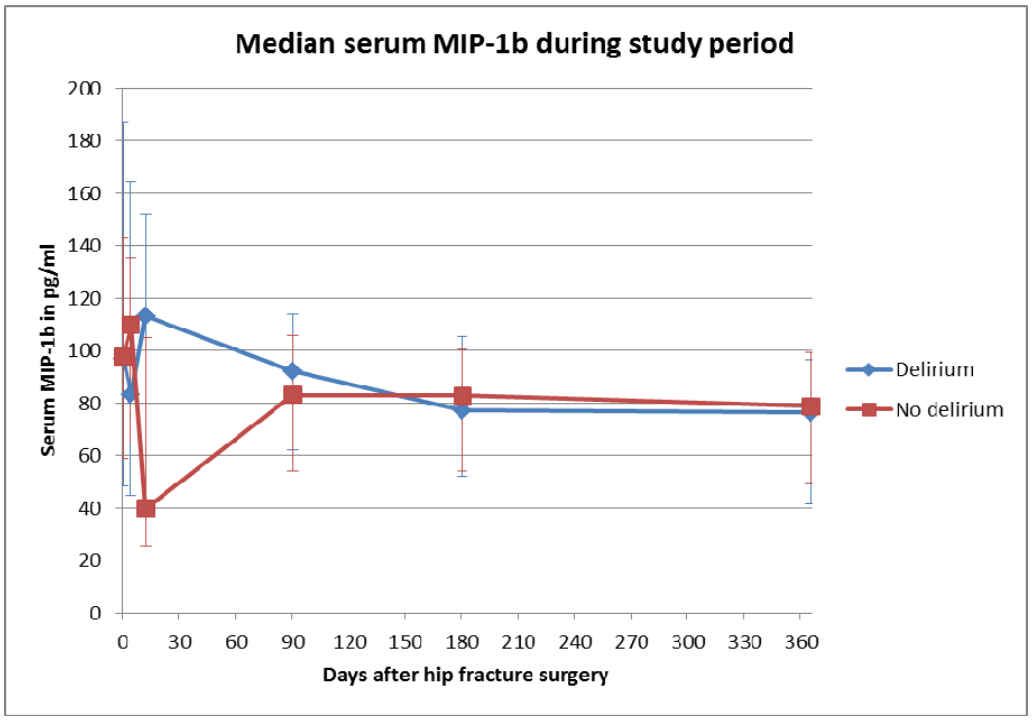
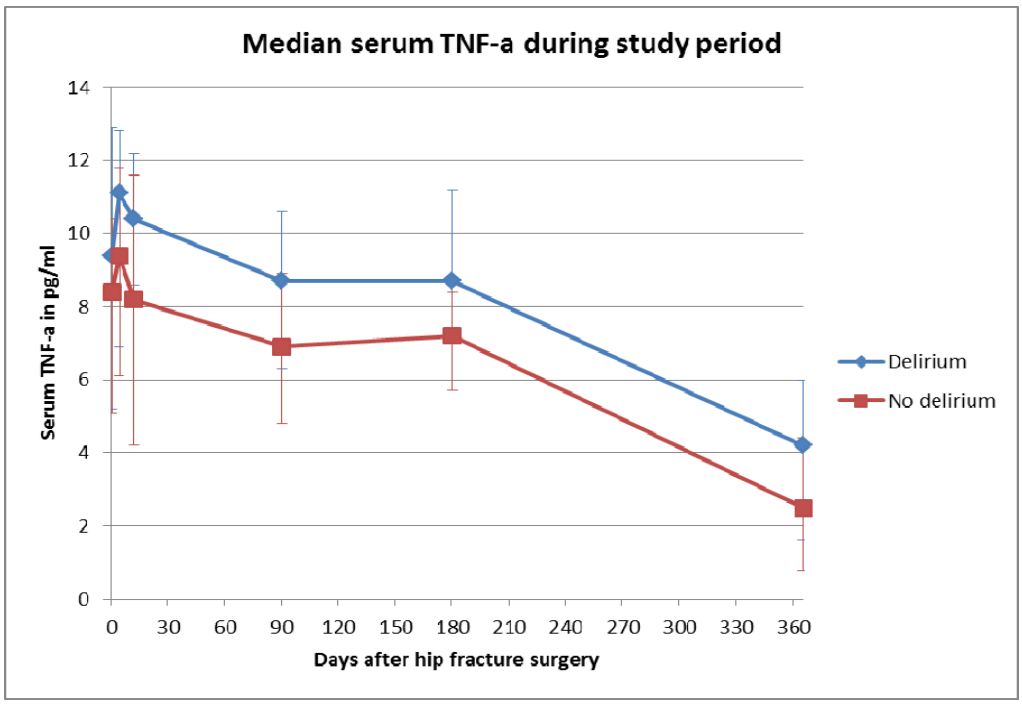


Figure 8.20 Median serum TNF-α during the study period in those with and without delirium at any stage



8.3.5 Subgroup analyses of inflammatory profile by delirium and dementia status

Since there were only N=10 participants with likely dementia (known or as defined by IQCODE ≥3.44) in the study, and only N=2 of those did not develop delirium, this analysis was expanded to include participants with mild cognitive impairment based on IQCODE ≥3.19 (Li, Jia et al. 2012). Levels of individual cytokines or chemokines were compared between subgroups with delirium superimposed on dementia/MCI (IQCODE ≥3.19), delirium only, dementia/MCI only and neither delirium nor dementia/MCI, at each timepoint with Kruskal-Wallis test.

8.3.5.1 Inflammatory profile in the perioperative period by delirium and dementia status

At baseline, there was a significant difference in MIP-1α between subgroups, with higher MIP-1α in those with neither delirium nor MCI/dementia (delirium on dementia/MCI N=15, 0pg/ml (0-24.0), delirium only N=13, 0pg/ml (0-53.6), dementia/MCI only N=10, 0pg/ml (0-7.5), neither N=38, 36.8pg/ml (0-91.5), Kruskal-Wallis p=0.027). Pairwise comparisons did not demonstrate any significant differences. There was also a difference in the pro/anti-inflammatory ratio between subgroups, with the highest ratios in those with delirium superimposed on dementia/MCI, similar ratios in those with delirium only or dementia/MCI only, and the lowest ratios in those with neither (delirium on dementia/MCI N=14, 28.4pg/ml (23.2-45.0), delirium only N=13, 26.1pg/ml (20.5-37.3), dementia/MCI only N=10, 25.6pg/ml (14.2-33.0), neither N=38 20.0pg/ml (12.5-30.5), Kruskal-Wallis p=0.045). Pairwise comparisons did not demonstrate any significant differences, but there was a trend towards a difference between delirium superimposed on dementia/MCI and neither (adjusted p=0.081).

At day 4, there were no significant differences in any of the individual cytokines or chemokines between subgroups. There was a difference in the pro/anti-inflammatory ratio, however, with the same pattern as at baseline (delirium on dementia/MCI N=16, 39.7pg/ml (27.9-46.4), delirium only N=15, 30.5pg/ml (22.0-36.7), dementia/MCI only N=13, 33.6pg/ml (21.0-54.8), neither N=37 21.6pg/ml (15.9-32.7), Kruskal-Wallis p=0.009). Pairwise comparisons revealed that this was due to the difference between those with delirium superimposed on dementia/MCI and those with neither (adjusted p=0.007).

At day 10-14, there was a significant difference between subgroups in serum IL-6, with higher levels in both delirium subgroups (delirium on dementia/MCI N=9, 9.8pg/ml (7.4-16.5), delirium only N=7, 8.7pg/ml (6.7-19.9), neither N=13, 5.2pg/ml (2.6-6.4), dementia/MCI only N=5, 3.7pg/ml (2.1-7.6), Kruskal-Wallis p=0.043). Pairwise comparisons did not demonstrate any significant differences. There was no significant difference in pro/anti-inflammatory ratios between subgroups at day 10-14, but the same pattern was evident between groups.

8.3.5.2 Inflammatory profile in the follow-up period by delirium and dementia status

At 3 months, there was a significant difference between subgroups in IL-6, with the highest levels in those with delirium superimposed on dementia/MCI (delirium on dementia/MCI N=17, 7.9pg/ml (4.8-12.8), delirium only N=17, 4.3pg/ml (3.3-8.5), dementia/MCI only N=10, 5.4pg/ml (3.6-11.1), neither N=35, 3.9pg/ml (3.4-5.4), Kruskal-Wallis p=0.044). Pairwise comparisons revealed that this was due to the difference between those with delirium superimposed on dementia/MCI and those with neither (adjusted p=0.030). There was also a significant difference between subgroups in serum IL-8, with higher levels in both delirium subgroups (delirium on dementia/MCI N=17, 16.1pg/ml (13.0-28.5), delirium only N=17, 20.5pg/ml (15.1-26.6), dementia/MCI only N=10, 11.4pg/ml (10.7-17.5), neither N=35, 13.2pg/ml (9.6-19.3), Kruskal-Wallis p=0.013). Pairwise comparisons revealed that this was due to the difference between those with delirium only and neither delirium nor dementia/MCI (adjusted p=0.047).

At 6 months, there was a difference between subgroups in IL-6, with the same pattern as at 3 months (delirium on dementia/MCI N=13, 7.8pg/ml (4.2-10.0), delirium only N=14, 3.5pg/ml (2.7-11.4), dementia/MCI only N=11, 5.4pg/ml (5.0-6.6), neither N=34, 3.9pg/ml (2.3-4.5), Kruskal-Wallis p=0.015). Pairwise comparisons revealed that there was a significant difference between those with delirium superimposed on dementia/MCI and those with neither delirium nor dementia/MCI (adjusted p=0.030). There was also a difference between subgroups in IL-8 at 6 months, again with higher levels in both delirium groups (delirium on dementia/MCI N=13, 17.8pg/ml (13.1-29.1), delirium only N=14, 17.3pg/ml (13.3-20.7), dementia/MCI only N=11, 14.0pg/ml (10.5-19.2), neither N=34, 11.5pg/ml (8.5-16.7), Kruskal-Wallis p=0.019). Pairwise comparisons again revealed that there was a

significant difference between those with delirium superimposed on dementia/MCI and those with neither delirium nor dementia/MCI (adjusted p=0.043).

There were no significant differences between these subgroups in any of the cytokines or chemokines at 12 months.

8.3.6 Correlations between serum inflammatory markers and measures of delirium, and with cortisol

8.3.6.1 Correlations between serum inflammatory markers and contemporaneous measures of delirium during the perioperative period

At baseline, DRS-R98 severity score correlated inversely with serum MCP-1 (rho= -0.234, p=0.032). There was also a trend towards positive correlations between DRS-R98 and serum IL-6 (rho=0.202, p=0.065). OSLA score at baseline correlated inversely with serum MIP-1α (rho= -0.237, p=0.030). Baseline MMSE and EDTB-1 scores did not correlate with any of the inflammatory markers.

At day 4, DRS-R98 correlated inversely with MIP-1β (rho= -0.321, p=0.003). There was a trend towards an inverse correlation between DRS-R98 and IL-1ra (rho= -0.199, p=0.073), and a positive correlation with IL-6 (rho=0.194, p=0.080). OSLA score correlated inversely with IL-1ra (rho= -0.235, p=0.035) and MIP-1β (rho= -0.228, p=0.041). MMSE did not correlate with any of the inflammatory markers, though there were trends towards positive correlations with IL-5 and MIP-1β, and an inverse correlation with IL-6. There were trends towards correlations between EDTB-1 score and IL-1ra, IL-10 and MIP-1β.

At day 10-14, there were no significant correlations between DRS-R98 severity score and any of the inflammatory markers; there was a trend towards correlation between DRS-R98 and IL-6 (rho=0.329, p=0.058). There was a trend towards correlation between OSLA score and IL-1ra (rho=0.317, p=0.072) and TNF-α (rho=0.325, p=0.065). Neither MMSE nor EDTB-1 score correlated with any of the markers.

8.3.6.2 Correlations between serum inflammatory markers and contemporaneous measures of delirium during the follow-up period

At 3 months, serum IL-6 was positively correlated with DRS-R98 severity score (rho=0.449, p<0.001), and inversely correlated with MMSE score (rho= -0.399, p<0.001). Serum IL-8 also correlated positively correlated with DRS-R98 (rho=0.224, p=0.046). TNF-α was positively correlated with DRS-R98 severity score (rho=0.294, p=0.008), and inversely correlated with MMSE score (rho= -0.261, p=0.020). Neither EDTB-1 score nor OSLA score correlated with any of the inflammatory markers.

At 6 months, DRS-R98 severity score correlated positively with serum IL-1β (rho=0.239, p=0.042), IL-1ra (rho=0.286, p=0.014), IL-6 (rho=0.446, p<0.001), IL-8 (rho=0.291, p=0.012) and TNF-α (rho=0.406, p<0.001). MMSE score was inversely correlated with IL-6 (rho= -0.364, p=0.002). OSLA score correlated positively with IL-6 (rho=0.236, p=0.045), IL-10 (rho=0.246, p=0.036) and TNF-α (rho=0.400, p<0.001). EDTB-1 score correlated positively with MCP-1 (rho=0.369, p=0.002).

At 12 months, DRS-R98 severity score correlated positively with serum IL-6 (rho=0.323, p=0.007) and TNF-α (rho=0.239, p=0.050). MMSE score correlated inversely with IL-6 (rho= -0.304, p=0.012). OSLA score correlated positively with IL-6 (rho=0.288, p=0.017) and IL-8 (rho=0.314, p=0.009). EDTB-1 score did not correlate with any of the inflammatory markers.

8.3.6.3 Correlations between baseline serum inflammatory markers and CSF inflammatory markers

There were N=41 participants who had both a baseline serum sample and a CSF sample in which CSF cytokines were measured by a different Luminex assay as described in chapter 5. Of the cytokines measured, IL-5 did not correlate (rho=0.199, p=0.212), IL-6 was positively correlated (rho=0.490, p=0.001), IL-8 did not correlate (rho=0.076, p=0.638), IL-10 was positively correlated (rho=0.347, p=0.026), and TNF-α did not correlate (rho= -0.239, p=0.133).

8.3.6.4 Correlations among serum inflammatory markers during the perioperative period

Tables 8.10 to 8.11 in appendix 10 show Spearman's Rho correlations among serum inflammatory markers measured at baseline, and on days 4 and 10-14. At baseline, the majority of inflammatory markers correlated with each other, except MIP-1α which only correlated inversely with IL-1β. By postoperative day 4, MIP-1α correlated inversely with many of the other inflammatory markers. By postoperative day 10-14, there were fewer significant correlations, but the pro-inflammatory IL-6, IL-8 and TNF-α correlated with each other.

8.3.6.5 Correlations among serum inflammatory markers during the follow-up period

Tables 8.12 to 8.14 in appendix 10 show Spearman's Rho correlations among serum inflammatory markers measured at 3, 6 and 12 months after hip fracture. There were fewer significant correlations between the inflammatory markers during the follow-up period, particularly at 12 months after hip fracture; TNF-α correlated with many of the pro-inflammatory cytokines and chemokines, and IL-6 with IL-8.

8.3.6.6 Correlations between inflammatory markers, serum cortisol and cortisol AM:PM ratio

At baseline, serum cortisol correlated positively with serum IL-1β (rho=0.233, p=0.033), IL-1ra (rho=0.311, p=0.004) and IL-10 (rho=0.226, p=0.039). Cortisol AM:PM ratio at baseline correlated inversely with serum TNF-α (rho= -0.781, p=0.050), meaning a lower and more abnormal cortisol diurnal rhythm with higher TNF-α. On postoperative day 4, serum cortisol correlated inversely with MCP-1 (rho= -0.251, p=0.022), and there was a trend towards positive correlation with IL-6 (rho=0.213, p=0.053). Cortisol AM:PM ratio on day 4 correlated with IL-10 (rho=0.300, p=0.017). On day 10-14, serum cortisol did not correlate with any of the inflammatory markers. Cortisol AM:PM ratio on day 10-14 correlated inversely with serum IL-1β (rho= -0.578, p=0.001).

At 3 and 12 months, neither serum cortisol nor cortisol AM:PM ratio correlated with any of the inflammatory markers. At 6 months, serum cortisol did not correlate with any of the

inflammatory markers, but cortisol AM:PM ratio correlated inversely with IL-1β (rho= -0.360, p=0.008), IL-6 (rho= -0.328, p=0.015) and IL-10 (rho= -0.350, p=0.009).

8.4 Discussion

This study lends further support to the hypothesis that there is an exaggerated inflammatory response in delirium, with higher pro-inflammatory cytokines and chemokines, but it did not find lower levels of anti-inflammatory cytokines in delirium. The results support an imbalance in pro/anti-inflammatory ratio in delirium, with a shift towards a pro-inflammatory state. I will now discuss the key results for the different cytokine and chemokine families.

8.4.1 Interleukin-1 family

This study found elevated levels of IL-1ra in the delirium group at baseline and at day 10-14 postoperatively. Serum IL-1ra has been shown to be elevated in delirium in critical illness (van den Boogaard, Kox et al. 2011), and in delirium after hip fracture in a cohort related to this hip fracture cohort (Cape, Hall et al. 2014). It has also been shown to be a protective modifier of delirium risk (Adamis, Lunn et al. 2009). IL-1ra is the endogenous antagonist of the key pro-inflammatory cytokine IL-1β, and it is released after IL-1β. It is thought to have a neuroprotective role (Loddick, Wong et al. 1997), and is in fact being trialled as a therapy in subarachnoid haemorrhage and traumatic brain injury (Galea, Ogungbenro et al. 2011, Clinical Trials Register 2013). On day 4, there was an inverse correlation between OSLA score and IL-1ra, suggesting higher IL-1ra with more normal level of arousal, and a trend to an inverse correlation with delirium severity, which may indicate a greater IL-1ra response at this point as protective or restorative. Elevated IL-1ra seen here may therefore be in response to IL-1β release, although levels of IL-1β largely did not change, except in the two patients who still had delirium at day 10-14 postoperatively, where levels were very high. Although these were small numbers and the analyses must be viewed as exploratory, the relationship remained after adjusting for confounders. IL-1β is often difficult to detect, although in this study the majority of samples were above the minimum detectable concentration. I did not measure IL-1α, a further member of the IL-1 family which may have a role here.

8.4.2 Interleukin-6

This study has replicated the findings of previous studies of elevated IL-6 in delirium after hip fracture (Beloosesky, Hendel et al. 2007, van Munster, Korevaar et al. 2008, MacLullich, Edelshain et al. 2011). After adjusting for potential confounders including illness severity,

comorbid disease burden and prior dementia, IL-6 was still associated with delirium when measured towards the end of a delirium episode, 10-14 days after hip fracture surgery. IL-6 was also markedly elevated in patients with active delirium on postoperative day 4, and after adjusting for confounders there was a trend towards a significant relationship remaining with delirium. There were trends towards positive correlations between IL-6 and DRS-R98 delirium severity score, also suggesting more severe the delirium with greater IL-6 elevation. IL-6 also remained elevated in the delirium group at 3 months post-hip fracture, although this was no longer significant after adjusting for covariates, It also correlated positively with DRS-R98 score and inversely with MMSE score throughout the follow-up period (higher IL-6 with worse delirium and cognition) and positively with OSLA score at 6 and 12 months (higher IL-6 with more abnormal arousal), suggesting sustained elevation may be related to persistence of delirium symptoms or worse cognition.Subgroup analyses found that the highest levels were in those with delirium superimposed on dementia/MCI, so it may be that persistence of IL-6 production was due to the cumulative effect of delirium and dementia/MCI, or that the immune response was dysregulated in this subgroup of patients.

IL-6 is a key player in the acute phase response, and is released in reponse to bacterial endotoxin and other inflammatory insults (Reichenberg, Yirmiya et al. 2001). It is a pleiotropic cytokine with endocrine, autocrine and paracrine roles, and it is produced by several different types of immune cell and adipocytes. It stimulates activity of the hypothalamic-pituitary-adrenal axis, and production of IL-6 is suppressed by glucocorticoids. In this study, IL-6 did not correlate with any of the cortisol measures, with only a trend towards a positive correlation between the two serum measures on postoperative day 4. IL-6 has a circadian rhythm of release, which correlates with level of sleepiness, and it has been suggested to have a pathological role in disorders characterised by excessive daytime sleepiness (Vgontzas, Bixler et al. 2005). Delirium is almost universally associated with sleep-wake cycle disturbance (Meagher, Moran et al. 2007), and IL-6 could plausibly have a role in this disturbance. In addition to hip fracture, it has been found to be elevated in delirium in other settings including delirium following cardiac surgery (Plaschke, Fichtenkamm et al. 2010), non-cardiac surgery (Liu, Ya-wei et al. 2013, Capri, Yani et al. 2014), in elderly medical patients (de Rooij, van Munster et al. 2007) and in critical illness (van den Boogaard, Kox et al. 2011). It would be very interesting to examine IL-6 levels in delirium subtypes, to see whether it is higher in those with hypoactive delirium as opposed to

hyperactive, or whether it correlates with the degree of sleep-wake cycle disturbance exhibited.

8.4.3 Tumour Necrosis Factor-α

TNF-α is an early and key player in the acute phase response, released in response to bacterial endotoxin and other inflammatory stimuli (Reichenberg, Yirmiya et al. 2001, Grigoleit, Oberbeck et al. 2010). It has been shown to be elevated in delirium after cardiac surgery (Kazmierski, Banys et al. 2014) and delirium in critical illness (van den Boogaard, Kox et al. 2011, Ritter, Tomasi et al. 2014), but in each case this was no longer significant after controlling for covariates. TNF-α levels were generally higher in the delirium group during the perioperative period, and this was significant for those with active delirium on postoperative day 4. TNF-α also stayed significantly elevated in the delirium group throughout the year follow-up period, and correlated positively with delirium severity (DRS-R98), level of arousal (OSLA) and inversely with MMSE score, in keeping with higher TNF-α with worse delirium and global cognition, and more abnormal arousal. This is the first time that such a sustained inflammatory response has been demonstrated in delirium. The relationship between TNF-α and delirium was no longer significant after adjusting for covariates, however, and it is possible that it is a function of illness severity or comorbidity.

8.4.4 Balance of pro/anti-inflammatory cytokines

This study found a higher ratio of pro/anti-inflammatory cytokines in those with delirium, replicating the findings of Cerejeira *et al* in patients with delirium after elective arthroplasty (Cerejeira, Nogueira et al. 2012). Given the results of the analyses for individual cytokines, this is likely to be predominantly due to higher levels of pro-inflammatory cytokines. There was also a greater postoperative change in pro-inflammatory cytokines in those with delirium, suggesting that those with delirium had a more exaggerated response to the surgical insult, although it is possible that this was due to perioperative complications. Subgroup analyses suggested that the imbalance in the pro/anti-inflammatory ratio was greatest in those with delirium superimposed on dementia/MCI throughout the perioperative period. This may be due to the cumulative effect of delirium and dementia, both bringing with it an inflammatory pathology, or that those with delirium and dementia/MCI are more likely to produce a greater pro-inflammatory response to trauma and surgery.

8.4.5 Chemokines

This study replicated previous studies which demonstrated elevated serum IL-8 in delirium after hip fracture (Beloosesky, Hendel et al. 2007, van Munster, Korevaar et al. 2008) and in other settings such as ICU (van den Boogaard, Kox et al. 2011). This study is the first to show persistent elevation of IL-8 after a month post-hip fracture (Beloosesky, Hendel et al. 2007), and IL-8 levels here were significantly higher in the delirium group up to 6 months post-hip fracture. At 3 months, this relationship was still significant after adjusting for potential confounders including age, gender, prior dementia and comorbidity. This might be related to persistent symptoms of delirium, and serum IL-8 level was positively correlated with DRS-R98 severity score at both 3 and 6 months. Subgroup analysis found comparable higher levels in both subgroups with delirium only and delirium superimposed on dementia/MCI, suggesting that it may be more related to delirium than the cumulative effect of both pathologies or the influence of delirium on dementia. IL-8 is generally pro-inflammatory, with one of its main roles being chemotaxis of neutrophils (Semple, Kossmann et al. 2010). It is primarily produced by monocytes and macrophages, and is produced early in the inception of an inflammatory response, but may also persist for days or weeks as seen here (Remick 2005). Its persistent elevation in the delirium group may be due to continued or new foci of inflammation.

This study is one of the first to measure macrophage inflammatory protein (MIP)-1α and MIP-1β in delirium. MIP-1α and MIP-1β are chemotactic proteins produced by neutrophils to recruit and activate macrophages (Solana, Tarazona et al. 2012). As a group, these and other chemokines were shown to be elevated in delirium after cardiac surgery (Rudolph, Ramlawi et al. 2008). MIP-1β was significantly lower in patients with active delirium preoperatively and was inversely correlated with delirium severity and abnormal level of arousal on day 4, but was thenhigher in the whole delirium group at day 10-14. Both of these relationships were independent of age, gender, prior dementia, illness severity and comorbidity. When those with active delirium were compared with those without at days 4 and 10-14, there was no difference in levels. It may be that those who developed delirium preoperatively had a poor initial immune response to the trauma with lack of macrophage recruitment, but that this occurred later on and persisted as delirium was resolving, as seen in the higher levels in the delirium group at day 10-14, or it may have been related to intercurrent infective illness and macrophage activation at a later stage postoperatively. The results in general at day 10-14 are

interesting; patients who were still in the Orthopaedic unit at 10-14 days postoperatively were often those who had had a complicated postoperative course and were not well enough to go to the Orthogeriatric Rehabilitation unit yet, or those who were about to go home. Higher levels were seen at that timepoint in the chemokines MCP-1 (non-significant) and MIP-1β in the group who had been delirious. This may be because the patients in the delirium group assessed at that time were more unwell, or because the inflammatory response in more prolonged delirium evolves with more chemokine activity, recruiting monocytes and macrophages.

Where levels of cytokines were measured in CSF and serum at the baseline timepoint, IL-6 and IL-10 were correlated, whereas IL-5, IL-8 and TNF-α were not correlated. This suggests that the central and peripheral inflammatory profile may be different, however there were only small numbers and a different Luminex assay was used, which limits the conclusions that can be drawn. Future studies could assay paired serum and CSF together. Correlations among the serum inflammatory markers during the perioperative period found that generally pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IL-8, TNF-α) were positively correlated and T-helper 2 cytokines IL-5 and IL-10 were correlated, which adds to the biological validity of the findings. Interestingly, IL-1β and its endogenous antagonist IL-1ra were only correlated at baseline, perhaps due to different metabolism or clearance of one of the markers. Also, although many of the chemokines correlated with each other, MIP-1α did not correlate with MIP-1β throughout the perioperative period, although similar pathways would be expected in their generation; the results for these chemokines should therefore be viewed with some caution, particularly MIP-1α which did not correlate with many other inflammatory markers, suggesting there may have been a problem with its detection. During the follow-up period, where levels of many of the inflammatory markers were much lower, there are fewer correlations. Levels of IL-6, IL-8 and TNF-α, which remained elevated in the delirium group, were all positively correlated, which may suggest a persistent pro-inflammatory shift. Finally, although there were some correlations between cortisol measures and inflammatory markers, these were not very consistent. At baseline, there was a positive correlation between the IL-1 family and cortisol, and inverse between TNF-α and AM:PM ratio, in keeping with a greater acute phase response with more activation of the HPA axis and loss of diurnal rhythm. After the acute phase, the relationships are less clear, suggesting a possible divergence of stress and inflammatory pathways.

There are some important limitations to this study which should be discussed. Although participants were assessed regularly throughout the perioperative period, the fluctuating nature of delirium means that some brief episodes may have been missed. During the follow-up period, some participants in both groups may have had episodes of delirium during intercurrent illnesses. It is difficult to capture the complexity of the relationship between the inflammatory response of elderly patients to the dual insults of hip fracture and surgery, and the fluctuating syndrome of delirium, which is often superimposed on dementia. Some of these cytokines are fleeting and change over minutes to hours, whereas some persist longer such as IL-8. By examining levels of these inflammatory proteins in the delirium group as a whole and comparing those with and without active delirium on a day-to-day basis, and by seeking trends in cytokine and chemokine families as well as the profiles of individual inflammatory proteins, this study has attempted to map the inflammatory profile of delirium after hip fracture. The significant relationship between some inflammatory markers after controlling for important confounding variables suggests that there is an altered inflammatory response in delirium. It is plausible that this may be causative, in the case of IL-6, and the new finding of persistent elevation of pro-inflammatory cytokines and chemokines after delirium may be related to persistent delirium symptoms or to cognitive or physical sequaelae of delirium.

Delirium is a heterogeneous syndrome, often with multiple contributing precipitants and mechanisms. In order to overcome the "noise" generated by these multiple mechanisms, larger studies are now required that can take account of multiple covariates, including functional level, waiting time for surgery and anaesthetic type, which could not be accounted for here. In the case of anaesthetic type, no consistent difference in inflammatory response to surgery could be found in the literature for those receiving general or regional anaesthesia. A small study comparing spinal plus epidural to general anaesthesia in total hip replacement found higher TNF-α levels 6h and 20h after surgery, which was just significant at 6h, but no difference in IL-6 and very little detectable IL-1β (Hogevold, Lyberg et al. 2000). A further small study found no difference in IL-6, TNF-α or CRP at 24h post-operatively in those undergoing haemorrhoidectomy under spinal or general anaesthesia (Buyukkocak, Caglayan et al. 2006). Kudoh *et al* in their trial of regional versus general anaesthesia in patients with schizophrenia found lower IL-6 levels at the end of surgery and 24h later in their group with schizophrenia who received epidural anaesthesia than in the control group who received general anaesthesia, but no difference at all between the two groups with schizophrenia who

received general versus epidural anaesthesia. It is therefore difficult to conclude that anaesthetic type was responsible for this difference (Kudoh, Takese et al. 2003).

This study had some predefined hypotheses, but there was also an exploratory element, in order to generate hypotheses for future work.Future studies could examine the relationship between these cytokines and chemokines and delirium subgroups and symptom profiles, particularly the relationship between IL-6 and sleep-wake cycle disturbance, including blood sampling at two or three points during the day to examine circadian rhythm, with comtemporaneous assessment of delirium symptoms and severity. Additional members of the IL-1 family such as IL-1α could be measured to complement measurements of IL-1β and IL-1ra. Soluble receptors of some cytokines such as TNF and IL-6 can be measured, and this may give an idea of the relationship between the inflammatory ligands and their receptors in delirium. Also, there has been little previous research on the function of cells of the immune system such as neutrophils and macrophages in delirium, which would need to be examined in the context of immunosenescence.

In conclusion, this study has found evidence of elevated pro-inflammatory cytokines and chemokines in delirium after hip fracture, with a shift towards a pro-inflammatory state. There was persistent elevation in the delirium group of IL-6, IL-8 and TNF-α during the months after hip fracture, which for IL-8 at 3 months was independent of age, gender, prior dementia and co-morbidity. For IL-6, those with delirium superimposed on dementia/MCI had a cumulative or exaggerated response, whereas for IL-8, both delirium subgroups had higher levels. This persistent inflammatory response should be examined further in the context of the physical, functional and cognitive sequaelae of delirium.

9 Chapter 9: The relationships among persistent delirium, cognition and the HPA axis

9.1 Introduction

There is evidence from several sources of hypothalamic-pituitary-adrenal (HPA) axis dysfunction in delirium. This is discussed in more detail in Section 1.5. An aberrant response to stress has been suggested, leading to an increased and prolonged cortisol response to stressors, and possibly a loss of diurnal rhythm (McIntosh, Bush et al. 1985, Olsson 1999). Studies have shown elevated serum cortisol in delirium in several settings, and elevated CSF cortisol in delirium after hip fracture, but the relationship is sometimes lost after adjusting for prior dementia (Mu, Wang et al. 2010, Pearson, de Vries et al. 2010, Bisschop, de Rooij et al. 2011, Colkesen, Giray et al. 2013). Cortisol levels may be elevated for at least 8 weeks after hip fracture. Roberts *et al* found median morning serum cortisol levels of 0.38μM (380nM/L) in mobile elderly controls, 0.38μM (380nM/L) in immobile elderly controls, 0.60μM (600nM/L) two weeks after hip fracture and 0.58μM (580nM/L) eight weeks after hip fracture (Roberts, Barton et al. 1990). However, it is not known if this is sustained, and whether there is any relationship between persistent delirium and derangement of the HPA axis.

Persistent delirium is common in many settings. A systematic review of studies of persistent delirium in hospitalised patients reported combined rates of 44.7% at hospital discharge, 32.8% at 1 month, 25.6% at 3 months and 21% at 6 months (Cole, Ciampi et al. 2009). In a cohort of patients with acute hip fracture where 41% developed delirium perioperatively, Marcantonio found rates of persistent delirium of 39% at hospital discharge, 32% at 1 month and 6% at 6 months (Marcantonio, Flacker et al. 2000). Risk factors for persistent delirium in general medical patients and palliative care patients include older age, male gender, dementia, infection, terminal illness, brain cancer, visual impairment, functional impairment, high co-morbidity and use of physical restraints during delirium (Inouye, Zhang et al. 2007, Boettger, Jenewein et al. 2014). Outcomes for those with persistent delirium are consistently worse than for those whose delirium resolves (Cole, Ciampi et al. 2009), including the risk of

increased mortality (Kiely, Marcantonio et al. 2009). However, the pathophysiological mechanisms of persistent delirium, which often continues after the original trigger has been treated, are poorly understood, and little is known of the role of the HPA axis.

Among the adverse outcomes of delirium and persistent delirium is an increased risk of dementia (MacLullich, Beaglehole et al. 2009, Witlox, Eurelings et al. 2010, Davis, Muniz Terrera et al. 2012). Patients with dementia often deteriorate cognitively and functionally after a hospital admission or episode of delirium, and a delirium episode has been shown to accelerate the trajectory of cognitive decline in out-patients with Alzheimer's Dementia (Fong, Jones et al. 2009). It is currently not clear whether the increased risk of incident dementia following delirium is due to the unmasking of a dementia process which had begun but had not yet been recognised, or whether new pathological processes are begun in the brain. Incident dementia with a preceding documented episode of delirium in an epidemiological study has been shown to have no relationship with standard neuropathological markers (Davis, Muniz Terrera et al. 2012). Critical illness has been shown in a large study to be followed by significant long-term cognitive impairment, even in young, fit people, and longer delirium duration was associated with worse global cognition and executive function in that study (Pandharipande, Girard et al. 2013). Detailed studies of the cognitive sequelae of delirium in elderly patients and of their pathophysiological mechanisms are few.Witlox *et al* found in a cohort of patients with hip fracture that at three months post-fracture, the delirium group had worse episodic memory, global cognition and more cognitive decline (Witlox, Slor et al. 2013). Postoperative cognitive dysfunction is an important and related construct, but it is poorly defined and most of the studies of this construct did not screen for delirium (Nadelson, Sanders et al. 2014).

There is also a relationship between cortisol and long-term cognitive impairment and dementia as discussed in more detail in section 1.5.8, although there is some heterogeneity of findings in the literature. There is evidence supporting hyperactivity of the HPA axis in dementia, with positive dexamethasone suppression tests (Robertsson, Blennow et al. 2001), and this is related to disease severity and cerebral atrophy (Giubilei, Patacchioli et al. 2001). The hippocampus is the primary site of higher cortical feedback of the HPA axis and has a dense population of glucocorticoid receptors. It is also important in long-term potentiation and memory formation, and is atrophied in Alzheimer's dementia (Olsson 1999, Ferrari and Magri 2008).

This study therefore examined the relationships between both elevated cortisol in the perioperative period, and sustained derangement of the HPA axis, with persistent delirium, and between sustainment of HPA axis activity following a delirium episode. It also examined the relationships between delirium during the perioperative period, the HPA axis and cognition in the year after hip fracture.

The questions addressed in this study were as follows:

1) Is the elevation in cortisol seen after hip fracture sustained during the subsequent year in the whole cohort?
2) Are the higher levels of morning cortisol and loss of cortisol diurnal rhythm seen in the delirium group during the postoperative period sustained during the year after hip fracture?
3) Is any sustained dysregulation of the HPA axis in the year after hip fracture, with higher morning cortisol and loss of diurnal rhythm, greater in those with persistent delirium in the year after hip fracture, compared to those whose delirium resolves and those who never develop delirium?
4) Is any sustained dysregulation of the HPA axis in the year after hip fracture greater in those with delirium superimposed on dementia or mild cognitive impairment (MCI), compared to those with delirium only, dementia or MCI only, and neither?
5) Is the trajectory of cognitive decline different in the delirium group, the delirium superimposed on dementia or MCI group, and the no delirium group, and is it worse in those with delirium only and delirium superimposed on dementia/MCI?
6) Is the trajectory of cognitive decline different for those with high cortisol and low cortisol during the perioperative period?
7) Is cognitive decline over the year after hip fracture greater in those with (a) higher cortisol during the baseline perioperative period, and in those with (b) greater loss of diurnal rhythm, with elevated afternoon salivary cortisol levels and lower AM:PM ratio at baseline.?
8) Is cognitive decline greater in those with (a) sustained elevation of cortisol during the year after hip fracture, and (b) a more abnormal cortisol rhythm during the year after

hip fracture, as shown by elevated afternoon salivary cortisol and lower AM:PM ratio at follow-up?

9.2 Methods

9.2.1 Baseline assessment

Patients with acute hip fracture were recruited and assessed during the perioperative period as described in chapter 2. At baseline, the Informant Questionnaire on Cognitive Decline in the Elderly was used as a proxy measure for premorbid dementia (Jorm and Jacomb 1989). Those with an average IQCODE score of ≥3.44 (Harwood, Hope et al. 1997) or a prior diagnosis of dementia were deemed to have evidence of prior dementia, and those with an average IQCODE score of ≥3.19 were deemed to have evidence of mild cognitive impairment (MCI) (Li, Jia et al. 2012). The National Adult Reading Test was performed during the perioperative period and was used as an estimate of crystallised intelligence (Nelson 1982). The Mini-Mental State Examination (Folstein, Folstein et al. 1975), which is a commonly used test of global cognition, was used as part of the delirium assessment performed at each visit.

9.2.2 Follow-up assessments

Follow-up visits were performed at 3, 6 and 12 months after hip fracture. Prior to arranging each follow-up visit, I contacted the participant's General Practitioner to check whether the participant was still living and well enough to be contacted to arrange a follow-up visit. The participant or, if appropriate, their next of kin was then contacted and a visit arranged. These took place either at the participant's home or at the Wellcome Trust Clinical Research Facility at the Royal Infirmary of Edinburgh. The majority of visits were conducted by me, and a small proportion (17 visits out of a total of 237) by two Community Research Nurses, Catherine Beveridge and Avril Cairns, during the last four months of the study when I was on maternity leave. The nurses accompanied me on several visits each while training, I provided training on delirium assessment and cognitive testing, and they each completed one visit themselves in my presence.

9.2.2.1 Assessment for persistent delirium

Participants were assessed for any persisting features of delirium, or a new diagnosis of delirium, at each follow-up visit at 3, 6 and 12 months. Delirium assessment followed the same structure as during the perioperative period, as described in Chapter 2. This involved a semi-structured interview with cognitive testing using the MMSE, delirium severity

assessment using the Delirium Rating Scale Revised '98 (DRS-R98), testing of attention with the Edinburgh Delirium Test Box-1 (EDTB-1), assessment of level of arousal with the Richmond Agitation Sedation Score (RASS) and the Observational Scale of Level of Arousal (OSLA), and completion of the Confusion Assessment Method (CAM) algorithm. New delirium was defined as CAM positive fulfilling DSM-IV criteria, and persistent delirium features was defined as the ongoing presence of at least one CAM positive feature, or the presence of an abnormal RASS score (any score other than 0) in a participant who was delirious during the perioperative period or developed syndromal delirium during the follow-up period. The DRS-R98 score was not used to define persistent delirium because this may have been confounded by cognitive impairment, due to the inclusion of memory questions.

9.2.2.2 Assessment of cognition

Participants also completed a cognitive test battery at each follow-up visit. The cognitive test battery took 45-60 minutes to complete; this was curtailed if participants were unable to complete it due to fatigue, frailty or advanced dementia, and some tests were omitted if participants' vision was too poor. In particular, a colour vision test was performed prior to administration of Victoria Stroop, and the test omitted if the participant was unable to distinguish between the colours. A full description of the cognitive test battery is in section 2.7.1. A list of the included tests and the score used in statistical analysis is in table 9.1.

Table 9.1 A summary of the follow-up cognitive test battery

Test	Score(s) used
Digit Span Forwards	Total score
Digit Span Backwards	Total score
Victoria Stroop	Difference score
	Interference score
HVLT-R List Learning	Total recall
	Recognition Discrimination Index
Visual Reproduction	Recall I score
	Recall II score
	% retention
Digit Symbol Substitution Test	Number in 90 seconds
Verbal Fluency	FAS (letters F, A and S) total in 60 seconds
	Animals total in 60 seconds

9.2.2.3 Assessment of function and quality of life

At each follow-up visit, information was collected on current medication, new medical problems, and current mobility and living situation. The Katz PADL (Katz, Ford et al. 1963) and Lawton IADL (Lawton and Brody 1969) scores were completed to assess current functional level (Appendix 5). The 15-item Geriatric Depression Scale (Appendix 6) was used to assess for co-morbid depression (Brown, Woods et al. 2007). The EQ-5D questionnaire (Appendix 7) was used to estimate quality of life (Cheung, Oemar et al. 2009).

9.2.3 Sample collection

Morning serum samples and diurnal saliva samples were collected for measurement of cortisol at each follow-up visit at 3, 6 and 12 months. They were processed and stored as described in section 2.5. Samples were processed at the Wellcome Trust Clinical Research Facility, Royal Infirmary of Edinburgh by research nurses, or by me at the Queens Medical Research Institute, Royal Infirmary of Edinburgh out of hours. Cortisol was measured in duplicate using ELISA as described in sections 2.10 and 2.11. I performed all cortisol assays.

9.2.4 Statistical analysis

Normality of distribution was tested for each variable using histogram plots and Kolgomorov-Smirnov tests.

9.2.4.1 Morning serum cortisol and AM:PM ratio

To explore the pattern of cortisol levels in the whole cohort in the year after hip fracture, median cortisol levels in morning serum and morning and afternoon saliva were plotted on line graphs.

In order to test whether levels of cortisol remained higher and whether there was still loss of cortisol diurnal rhythm during the follow-up period in those who had developed delirium at baseline, group comparisons were performed between those who did or did not develop delirium at baseline for cortisol measures at each follow-up timepoint, with Mann-Whitney U test. Participants with new syndromal delirium were excluded from this analysis since they may have had new dysregulation of the HPA axis.

To test whether persistent delirium influenced persistent dysregulation of the HPA axis, participants were divided into three groups. “Resolved delirium” included those who had developed syndromal delirium during the perioperative period or at follow-up assessment visits but which had subsequently fully resolved with no CAM positive features and a RASS of 0, “persistent delirium” where participants who had had syndromal delirium still had at least one CAM positive feature or a RASS other than 0 at follow-up but did not have new syndromal delirium, and “never delirium” where participants never developed (or at that point hadn’t yet developed) syndromal delirium. Group comparisons were performed for cortisol measures at each follow-up timepoint with Kruskal-Wallis test (post hoc pairwise comparisons).

To test whether persistent dysregulation of the HPA axis was greater in those with prior delirium superimposed on dementia/MCI, participants were divided into groups with delirium at any stage superimposed on dementia/MCI, delirium only, dementia/MCI only and neither. Those with new syndromal delirium at follow-up were excluded from these analyses to reduce any influence of active delirium, but were included at later timepoints if they no

longer met criteria for syndromal delirium. Group comparisons for cortisol measures at follow-up were performed with Kruskal-Wallis test (post hoc pairwise comparisons).

To explore the relationship between persistent delirium features and cortisol, DRS-R98 severity score, and the EDTB-1 score (as a measure of inattention) and OSLA score (as a measure of abnormal arousal) were correlated with contemporaneous measures of cortisol (morning serum cortisol, AM and PM salivary cortisol and the AM:PM cortisol ratio).

9.2.4.2 Cognitive tests

In order to account for any influence of persistent delirium on cognitive test performance at follow-up, group differences were tested in cognitive test scores between the "resolved", "persistent" and "never" groups with ANOVA (post hoc pairwise comparisons with Dunnett's T3 test) or Kruskal-Wallis test (post hoc pairwise comparisons), at each follow-up timepoint. Participants with new syndromal delirium were excluded from these analyses, but were included at later timepoints if they no longer met criteria for syndromal delirium.

To explore the relationship between cognition and cortisol, cognitive test scores were also correlated with contemporaneous measures of cortisol (morning serum cortisol, AM and PM salivary cortisol and the AM:PM cortisol ratio) with Spearman's Rho.

In order to explore the effects of cortisol, delirium status and other important co-variates such as dementia and age on the trajectory of cognition during the follow-up period, a linear latent growth model trajectory analysis was constructed. Since the cognitive test battery involved seven individual tests, a principal components analysis was performed with the results of the first administration of the cognitive tests at the 3 month follow-up assessment. Six variables were included in the principal components analysis, which were Digit Span total score (Digit Span Forwards plus Digit Span Backwards), Verbal Fluency FAS total score, HVLT-R List Learning Recognition Discrimination Index, Visual Reproduction Percent Retention, Digit Symbol Substitution Test total score and Victoria Stroop interference score. There were no significant outliers and tests of assumption were satisfactory; the Kaiser-Meyer-Olkin measure of sampling adequacy statistic was 0.637 and Bartlett's test of sphericity was highly significant. This showed that there was one principal component explaining 53% of the

variation at this wave, and we were therefore able to use a composite score of cognition for the trajectory analysis modelling.

Professor Ian Deary and Dr Zoë Tieges gave advice on performing and interpreting the principal components analysis and Dr Mike Allerhand performed the trajectory analysis, and gave advice on its interpretation.

9.3 Results

One-hundred and eight participants were recruited to the study. Ninety-seven completed the perioperative period of the study, and their GP was contacted regarding follow-up. Seven participants had died between the last visit of the perioperative period and the three month follow-up visit. Five participants declined to continue participating in the study, and one declined the three month visit but asked that they be contacted regarding the next visit, and did rejoin the study for the six and twelve month visits. Eighty-four participants took part in an assessment at three months. Between three and six months, four participants died, one declined to participate further and two were excluded (one due to new steroid prescription and one due to a move to a Nursing Home in the north of Scotland). Seventy-eight participants took part in an assessment at six months. Between six and twelve months, two participants died and one declined to participate further in the study. All samples and data were included for all participants until they withdrew, were excluded or died. Figure 9.1 further describes study attrition according to delirium status. Of forty-four participants who developed delirium during the perioperative period, twenty-nine (66%) completed the study; ten died and five withdrew consent to continue participation. Seventy-two percent (46/64) of the no delirium group completed the study.

The presence of a new diagnosis of delirium at follow-up was rare, as I was required to check with the participants' GP (or hospital team where they were still in hospital or had been readmitted) that they were well enough to participate in follow-up testing. However, persistence of delirium features was relatively common. At three months, N=1 participant had full syndromal delirium, N=19 had persistent features of delirium as outlined in section 9.2.4, N=16 had resolved delirium and N=48 had never had delirium. At six months, N=2 had syndromal delirium, N=8 had persistent features of delirium, N=22 had resolved delirium and N=46 had never had delirium. At twelve months, N=2 had syndromal delirium, N=7 had persistent features of delirium, N=21 had resolved delirium and N=45 had never had delirium. Those with persistent features of delirium were more likely to have underlying dementia or mild cognitive impairment.

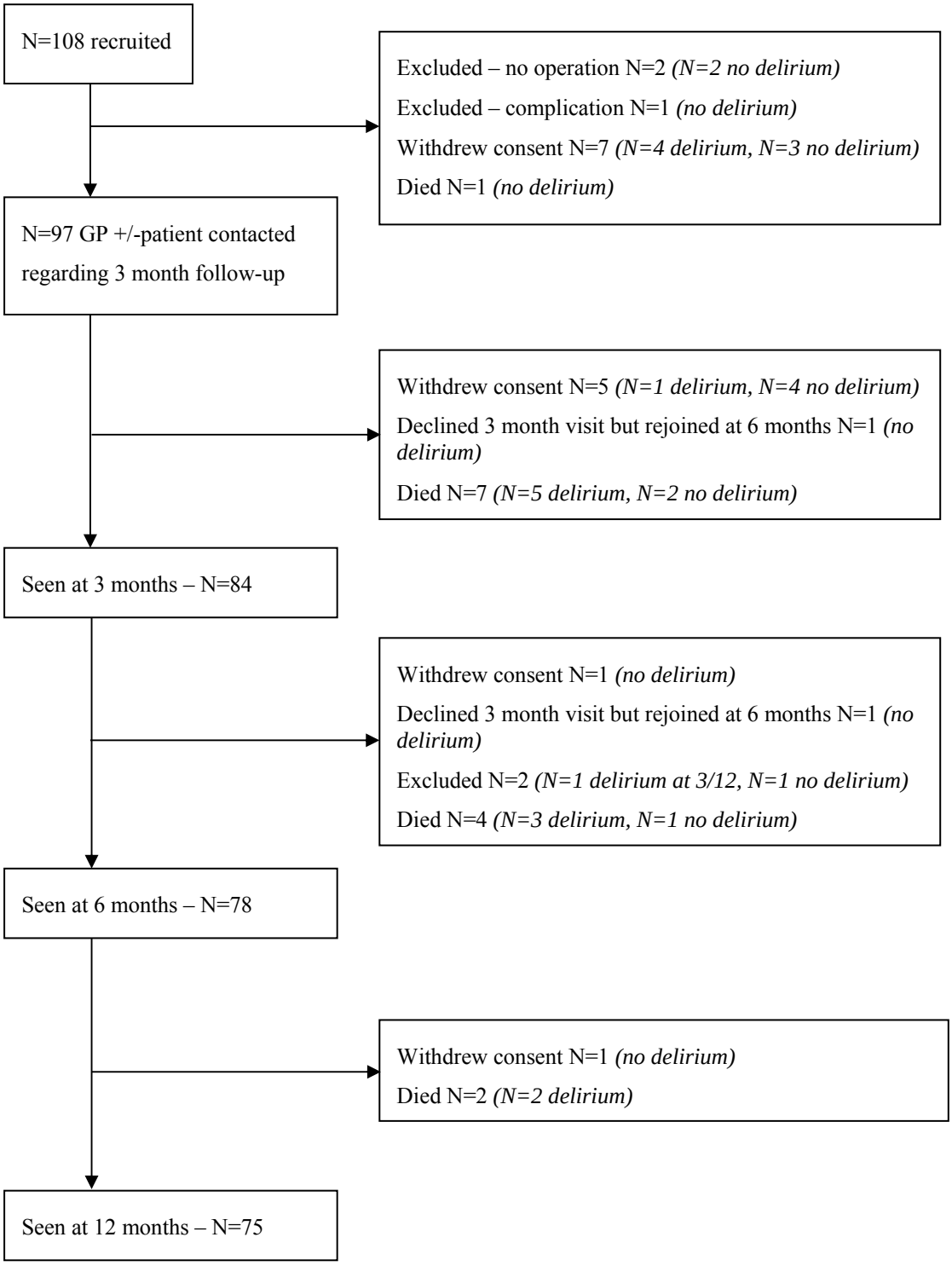
Table 9.2 Prior dementia status of those with persistent or resolved delirium, according to IQCODE

Timepoint	**Delirium status**	**Dementia status according to IQCODE**			
		Normal	**MCI**	**Dementia**	**p[a]**
3 months	Syndromal	0	1	0	**0.006**
	Persistent	8	4	6	
	Resolved	9	5	2	
	Never	37	10	1	
6 months	Syndromal	1	0	1	**0.016**
	Persistent	3	2	3	
	Resolved	11	10	1	
	Never	35	10	1	
12 months	Syndromal	0	0	2	**<0.001**
	Persistent	1	2	4	
	Resolved	13	6	1	
	Never	34	10	1	

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly, ≤3.19 = "normal", 3.20-3.43 = "mild cognitive impairment", ≥3.44 = "dementia"

[a]Pearson Chi-Square

Figure 9.1


N=108 recruited
Excluded – no operation N=2 (N=2 no delirium)
Excluded – complication N=1 (no delirium)
Withdrew consent N=7 (N=4 delirium, N=3 no delirium)
Died N=1 (no delirium)
N=97 GP +/-patient contacted regarding 3 month follow-up
Withdrew consent N=5 (N=1 delirium, N=4 no delirium)
Declined 3 month visit but rejoined at 6 months N=1 (no delirium)
Died N=7 (N=5 delirium, N=2 no delirium)
Seen at 3 months – N=84
Withdrew consent N=1 (no delirium)
Declined 3 month visit but rejoined at 6 months N=1 (no delirium)
Excluded N=2 (N=1 delirium at 3/12, N=1 no delirium)
Died N=4 (N=3 delirium, N=1 no delirium)
Seen at 6 months – N=78
Withdrew consent N=1 (no delirium)
Died N=2 (N=2 delirium)
Seen at 12 months – N=75

9.3.1 Function, depression and quality of life

Preoperatively, the group who went on to develop delirium were more functionally impaired in personal and instrumental activities of daily living (Table 9.3). Postoperatively, this difference was starker and persisted throughout the year after fracture. Only community-dwelling people were recruited to this study. At baseline, there was no difference between groups in place of living, however during the year after hip fracture there was a significant difference between groups, with fewer participants who had developed delirium returning to their own homes, and new Nursing Home or NHS in-patient continuing care admissions among this group.

There was no difference between groups with and without delirium at any stage in self-reported overall health status (EQ-VAS). However, within the EQ-5D subdimensions, at three months the delirium group self-reported significantly worse mobility and ability to do their usual activities, but no difference in ability to do self-care, pain / discomfort, nor anxiety / depression. At six months the delirium group still reported significantly more difficulty with their usual activities and there was a trend towards worse mobility and self care, but the other subdimensions were no different. At twelve months, there were no significant differences between groups in any of the subdimensions. This may have been due to some slow improvement in the function and mobility of the delirium group, attrition of some frailer and more functionally dependent participants, or adjustment in self-perception of quality of life within the delirium group to their new baseline.

Scores on the 15-item Geriatric Depression Scale were generally low. There was a significant difference between groups at six months only, with higher scores in the delirium group, but the median and interquartile range were within the range which may be interpreted as suggestive of possible depression.

Table 9.3 Functional status, place of living, quality of life and depressive symptoms throughout the year after hip fracture for the whole cohort and between those with and without delirium at any stage

	Whole group Median (IQR)	Delirium at any stage Median (IQR)	No delirium Median (IQR)	p value
Katz PADL score /6	Baseline: 6 (6-6) N=106 3 months: 6 (5-6) N=84 6 months: 6 (6-6) N=78 12 months: 6 (5-6) N=75	Baseline: 6 (5-6) N=43 3 months: 6 (3-6) N=36 6 months: 6 (3-6) N=32 12 months: 5 (3-6) N=30	Baseline: 6 (6-6) N=62 3 months: 6 (6-6) N=48 6 months: 6 (6-6) N=46 12 months: 6 (6-6) N=45	**0.024**[b] **0.002**[b] **<0.001**[b] **<0.001**
Lawton IADL score /8	Baseline: 7 (5-8) N=104 3 months: 5 (4-7) N=84 6 months: 6 (4-8) N=78 12 months:6 (4-8) N=75	Baseline: 6 (4-8) N=42 3 months: 4 (2-6) N=36 6 months: 4 (2-7) N=32 12 months: 4 (2-7) N=30	Baseline: 7 (6-8) N=61 3 months: 6 (5-7) N=48 6 months: 7 (5-8) N=46 12 months: 7 (5-8) N=45	**0.038**[b] **<0.001**[b] **<0.001**[b] **<0.001**[b]
Place of living Baseline	House/flat: N=92 Sheltered Housing: N=14	House/flat: N=38 Sheltered Housing: N=6	House/flat: N=54 Sheltered Housing: N=8	0.913[c]
Place of living 3 months	House/flat: N=60 Sheltered Housing: N=9 Nursing Home: N=2 Still in NHS rehab: N=13	House/flat: N=21 Sheltered Housing: N=1 Nursing Home: N=2 Still in NHS rehab: N=12	House/flat: N=39 Sheltered Housing: N=8 Nursing Home: N=0 Still in NHS rehab: N=1	**<0.001**[c]
Place of living 6 months	House/flat: N=60 Sheltered Housing: N=12 Nursing Home: N=7	House/flat: N=23 Sheltered Housing: N=3 Nursing Home: N=7	House/flat: N=37 Sheltered Housing: N=9 Nursing Home: N=0	**0.003**[c]
Place of living 12 months	House/flat: N=59 Sheltered Housing: N=10 Nursing Home: N=5 NHS IPCC: N=1	House/flat: N=21 Sheltered Housing: N=3 Nursing Home: N=5 NHS IPCC: N=1	House/flat: N=38 Sheltered Housing: N=7 Nursing Home: N=0 NHS IPCC: N=0	**0.019**[c]
EQ-5D EQ-VAS (0-100)	3 months: 70 (50-80), N=78 6 months: 70 (53-80), N=76 12 months: 70 (50-80), N=73	3 months: 70 (50-80), N=32 6 months: 70 (60-80), N=31 12 months: 60 (50-77), N=29	3 months: 70 (60-80), N=46 6 months: 70 (50-80), N=45 12 months: 70 (60-80), N=44	0.440[b] 0.705[b] 0.210[b]
Geriatric Depression Score 15-item	3 months: 3 (3-4), N=74 6 months: 3 (2-5), N=70 12 months: 3 (1-4), N=68	3 months: 3 (3-6), N=29 6 months: 3 (2-5), N=26 12 months: 3 (2-5), N=23	3 months: 3 (2-4), N=45 6 months: 2 (1-4), N=44 12 months: 2 (1-4), N=45	0.064[b] **0.006**[b] 0.178[b]

[a]Student's t test, [b]Mann-Whitney U test, [c]Pearson Chi-square

PADL: Personal Activities of Daily Living, IADL: Instrumental Activities of Daily Living, NHS IPCC: National Health Service In-Patient Continuing Care; EQ-5D: European Quality of Life Scale with 5 components; EQ-VAS: EQ-5D visual analogue scale; Geriatric Depression Score: ≥5 suggestive of depression, ≥10 almost always indicates depression

9.3.2 The HPA axis in the year after hip fracture

Figures 9.2 and 9.3 show the median cortisol levels in morning serum and morning and afternoon saliva in the whole cohort during the year after hip fracture. Table 9.4 shows the median values and interquartile range for the cohort. Morning serum levels steadily fell during the year after hip fracture. Salivary cortisol levels had fallen by 3 months post-hip fracture, rose again at 6 months and fell again by 12 months. This may have been due to intercurrent illness in some patients at 6 months.

Figure 9.2 Median morning serum cortisol level during the year after hip fracture for the whole cohort

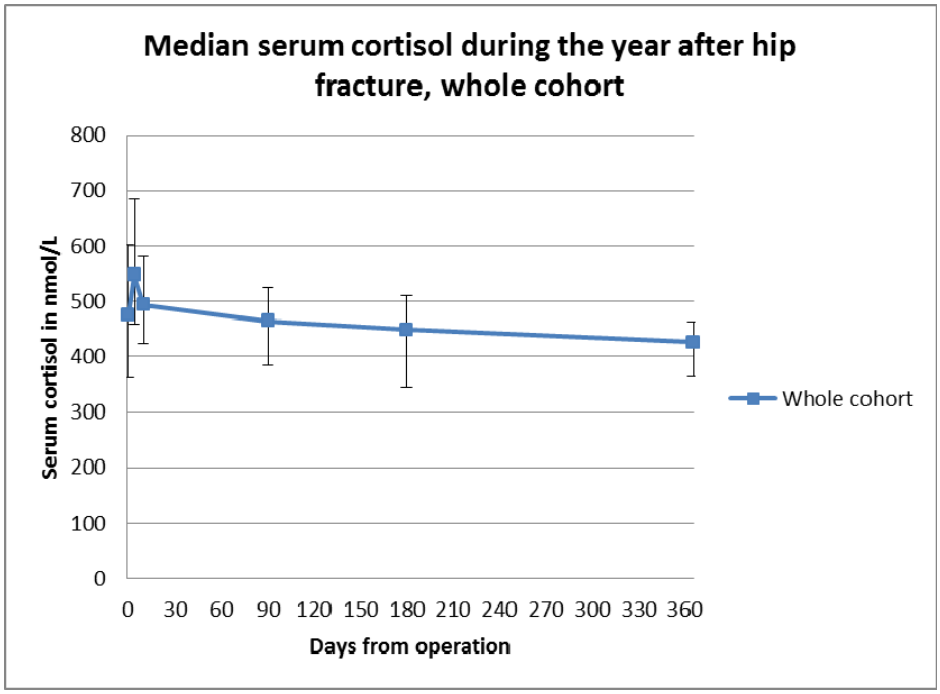
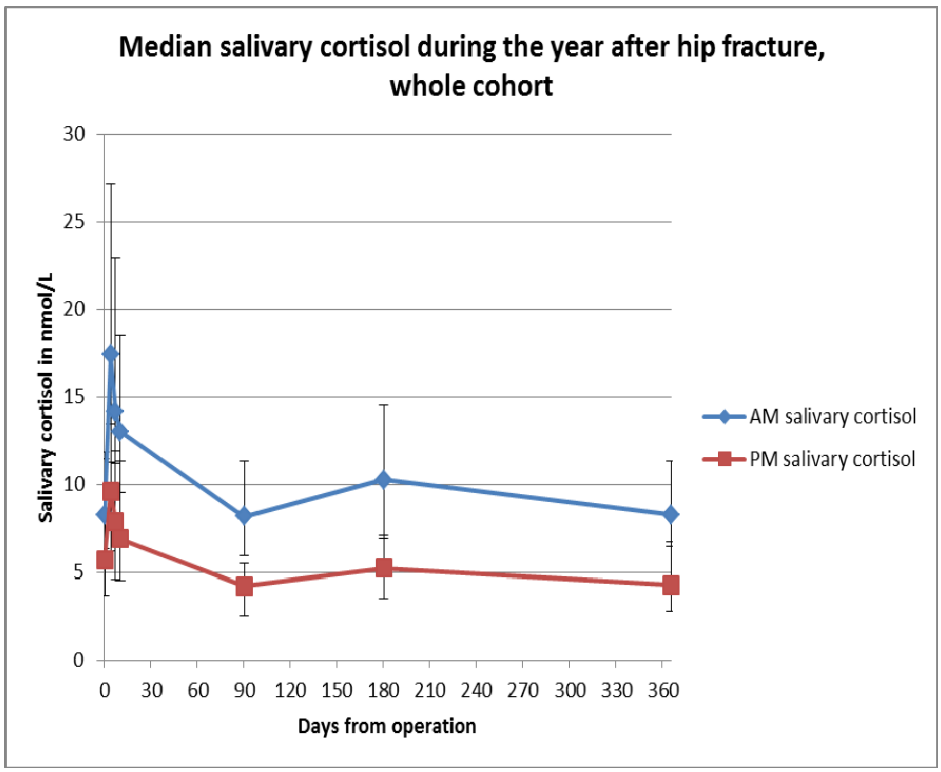


Figure 9.3 Median morning and afternoon salivary cortisol level during the year after hip fracture for the whole cohort



9.3.3 The HPA axis in the year after hip fracture and its relationship with delirium

Figures 9.4 and 9.5 show serum and diurnal salivary cortisol respectively, viewed over the study period in those who did and did not develop delirium during the perioperative period after hip fracture, excluding those who had new or syndromal delirium when seen at follow-up. At three months, serum cortisol level was similar to that observed at baseline at the time of anaesthetic. Peak morning serum cortisol gradually fell over the course of the year in both groups, but remained at a slightly higher level in the delirium group at each timepoint, although this difference was not significant (Table 9.4). Cortisol circadian rhythm appeared to still show derangement at three and six months in the group who had delirium perioperatively, with a trend towards higher afternoon salivary cortisol at three months in the delirium group ($p=0.061$). This was in the absence of any continuing or new syndromal delirium. By twelve months the levels in the two groups are very similar. This may be because the sustained derangement in the cortisol axis in the delirium group has "normalised", or it may be because those in the delirium group who survived to one year and were well enough to be seen and give samples were those with a lower cortisol level.

When the influence of persistent delirium features was explored, by comparing those with resolved delirium, persistent CAM features or RASS other than 0, or never delirium, in the absence of new or syndromal delirium, those with persistent features of delirium had significantly higher morning serum cortisol at three months (Table 9.5). This group tended to have higher cortisol levels at three and six months, and slightly higher afternoon salivary cortisol levels particularly at six months, but this was not significant. By twelve months after hip fracture, very few participants remained who had persistent features of delirium, and cortisol levels in serum and saliva were very similar between groups.

Table 9.4 Cortisol levels in serum and saliva at 3, 6 and 12 months for the whole group, and group comparisons of serum and salivary cortisol levels at 3, 6 and 12 months after hip fracture, between those who did or did not develop delirium during the perioperative period, excluding those with new or full syndromal delirium when seen at follow-up

Cortisol sample and timepoint	**Whole group median (IQR)**	**Median (IQR) serum or salivary cortisol in nmol/L**		
		Delirium during perioperative period	**No delirium**	**MWU p**
3 months morning serum	464.5 (384.0-526.0) N=80	475.5 (397.2-558.7) N=33	453.3 (377.3-498.9) N=46	638 0.229
3 months AM saliva	8.2 (5.9-11.3) N=65	9.4 (5.8-14.8) N=21	7.8 (6.0-10.7) N=44	383 0.268
3 months PM saliva	4.2 (2.6-5.5) N=56	5.4 (2.8-8.1) N=22	3.6 (2.3-4.8) N=34	263 0.061
3 months AM:PM ratio	2.2 (1.3-3.0) N=52	1.5 (1.3-3.0) N=19	2.2 (1.3-3.0) N=33	273 0.442
6 months morning serum	448.7 (345.4-511.0) N=73	457.2 (416.6-511.8) N=27	430.2 (337.8-485.4) N=45	511 0.262
6 months AM saliva	10.2 (6.9-14.5) N=62	11.9 (6.9-16.2) N=20	8.6 (6.9-13.6) N=41	331 0.231
6 months PM saliva	5.2 (3.5-7.1) N=60	6.0 (3.3-8.1) N=19	4.8 (3.5-6.6) N=41	328 0.328
6 months AM:PM ratio	1.9 (1.4-2.7) N=56	2.1 (1.5-2.7) N=17	1.7 (1.3-2.7) N=39	294 0.504
12 months morning serum	425.8 (366.4-462.6) N=68	432.4 (381.2-457.3) N=24	409.3 (340.5-478.8) N=44	497 0.686
12 months AM saliva	8.2 (6.4-11.3) N=63	7.3 (6.6-10.9) N=19	8.3 (5.9-11.4) N=43	390 0.778
12 months PM saliva	4.3 (2.8-6.7) N=58	4.7 (3.5-8.6) N=19	4.2 (2.8-5.8) N=39	313 0.341
12 months AM:PM ratio	1.6 (1.2-3.1) N=53	1.5 (1.2-3.4) N=15	1.7 (1.2-3.0) N=38	276 0.859

MWU: Mann Whitney U test

Figure 9.4 Median morning serum cortisol level throughout the study period, in those with and without delirium during the perioperative period, excluding at follow-up those with new or syndromal delirium

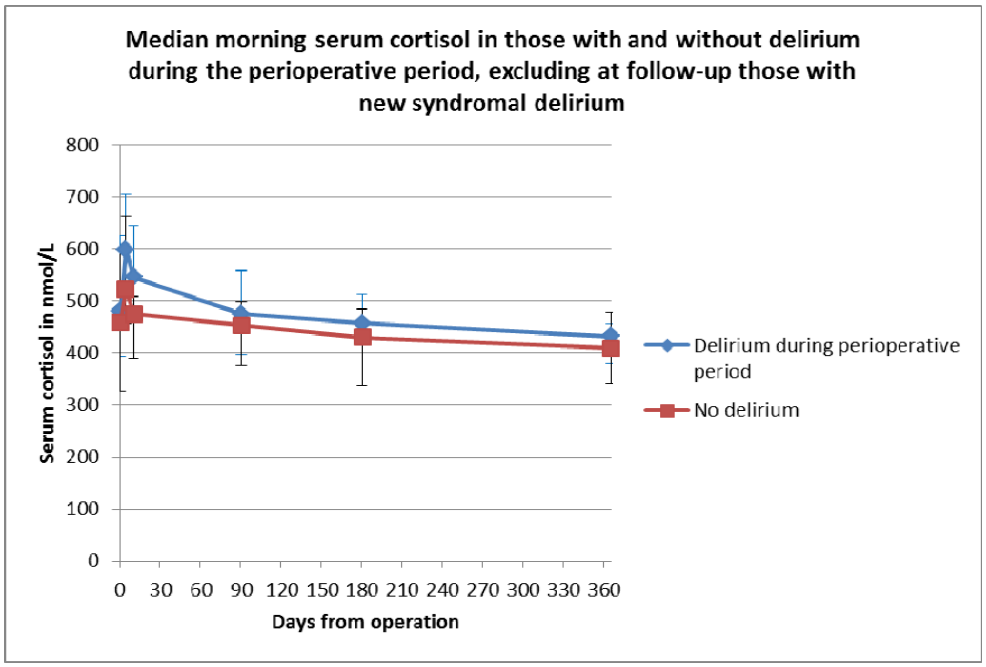


Figure 9.5 Diurnal salivary cortisol levels during the study period in those who did and did not develop delirium during the perioperative period, excluding at follow-up those with new or syndromal delirium

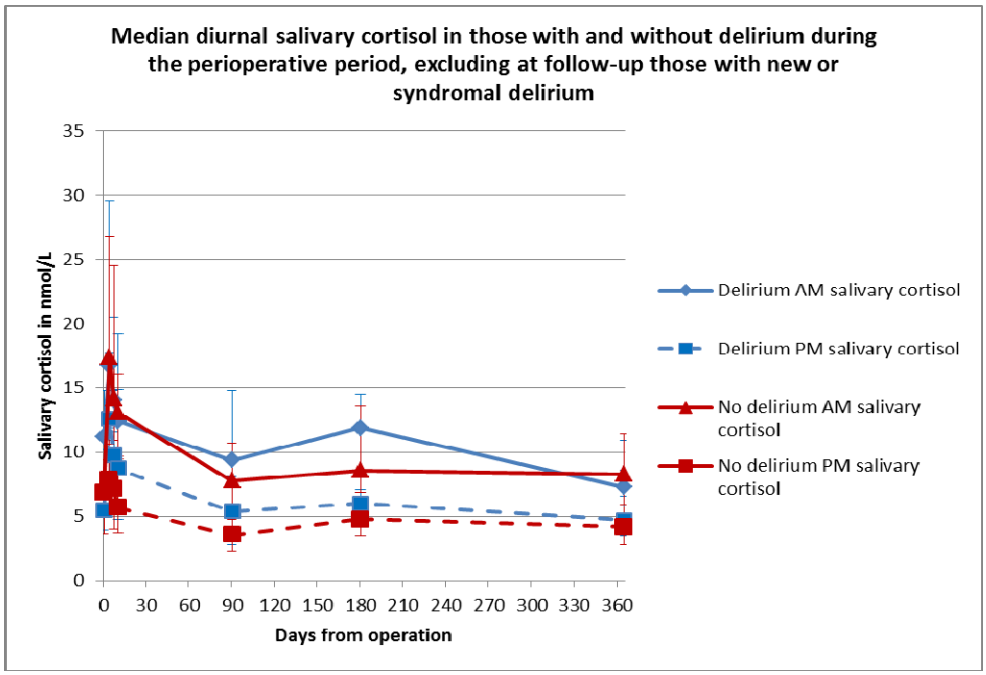


Table 9.5 Group comparisons of cortisol levels between those with delirium perioperatively which had resolved, those with persistent delirium features and those with no delirium, excluding those with new syndromal delirium at follow-up

Timepoint	**Median serum or salivary cortisol (IQR) in nmol/L**			
	Resolved	**Persistent excluding new**	**Never**	**K-W p**
3 months serum	440.7 (292.4-498.4) N=16	553.2 (447.1-625.7) N=18	446.8 (376.7-494.3) N=45	**0.005**
3 months AM saliva	8.9 (5.5-12.3) N=14	14.0 (5.7-15.9) N=7	7.8 (6.0-10.7) N=44	0.470
3 months PM saliva	5.4 (2.8-6.9) N=14	5.9 (2.3-11.3) N=8	3.6 (2.3-4.8) N=34	0.174
3 months AM:PM ratio	2.3 (1.1-3.2) N=13	1.4 (1.2-2.1) N=6	2.2 (1.3-3.0) N=33	0.502
6 months serum	457.2 (384.8-510.9) N=21	466.1 (385.6-755.7) N=6	430.2 (337.8-485.4) N=45	0.475
6 months AM saliva	11.9 (7.6-16.9) N=18	9.6 (6.4-9.6) N=2	8.6 (6.9-13.6) N=41	0.391
6 months PM saliva	6.0 (3.2-7.7) N=17	7.2 (5.9-7.2) N=2	4.8 (3.5-6.6) N=41	0.422
6 months AM:PM ratio	2.2 (1.6-2.8) N=15	1.3 (1.1-1.3) N=2	1.7 (1.3-2.7) N=39	0.180
12 months serum	436.9 (381.2-459.9) N=20	417.8 (338.8-449.6) N=4	409.3 (340.5-478.8) N=44	0.787
12 months AM saliva	7.3 (6.6-11.1) N=17	7.2 (6.2-7.2) N=3	8.4 (5.8-11.7) N=42	0.923
12 months PM saliva	4.7 (3.6-8.7) N=17	3.6 (2.3-3.6) N=2	4.2 (2.8-5.8) N=39	0.445
12 months AM:PM ratio	1.6 (1.2-3.4) N=14	N=1	1.7 (1.2-3.1) N=38	0.840

K-W: Kruskal-Wallis test

Figure 9.6 Median serum cortisol levels during the follow-up period in those with resolved delirium, persistent features of delirium and never delirium, excluding those with new syndromal delirium during the follow-up period

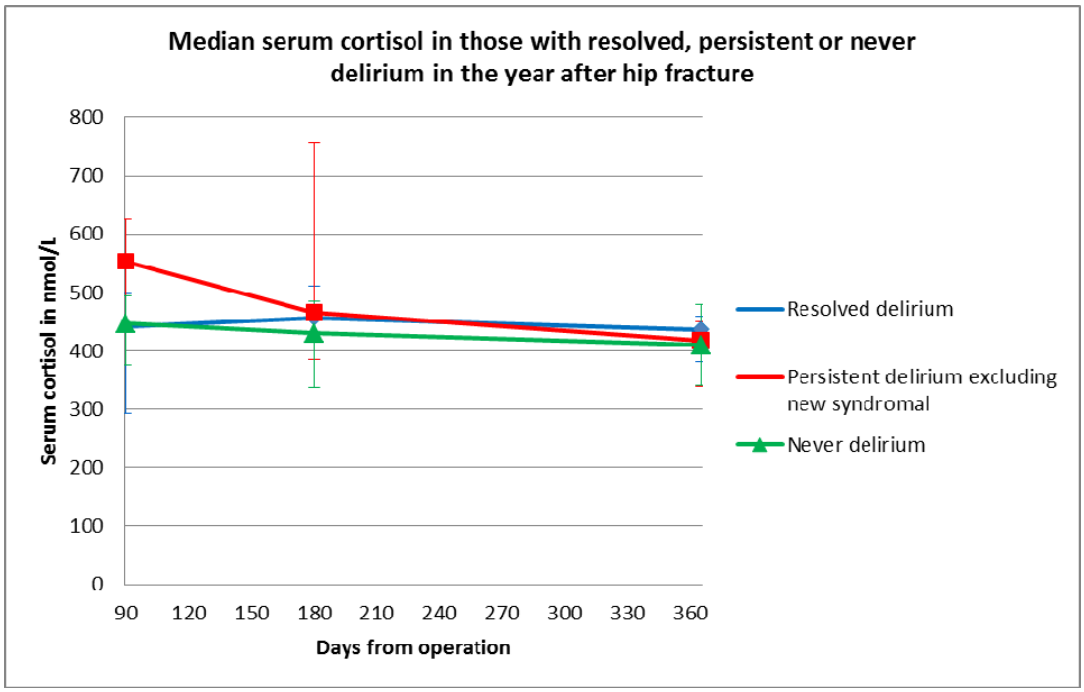
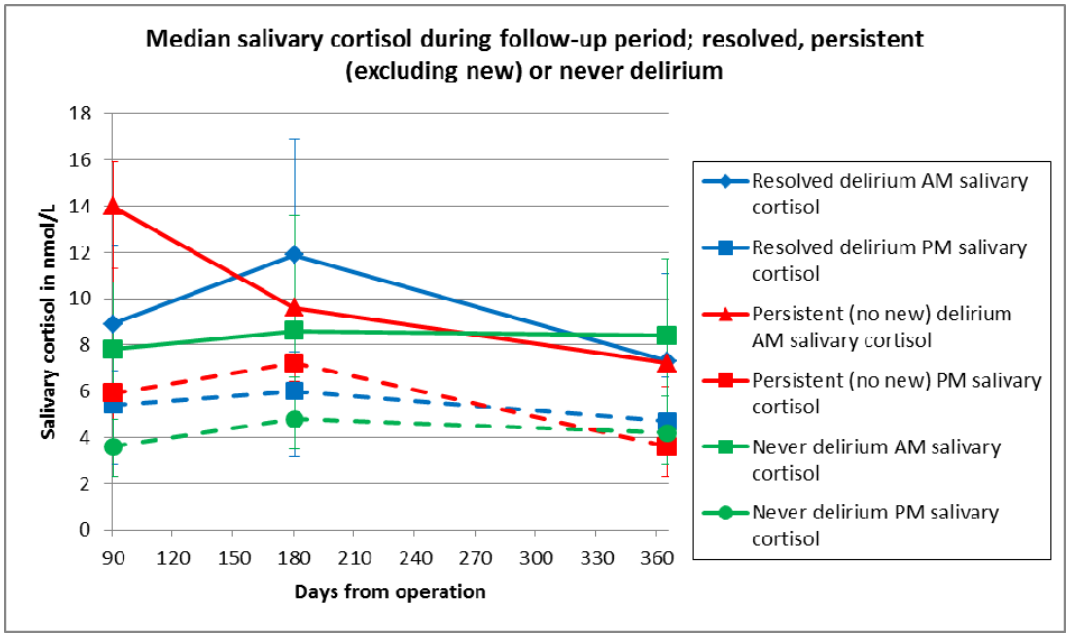


Figure 9.7 Median morning and afternoon salivary cortisol levels during the follow-up period in those with resolved delirium, persistent features of delirium and never delirium, excluding those with new syndromal delirium during the follow-up period



9.3.3.1 The HPA axis and persistence of delirium components

At 3 months, there was a correlation between morning salivary cortisol level and delirium severity as measured with the DRS-R98. There was also a moderate negative correlation between EDTB-1 score and morning serum cortisol, in keeping with higher serum cortisol being linked with worse inattention. At 6 months, there were significant correlations between afternoon salivary cortisol level and DRS-R98, EDTB-1 and OSLA, with a higher afternoon salivary cortisol level (suggesting loss of diurnal rhythm) correlated with worse delirium severity, inattention and abnormal level of arousal. DRS-R98 also correlated positively with both morning salivary and serum cortisol levels. At 12 months, contemporaneous cortisol measures were not correlated with any of the measures of delirium severity, inattention or arousal.

Table 9.6 Spearman's Rho correlations between cortisol measures at follow-up with contemporaneous measures of persistent delirium severity (DRS-R98 severity score), inattention (Edinburgh Delirium Test Box-1 score) and level of arousal (Observational Level of Arousal score)

Cortisol measure	3 months			6 months			12 months		
	DRS-R98	EDTB-1	OSLA	DRS-R98	EDTB-1	OSLA	DRS-R98	EDTB-1	OSLA
AM saliva	**R=0.26 p=0.04**	R=-0.16 p=0.21	R=0.08 p=0.54	**R=0.33 p=0.01**	R=-0.15 p=0.26	R=0.22 p=0.09	R=0.16 p=0.20	R=-0.09 p=0.47	R=0.06 p=0.67
PM saliva	R=0.07 p=0.61	R=0.11 p=0.43	R=-0.03 p=0.86	**R=0.33 p=0.01**	**R=-0.26 p=0.04**	**R=0.40 p<0.01**	R=0.09 p=0.52	R=-0.14 p=0.30	R=-0.05 p=0.74
Diurnal saliva ratio	R=0.09 p=0.54	R=-0.17 p=0.23	R=0.01 p=0.92	R=-0.06 p=0.67	R=0.20 p=0.16	R=-0.20 p=0.14	R=0.10 p=0.48	R=0.17 p=0.22	R=0.08 p=0.57
AM serum	R=0.16 p=0.16	**R=-0.31 p=0.01**	R=0.11 p=0.34	**R=0.34 p<0.01**	R=-0.20 p=0.11	R=0.19 p=0.11	R<-0.01 p=0.97	R=-0.04 p=0.77	R=0.08 p=0.53

R= Spearman's Rho

DRS-R98: higher score indicating more severe delirium

EDTB-1: lower score indicating more inattention, higher score indicating better performance

OSLA: higher score indicating more abnormal level of arousal

9.3.4 The HPA axis after hip fracture, and its relationship with delirium and dementia

There were no significant differences in serum or salivary cortisol levels based on delirium and dementia or mild cognitive impairment (MCI) status at 3, 6 or 12 months (Table 9.7). In line with my hypothesis, at 3 and 6 months, serum cortisol was highest in those with delirium superimposed on dementia/MCI, but this was not significant. Also in line with my hypothesis, afternoon salivary cortisol levels were higher in the groups with delirium superimposed on dementia/MCI and delirium only at 3 and 6 months, but this was not significant. At 12 months, serum and salivary cortisol levels were very similar between groups.

Table 9.7 Group comparisons of cortisol levels in serum and saliva at 3, 6 and 12 months after hip fracture, based on delirium and dementia/MCI status, excluding those with new syndromal delirium at follow-up

Timepoint	Median serum or salivary cortisol (IQR) in nmol/L				
	Delirium on MCI/dementia	Delirium only	MCI/dementia only	Neither	K-W p
3 months serum	513.7 (383.7-606.1) N=16	449.4 (404.5-550.9) N=17	469.7 (392.1-567.0) N=10	445.4 (371.9-488.1) N=35	0.272
3 months AM saliva	8.1 (5.5-10.8) N=10	12.6 (7.6-16.9) N=10	7.6 (6.0-11.3) N=11	8.0 (5.5-10.5) N=33	0.241
3 months PM saliva	5.4 (2.4-9.2) N=9	5.7 (2.9-8.9) N=12	2.7 (1.9-4.4) N=7	4.0 (2.3-4.9) N=27	0.252
3 months AM:PM ratio	1.5 (1.2-3.3) N=9	2.4 (1.2-3.2) N=9	2.2 (1.2-3.1) N=7	2.3 (1.3-3.0) N=26	0.851
6 months serum	475.6 (420.7-572.4) N=13	456.6 (347.1-510.9) N=13	442.6 (293.8-644.4) N=11	424.0 (337.8-471.9) N=34	0.615
6 months AM saliva	8.8 (6.5-16.9) N=9	13.6 (8.0-16.3) N=11	9.9 (7.0-17.8) N=10	8.6 (6.9-12.8) N=31	0.427
6 months PM saliva	5.9 (3.2-8.5) N=7	6.9 (3.3-8.1) N=11	4.3 (3.6-6.9) N=9	4.8 (3.5-6.7) N=32	0.779
6 months AM:PM ratio	1.8 (1.1-2.6) N=7	2.3 (1.6-2.9) N=10	1.7 (1.4-2.8) N=9	1.8 (1.2-2.6) N=30	0.665
12 months serum	430.3 (316.6-457.4) N=11	432.4 (395.7-456.4) N=12	379.3 (238.0-444.1) N=11	421.3 (369.0-488.2) N=33	0.510
12 months AM saliva	6.9 (6.5-13.2) N=8	8.0 (7.0-10.9) N=11	8.2 (5.5-14.2) N=11	8.4 (5.9-11.4) N=31	0.991
12 months PM saliva	4.6 (2.8-6.6) N=8	4.3 (3.4-8.8) N=10	4.4 (2.8-5.7) N=9	4.1 (2.7-6.9) N=30	0.880
12 months AM:PM ratio	1.5 (1.1-4.4) N=6	1.9 (1.4-3.4) N=8	1.9 (1.4-3.6) N=9	1.5 (1.2-3.0) N=29	0.870

K-W: Kruskal-Wallis test

9.3.5 Cognition, delirium and the HPA axis

There was no difference preoperatively in crystallised intelligence (NART) nor years in full time education between those who did or did not go on to develop delirium (Table 9.2). The delirium group was more likely to have evidence of prior dementia as assessed by the IQCODE. MMSE scores were lower in the delirium group preoperatively and throughout the year after hip fracture. MMSE scores rose slightly in the delirium group during the year after hip fracture; this may represent attrition of more impaired participants, a learning effect from repeated administration, or slow recovery from delirium. MMSE score preoperatively in the whole cohort was slightly lower than during the follow-up period, which may have represented the influence of delirium and in those without delirium the effects of analgesia, pain or anxiety.

Table 9.2 Baseline cognitive assessments of crystallised intelligence (NART), prior cognition (IQCODE) and serial assessment of global cognition (MMSE) over the year after hip fracture

	Whole group Mean (SD) or Median (IQR)	Delirium Mean (SD) or Median (IQR)	No delirium Mean (SD) or Median (IQR)	p value
NART at baseline /50	31.5 (10.7) N=83	30.3 (10.1) N=27	32.1 (10.9) N=56	0.494[a]
Years in education	11 (10-13) N=94	11 (10-13) N=37	11 (10-13) N=56	0.997[b]
IQCODE score at baseline	Total 49 (48-52) N=97 Average 3.06 (3.00-3.25) ≥3.44: N=11 3.19-3.43: N=22 ≤3.18: N=64	Total 51 (48-54) N=40 Average 3.13 (3.00-3.38) ≥3.44: N=9 3.19-3.43: N=11 ≤3.18: N=20	Total 48 (48-54) N=57 Average 3.00 (3.00-3.13) ≥3.44: N=2 3.19-3.43: N=11 ≤3.18: N=44	**0.001[b]** **<0.001[b]** **0.005**
MMSE score /30	Preoperative 27 (25-28) N=105 3 months: 29 (26-30) N=83 6 months: 29 (27-30) N=77 12 months: 29 (27-30) N=75	Preoperative: 25 (22-28) N=43 3 months: 26 (24-29) N=35 6 months: 27 (23-29) N=31 12 months: 28 (22-29) N=30	Preoperative: 28 (25-29) N=60 3 months: 29 (27-30) N=48 6 months: 29 (28-30) N=46 12 months: 29 (28-30) N=45	**<0.001[b]** **<0.001[b]** **<0.001[b]** **0.001[b]**

[a]Student's t test

[b]Mann Whitney U test

[c]Pearson Chi-Square

NART: National Adult Reading Test

IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly, "normal" ≤3.18, "MCI" 3.19-3.43, "dementia" ≥3.44

MMSE: Mini Mental State Examination

There was a large degree of missing data for the cognitive test battery. Although the battery was chosen to be relatively quick (administration took 45-60 minutes), many frailer participants found this too long and the testing was curtailed. The reasons for limiting the cognitive test battery were fatigue, distractibility, that participants felt they were not doing well and asked to finish or skip tests, and a small number of participants who had had prior dementia were too cognitively impaired later at follow-up to complete any or all of the tests. This, combined with the attrition through death (predominantly in the delirium group) or withdrawal (in both groups) mean that the numbers with complete cognitive test data are fairly small, and more of the fitter group who had not had delirium or had mild delirium were able to be tested. Results are therefore presented first as individual test scores for each cognitive test. These are raw cognitive test scores which have not been adjusted for IQCODE or NART. Tables 9.8 to 9.10 show mean or median scores on the cognitive test battery in the whole cohort at 3, 6 and 12 months after hip fracture. In order to explore the influence of persistent delirium on cognition, group differences in cognitive test scores were tested between participants who had had delirium which had resolved at reassessment ("resolved"), those who had persistent features of delirium ("persistent") and those who never developed syndromal delirium ("never"). Those participants with new or full syndromal delirium were excluded, as these were very small numbers (N=1 at 3 months, N=2 at 6 and 12 months) and may have had a significant confounding influence.

9.3.5.1 Digit span

Scores on digit span forwards and backwards stayed stable in the whole cohort throughout the follow-up period. At 3, 6 and 12 months there was a significant difference in digit span backwards total scores between those with resolved, persistent and never delirium. Scores were lower in those with persistent delirium, and post hoc pairwise comparisons suggested that this was due to the difference between the persistent and never groups (Tables 9.8-9.10).

9.3.5.2 Victoria Stroop

Victoria Stroop interference and difference scores improved slightly in the whole cohort between 3 and 6 months, which may have been a learning effect (Tables 9.8 and 9.9). There

was no significant difference between delirium groups in either score throughout the follow-up period.

9.3.5.3 HVLT-R List Learning

HVLT-R list learning total recall (the total number of words recalled in the first three learning trials) improved slightly in the whole cohort during the follow-up period. Different test sheets (of equivalent difficulty) were used so this is unlikely to be a learning effect. Recognition discrimination index (the number of true positive words from the original list recognised, minus semantically related and unrelated false negatives) stayed fairly stable in the whole cohort. There was a significant difference in both scores between delirium groups throughout the follow-up period, with the lowest scores in those with persistent delirium and scores in those with resolved delirium slightly lower than the never delirium group. At 3 months the scores on total recall were significantly different between all groups (Table 9.8), at 6 months both delirium groups were significantly different to the never group (Table 9.9), and at 12 months, the resolved delirium group was significantly different to the never delirium group (Table 9.10). At 3 and 12 months, recognition discrimination index was significantly lower in those with persistent delirium compared to those with never delirium. This suggests worse verbal learning and memory in those with persistent delirium, but also in those with resolved delirium.

9.3.5.4 Visual Reproduction

Scores on the visual reproduction test improved slightly in the whole cohort during the follow-up period. This may have been a learning effect, or may be due to attrition of more cognitively impaired participants. There were significant differences between delirium groups on immediate and delayed visual memory (recall I and II), with the worst scores in those with persistent delirium. At 3 months, post hoc testing suggested this was due to the difference between the persistent and never delirium groups (Table 9.8), whereas at 6 and 12 months, the difference was between those with resolved and never delirium, and there were small numbers in the persistent delirium group (Tables 9.9.and 9.10).

9.3.5.5 Digit Symbol Substitution Test

Scores on digit symbol substitution test remained stable in the whole cohort during the year after hip fracture. Scores tended to be lower in both delirium groups than in those who never developed delirium, in keeping with worse processing speed, but these differences were not significant (Tables 9.8-9.10).

9.3.5.6 Verbal Fluency

Verbal fluency scores for both letter (FAS) and category (animals) fluency stayed stable in the whole cohort throughout the follow-up period (Tables 9.8-9.10). Verbal fluency FAS score was significantly lower in those with persistent delirium at 6 months, with post hoc testing confirming significant differences compared with both resolved and never delirium groups. Verbal fluency animals scores were significantly different between groups at 3, 6 and 12 months (Tables 9.8-9.10). At 3 and 6 months, post hoc testing found a trend towards significance between persistent and never delirium groups, whereas at 12 months the persistent delirium group had significantly lower scores compared to the resolved and never delirium groups.

Table 9.8 Group differences in raw cognitive test scores at 3 months between participants who had delirium at baseline which had resolved, those with persistent delirium features, or who never developed delirium

Cognitive test	Whole group mean (SD) or median (IQR)	Delirium status at 3 months, mean (SD) or median (IQR)			
		Resolved	Persistent excluding new delirium	Never	p value
Digit Span forwards total	9 (8-10) N=69	8 (7-10) N=12	9 (7-10) N=12	9 (8-10) N=45	0.091[b]
Digit Span backwards total	6 (5-7) N=65	5 (4-7) N=12	5 (4-6) N=10	6 (5-7) N=43	**0.013[b]**
Victoria Stroop difference score	20.3 (12.4-30.9) N=65	22.7 (13.5-30.6) N=14	16.6 (1.6-29.9) N=11	19.7 (12.7-33.2) N=40	0.608[b]
Victoria Stroop interference score	1.17 (0.80-1.92) N=65	1.05 (0.87-1.73) N=14	1.03 (0.07-1.71) N=11	1.40 (0.91-2.01) N=40	0.312[b]
List learning total recall	19.1 (7.0) N=76	17.3 (5.0) N=15	11.7 (6.1) N=13	21.6 (6.1) N=48	**<0.001[a]**
List learning RDI	10 (8-11) N=72	9 (7-10) N=14	8 (5-10) N=12	10 (9-12) N=46	**0.003[b]**
Visual Reproduction recall I	56 (17) N=60	52 (16) N=11	42 (17) N=8	60 (16) N=41	**0.016[a]**
Visual Reproduction recall II	35 (19) N=54	33 (8) N=8	17 (15) N=7	38 (19) N=39	**0.019[a]**
Visual Reproduction % retention	57 (25) N=54	59 (14) N=8	36 (26) N=7	60 (26) N=39	0.054[a]
Digit symbol substitution test	33 (27-41) N=50	33 (27-38) N=9	26 (20-36) N=6	36 (29-43) N=35	0.140[b]
Verbal fluency FAS total	30 (13) N=74	32 (14) N=15	23 (15) N=11	31 (12) N=47	0.124[a]
Verbal fluency animals total	14 (6) N=74	14 (5) N=15	9 (7) N=11	15 (5) N=48	**0.007[a]**

[a]ANOVA, [b]Kruskal-Wallis test, Post hoc tests: pairwise comparisons for Kruskal-Wallis; post hoc Dunnett's T3 test for ANOVA

Table 9.9 Group differences in raw cognitive test scores at 6 months between participants who had delirium at baseline which had resolved, those with persistent delirium features, or who never developed delirium

Cognitive test	Whole group mean (SD) or median (IQR)	Delirium status at 6 months, mean (SD) or median (IQR)			
		Resolved	Persistent excluding new delirium	Never	p value
Digit Span forwards total	9 (8-10) N=68	9 (8-9) N=19	8 (6-9) N=4	9 (6-9) N=44	0.127[b]
Digit Span backwards total	6 (5-7)N=68	5 (4-7) N=19	5 (3-7) N=4	6 (5-7) N=44	**0.041[b]**
Victoria Stroop difference score	17.3 (11.2-26.7) N=64	18.6 (9.8-26.9) N=18	10.2 (-13.8-64.0) N=5	17.7 (13.1-26.9) N=41	0.214[b]
Victoria Stroop interference score	1.13 (0.68-1.55) N=64	1.23 (0.36-1.59) N=18	0.55 (-0.39-2.68) N=5	1.13 (0.69-1.60) N=41	0.341[b]
List learning total recall	20.0 (7.1) N=72	17.7 (5.6) N=20	10.2 (7.5) N=6	22.7 (5.9) N=45	**<0.001[a]**
List learning RDI	9 (7-11) N=70	9 (7-11) N=20	7 (6-9) N=5	9 (8-11) N=44	0.063[b]
Visual Reproduction recall I	61 (17) N=56	55 (15) N=15	37 (13) N=2	66 (13) N=38	**0.001[a]**
Visual Reproduction recall II	42 (18) N=55	35 (17) N=15	23 (16) N=2	47 (15) N=37	**0.011[a]**
Visual Reproduction % retention	66 (21) N=55	60 (22) N=15	59 (21) N=2	70 (17) N=37	0.202[a]
Digit symbol substitution test	32 (27-41) N=52	29 (25-34) N=12	29 (15-29) N=2	36 (29-42) N=38	0.182[b]
Verbal fluency FAS total	31 (13) N=72	34 (11) N=19	15 (12) N=6	33 (13) N=46	**0.003[a]**
Verbal fluency animals total	14 (5) N=72	13 (4) N=19	9 (7) N=6	15 (5) N=46	**0.004[a]**

[a]ANOVA, [b]Kruskal-Wallis test, Post hoc tests: pairwise comparisons for Kruskal-Wallis; post hoc Dunnett's T3 test for ANOVA

Table 9.10 Group differences in raw cognitive test scores at 12 months between participants who had delirium at baseline which had resolved, those with persistent delirium features, or who never developed delirium

Cognitive test	Whole group mean (SD) or median (IQR)	Delirium status at 12 months, mean (SD) or median (IQR)			
		Resolved	Persistent excluding new delirium	Never	p value
Digit Span forwards total	9 (8-10) N=70	8 (8-10) N=21	8 (4-8) N=3	9 (8-11) N=45	0.195[b]
Digit Span backwards total	6 (5-7) N=68	6 (5-7) N=21	5 (1-5) N=3	7 (5-8) N=44	**0.029[b]**
Victoria Stroop difference score	17.8 (10.4-26.8) N=65	17.6 (9.8-30.1) N=19	20.4 (-19.9-20.4) N=3	17.8 (10.7-25.2) N=42	0.916[b]
Victoria Stroop interference score	0.87 (0.60-1.57) N=65	0.71 (0.42-1.55) N=19	0.88 (-0.34-0.88) N=3	0.97 (0.69-1.69) N=42	0.494[b]
List learning total recall	21.0 (6.8) N=70	18.6 (5.1) N=20	9.0 (7.0) N=4	23.4 (5.6) N=45	**<0.001[a]**
List learning RDI	10 (8-11) N=69	9 (7-11) N=20	6 (3-8) N=4	10 (9-12) N=45	**0.002[b]**
Visual Reproduction recall I	65 (15) N=53	56 (16) N=15	N=1	70 (13) N=37	**0.004[a]**
Visual Reproduction recall II	49 (20) N=52	34 (18) N=15	N=1	55 (18) N=36	**0.002[a]**
Visual Reproduction % retention	72 (22) N=52	59 (24) N=15	N=1	77 (19) N=36	**0.027[a]**
Digit symbol substitution test	34 (26-43) N=52	31 (25-37) N=12	N=1	37 (27-43) N=39	0.160[b]
Verbal fluency FAS total	32.0 (13.3) N=70	32.2 (12.9) N=19	20.2 (11.0) N=5	33.4 (13.1) N=45	0.102[a]
Verbal fluency animals total	14.3 (5.4) N=70	13.8 (4.0) N=19	5.4 (3.1) N=5	15.7 (5.0) N=45	**<0.001[a]**

[a]ANOVA, [b]Kruskal-Wallis test, Post hoc tests: pairwise comparisons for Kruskal-Wallis; post hoc Dunnett's T3 test for ANOVA

9.3.6 Correlations

Spearman's Rho correlations between cognitive test scores and cortisol measures from the same timepoint are shown in Tables 9.11a and 9.11b. AM:PM salivary cortisol ratio did not correlate with any cognitive test scores at 3 or 12 months, and only correlated with verbal fluency FAS score at 6 months (Rho 0.291, p=0.033), in keeping with with a better verbal fluency score with a higher and therefore more intact cortisol diurnal rhythm. There was no consistent pattern in correlations between cognitive test scores and cortisol measures, but scores on HVLT-R list-learning and Visual Reproduction tended to correlate inversely with cortisol (higher cortisol level with worse verbal and visual memory scores). At 6 and 12 months, higher serum cortisol correlated inversely with Digit Symbol Substitution Test, in keeping with higher cortisol with worse processing speed. At 3 months, higher morning cortisol correlated inversely with Verbal Fluency Animals score, in keeping with higher cortisol with worse category verbal fluency.

Table 9.11a Correlations between contemporaneous cortisol measures and cognitive test scores, Digit Span, Victoria Stroop and List Learning

Cognitive test	3 months cortisol measures, Rho, p value			6 months cortisol measures, Rho, p value			12 months cortisol measures, Rho, p value		
	AM saliva	PM saliva	Serum	AM saliva	PM saliva	Serum	AM saliva	PM saliva	Serum
Digit Span forwards total	ρ -0.160 p=0.234	ρ -0.016 p=0.914	ρ 0.067 p=0.590	ρ -0.191 p=0.155	ρ -0.149 p=0.274	ρ -0.128 p=0.300	ρ -0.006 p=0.964	ρ -0.161 p=0.232	ρ 0.039 p=0.758
Digit Span backwards total	**ρ -0.270 p=0.046**	ρ 0.070 p=0.639	ρ -0.087 p=0.498	ρ -0.167 p=0.214	ρ 0.027 p=0.842	ρ -0.239 p=0.052	ρ 0.019 p=0.887	ρ -0.005 p=0.969	ρ 0.013 p=0.920
Victoria Stroop difference score	ρ 0.098 p=0.483	ρ 0.005 p=0.971	**ρ -0.266 p=0.035**	ρ 0.019 p=0.892	ρ -0.134 p=0.340	ρ 0.102 p=0.426	ρ -0.067 p=0.629	ρ -0.133 p=0.346	ρ -0.092 p=0.480
Victoria Stroop interference score	ρ 0.099 p=0.477	ρ -0.088 p=0.553	**ρ -0.316 p=0.012**	ρ -0.003 p=0.984	ρ -0.070 p=0.618	ρ 0.099 p=0.440	ρ -0.114 p=0.405	ρ -0.197 p=0.161	ρ -0.160 p=0.217
List learning total recall	**ρ -0.314 p=0.012**	**ρ -0.295 p=0.030**	ρ -0.062 p=0.601	ρ -0.213 p=0.104	**ρ -0.341 p=0.009**	ρ -0.204 p=0.089	**ρ -0.262 p=0.041**	**ρ -0.316 p=0.017**	ρ -0.052 p=0.681
List learning RDI	**ρ -0.374 p=0.003**	ρ -0.137 p=0.328	ρ -0.093 p=0.449	ρ -0.024 p=0.859	ρ -0.108 p=0.419	ρ -0.073 p=0.551	**ρ -0.266 p=0.038**	ρ -0.220 p=0.099	ρ -0.075 p=0.551

ρ: Spearman's Rho correlation co-efficient

Table 9.11b Correlations between contemporaneous cortisol measures and cognitive test scores, Visual Reproduction, DSST and Verbal Fluency

Cognitive test	3 months cortisol measures, Rho, p value			6 months cortisol measures, Rho, p value			12 months cortisol measures, Rho, p value		
	AM saliva	PM saliva	Serum	AM saliva	PM saliva	Serum	AM saliva	PM saliva	Serum
Visual Reproduction recall I	**ρ -0.343 p=0.012**	**ρ -0.327 p=0.028**	ρ 0.028 p=0.837	ρ -0.161 p=0.275	**ρ -0.304 p=0.032**	ρ -0.101 p=0.464	ρ -0.170 p=0.258	ρ -0.175 p=0.238	**ρ -0.286 p=0.042**
Visual Reproduction recall II	**ρ -0.376 p=0.008**	ρ -0.270 p=0.088	ρ 0.041 p=0.769	ρ -0.217 p=0.142	ρ -0.226 p=0.119	ρ -0.179 p=0.196	ρ -0.135 p=0.376	ρ -0.241 p=0.106	ρ -0.030 p=0.834
Visual Reproduction % retention	ρ 0.284 p=0.051	ρ -0.235 p=0.139	ρ -0.048 p=0.730	ρ -0.199 p=0.180	ρ -0.107 p=0.463	ρ -0.222 p=0.107	ρ -0.033 p=0.832	ρ -0.209 p=0.163	ρ 0.145 p=0.315
Digit symbol substitution test	ρ -0.280 p=0.069	ρ -0.196 p=0.232	ρ -0.261 p=0.070	ρ -0.167 p=0.262	ρ -0.187 p=0.203	**ρ -0.304 p=0.030**	ρ -0.265 p=0.079	ρ -0.028 p=0.850	**ρ -0.294 p=0.038**
Verbal fluency FAS total	ρ -0.144 p=0.271	ρ -0.008 p=0.954	ρ -0.073 p=0.546	ρ 0.093 p=0.486	ρ -0.089 p=0.513	ρ 0.006 p=0.961	ρ 0.228 p=0.079	ρ 0.098 p=0.472	ρ 0.092 p=0.466
Verbal fluency animals total	**ρ -0.316 p=0.012**	ρ -0.123 p=0.381	**ρ -0.328 p=0.005**	ρ 0.029 p=0.828	ρ -0.087 p=0.521	ρ -0.208 p=0.084	ρ 0.074 p=0.576	ρ 0.091 p=0.505	ρ -0.104 p=0.410

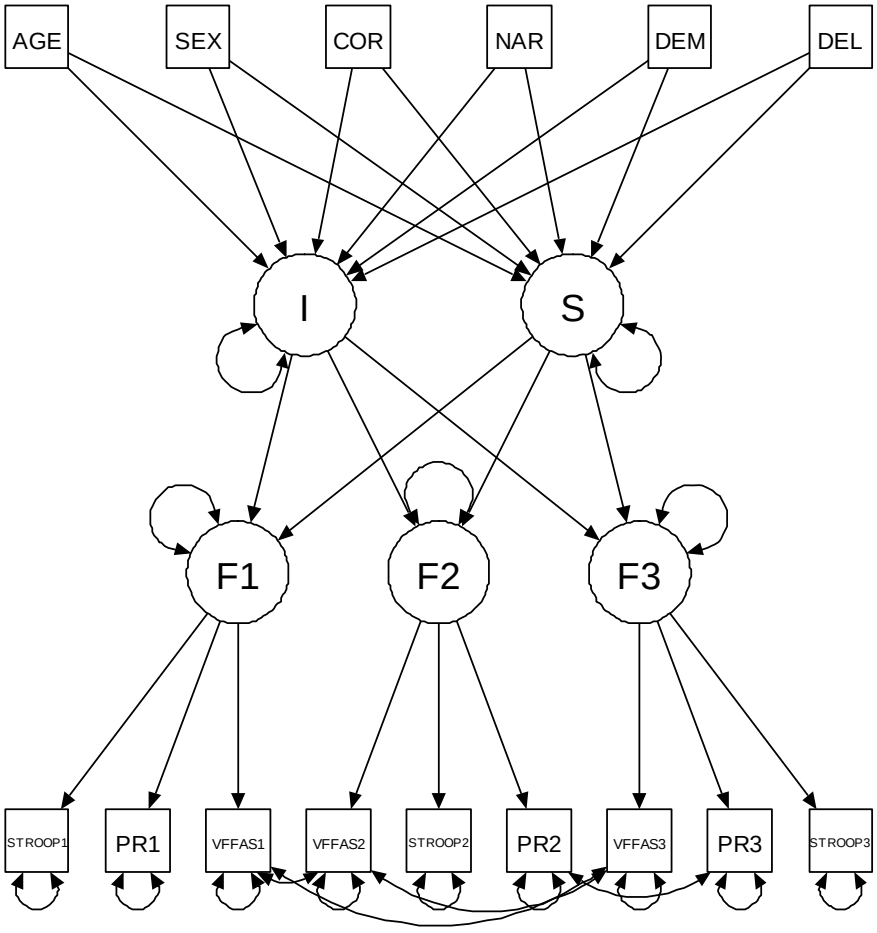
ρ: Spearman's Rho correlation co-efficient

9.3.7 Trajectory analysis

The trajectory analysis was performed by Dr Mike Allerhand at the Centre for Cognitive Ageing and Cognitive Epidemiology. The six cognitive scores used for the trajectory analysis were: Digit Span total score (Digit Span Forwards plus Digit Span Backwards), Verbal Fluency FAS total score, HVLT-R List Learning Recognition Discrimination Index, Visual Reproduction Percent Retention, Digit Symbol Substitution Test total score and Victoria Stroop interference score. The sample distributions of the six measures were assessed graphically and judged to be acceptably normal. The measures were standardised into units of the respective baseline (3 months) standard deviation, and centred on the respective baseline mean. Sample estimates of the mean and its standard error at each wave indicated that three of the cognitive measures did not have any systematic change over the three waves (3/6/12 months). These were dropped leaving three variables for subsequent analysis: Verbal Fluency FAS total score, Visual Reproduction Percent Retention, and Victoria Stroop interference score. A preliminary principal components analysis of these three variables indicated 53%, 48%, and 41% of the total variation at waves 1, 2, and 3 respectively could be explained by one factor at each wave. Preliminary growth curves fitted to each variable indicated no significant curvature. A linear (straight line) latent growth curve model was fitted to the three variables over three waves. The model's path diagram is shown in Figure 9.8. Contemporaneous loadings and intercepts were held equal for longitudinal measurement invariance. Time-invariant covariates were added as controls and to test their intercept and slope effects. Each covariate was centered on its sample mean. Model parameters were estimated using full information maximum likelihood by the Mplus program.The model fitted acceptably well, (RMSEA=0.08, CFI=0.85). An extract of the parameter estimates is shown in Table 9.12.

Using the three cognitive measures (Victoria Stroop, Visual Reproduction percent retention, and Verbal Fluency FAS score) there was a significant positive slope (0.255, $p<0.001$), indicating improvement of cognitive functioning over time. The units of change were standard deviations of Victoria Stroop interference score at wave 1 (3 month follow-up) per 6 months. This was non-significant using all six cognitive measures. None of the covariates, including CSF cortisol, day 4 cortisol AM:PM ratio or delirium status, had a significant effect on the slope. National Adult Reading Test and dementia status had a significant effect on the intercept.

Figure 9.8 Path diagram of trajectory analysis



F1/F2/F3: Composite cognitive ability at each wave, based on the three measures.

I: Intercept

S: Slope

Age (at baseline) and sex are time-invariant covariates centred on their respective means.

COR: Baseline cortisol level in cerebrospinal fluid or day 4 AM:PM ratio in saliva

NAR: National Adult Reading Test

DEM: Dementia status

DEL: Delirium status

Table 9.12 Parameter estimates of the trajectory analysis, CSF cortisol model

		EST	SE	SE/EST	p value
Intercepts	Slope	0.255	0.073	3.485	<0.001
Intercept on	Age	-0.002	0.011	-0.188	0.851
Intercept on	Sex	0.084	0.197	0.426	0.670
Intercept on	CSF cortisol	0.003	0.005	0.656	0.512
Intercept on	NART	0.035	0.010	3.475	0.001
Intercept on	Dementia	1.013	0.411	2.468	0.014
Intercept on	Delirium	0.255	0.208	1.226	0.220
Slope on	Age	0.005	0.006	0.766	0.444
Slope on	Sex	-0.115	0.111	-1.031	0.303
Slope on	CSF cortisol	0.001	0.003	0.259	0.796
Slope on	NART	-0.009	0.006	-1.367	0.172
Slope on	Dementia	-0.354	0.259	-1.368	0.171
Slope on	Delirium	-0.077	0.116	-0.666	0.505

EST:

SE: standard error

NART: National Adult Reading Test

CSF cortisol: Cerebrospinal fluid cortisol at baseline

Table 9.13 Parameter estimates of the trajectory analysis, salivary cortisol AM:PM ratio model

		EST	SE	SE/EST	p value
Intercepts	Slope	0.254	0.074	3.429	0.001
Intercept on	Age	<0.001	0.011	0.047	0.963
Intercept on	Sex	0.047	0.190	0.248	0.804
Intercept on	AM:PM cortisol	0.021	0.061	0.340	0.734
Intercept on	NART	0.038	0.010	3.942	<0.001
Intercept on	Dementia	1.083	0.398	2.721	0.007
Intercept on	Delirium	0.248	0.209	1.184	0.236
Slope on	Age	0.006	0.006	0.861	0.389
Slope on	Sex	-0.126	0.109	-1.159	0.247
Slope on	AM:PM cortisol	0.004	0.034	0.110	0.913
Slope on	NART	-0.009	0.006	-1.332	0.138
Slope on	Dementia	-0.326	0.248	-1.314	0.189
Slope on	Delirium	-0.088	0.116	-0.752	0.452

EST:

SE: standard error

NART: National Adult Reading Test

AM:PM cortisol: Postoperative day 4 morning:afternoon salivary cortisol ratio

9.4 Discussion

This study has demonstrated several interesting findings regarding the hypothalamic-pituitary-adrenal (HPA) axis after delirium, and the cognitive profile of persistent and resolved delirium post-hip fracture.

In the whole cohort, median serum cortisol levels were still elevated at 3 months, being only slightly lower than preoperative levels. Cortisol then steadily fell throughout the year after hip fracture. Cortisol has previously been shown to be elevated for eight weeks after hip fracture (Roberts, Barton et al. 1990), but this study suggests that this elevation might persist for even longer.

Although persistent syndromal delirium was rare in this study, persistent features of delirium were common at 3 and 6 months after hip fracture, and this group of patients had evidence of persistent activation of the HPA axis and worse performance on cognitive tests. At 3 months, morning serum cortisol was still elevated at "stress" levels (similar to perioperative levels) in those who had evidence of persistent delirium in the absence of full syndromal delirium. At 3 months, the results of correlations between HPA axis measures and delirium components were in keeping with worse delirium and worse inattention with higher peak cortisol levels, suggesting persistence of delirium features is associated with persistent high cortisol. At 6 months, this relationship with delirium severity scores and ongoing high morning cortisol persisted, and also, worse scores on delirium testing (severity, inattention and abnormal arousal) were correlated with higher afternoon salivary cortisol, suggesting those with persistent delirium features may have persistent loss of diurnal rhythm. There was a suggestion of a sustained increase in HPA axis activity and loss of diurnal rhythm in those who had had delirium during the baseline perioperative period, but this was not significant.

Those with features of persistent delirium performed poorly on many of the cognitive tests, with evidence of impairment in working memory (digit span backwards), verbal learning and memory (HVLT-R list learning), visual memory (visual reproduction) and executive function (verbal fluency). These impairments may have been due to persisting inattention or short-term memory impairments related to their persistent delirium, or due to an abnormal level of arousal. A phenomenological study of persistent versus resolving delirium has shown that short and long-term memory impairment as well as inattention, disorientation, delusions,

agitation and thought process abnormalities persist (Meagher, Adamis et al. 2012). Since I was unable to fully test participants' cognition at baseline due to the study design, it is difficult to know whether some or all of these impairments were pre-existing, due to known or undiagnosed dementia. Alternatively, these impairments may have been new and a consequence of their delirium, or of preceding and/or sustained HPA axis hyperactivity. Those who had had delirium at baseline but which had resolved showed impairment in verbal learning and memory and visual memory. These participants no longer exhibited any features of delirium, and had a normal level of arousal. As discussed above, these impairments may have been pre-existing or may be new and related to their delirium or from exposure to dysregulated cortisol. Although not always consistent, the results of the correlations between cognitive test scores and cortisol measures suggest some of the impairments may be related to HPA axis activation, with higher morning cortisol levels correlating with worse verbal and visual memory, processing speed and category fluency, and higher afternoon cortisol levels with worse verbal memory scores. Loss of cortisol diurnal rhythm with higher afternoon cortisol levels was also associated with more executive dysfunction at 6 months.

These findings are broadly consistent with a different study of neuropsychological and cognitive outcomes of delirium after hip fracture, which tested patients three months after hip fracture (Witlox, Slor et al. 2013). They also excluded patients with syndromal delirium at follow-up, and found that 10/22 patients who had had delirium perioperatively had at least one positive CAM feature, compared with 3/26 of those who had not developed delirium. They found that the delirium group had poorer global cognition (Mini Mental State Examination), episodic memory (Fifteen Words Test) and more cognitive decline (IQCODE based on the past 3 months). In those with no CAM positive features, they also found poorer performance on the Fifteen Words Test, which is similar in structure to HVLT-R List Learning, than non-delirious controls (Witlox, Slor et al. 2013).

Over the year after hip fracture there was in fact a general trajectory of cognitive improvement. This was not in keeping with my hypotheses, and there are several potential reasons for this finding. This may be a learning effect due to the repeated administration of some of the same cognitive tests at the 3, 6 and 12 month follow-up assessments. As shown in section 9.3.5, those with persistent delirium performed worse on most cognitive tests. The numbers of those with persistent features fell with each wave of follow-up. Therefore some participants were likely still recovering from their delirium, and when tested at the later

waves at 6 and 12 months may therefore have performed better than in previous waves. Some participants died during the study period, and these participants were predominantly in the delirium group, so numbers in the delirium subgroups of many of the analyses fell as the study progressed. These factors combined may have led to the overall improvement seen in the trajectory analysis. Postoperative cognitive improvement has been suggested to occur in the literature, in the context of recovery from delirium or early transient postoperative cognitive dysfunction, or where surgery alleviates chronic pain or disease (Nadelson, Sanders et al. 2014). Neither the development of delirium during the perioperative period nor baseline cortisol measures including cerebrospinal fluid cortisol at baseline and salivary AM:PM cortisol ratio on postoperative day 4 influenced cognitive trajectory during the year after hip fracture. Crystallised intelligence as estimated by the National Adult Reading Test and dementia status did influence the intercept of the model, suggesting these factors may have a more powerful influence on the trajectory of cognition in this setting.

The hypotheses of the study have been difficult to test, for several reasons. Attrition of participants, predominantly in the delirium group, meant that those who survived to be tested tended to be fitter and less likely to have had severe delirium at baseline, and many of the frailer or more cognitively impaired participants in the study could not complete the full cognitive test battery. Some participants may have had new episodes of delirium in the intervening months between assessments, which they were recovering from when next seen. These factors which have all led to missing data in the cognitive test battery has meant that the full picture of the cognitive sequaelae of delirium after hip fracture may not be represented in this study. Undoubtedly the functional, physiological and cognitive trajectory of a proportion of patients with hip fracture who develop no, mild or brief delirium, is of improvement over the year after hip fracture. However, some patients who develop severe delirium with significant comorbid illness and complications and suffer prolonged hospital stays, are more likely to have a deteriorating cognitive trajectory, or to plateau at a new lower level. The design of this study, to recruit community-dwelling patients with hip fracture, aimed to minimise attrition in order to gain as full a follow-up dataset of cognitive and HPA axis measures as possible. However, it may be that this has meant that the influence of severe, prolonged delirium on cognitive trajectory could not be assessed here. It may also be that assessment of cognition alone does not capture the full breadth of central nervous system related outcomes that follow delirium. For example, psychological or emotional distress may persist as a consequence of the delirium experience, and psychomotor changes or dyspraxia

might contribute to slower recovery of mobility after delirium. Another limitation which needs to be discussed is that although patients intended for spinal anaesthesia were included in the study, for various reasons some went on to have general anaesthesia and some had both. Although a recent systematic review and meta-analysis did not find any significant influence of anaesthetic type on incidence of post-operative delirium, it did find that general anaesthetic conferred a marginal, but non-significant increased risk of postoperative cognitive dysfunction, with an odds ratio of 1.34 (95% confidence interval 0.93-1.95) (Mason, Noel-Storr et al. 2010). It is therefore possible that anaesthetic type may have had a small influence on the results of cognitive testing at follow-up, although persistent delirium, prior dementia and crystallised intelligence are likely still to have had a greater influence. Ideally, anaesthetic type might have been included in the trajectory analysis but this would not have been possible with the participant numbers in the present study.

Future studies could use a simpler and more inclusive study design and focus on one or two areas of cognition such as verbal memory, working memory or executive function, using a brief cognitive battery which could be tested preoperatively in patients with hip fracture, along with detailed capture of delirium, and follow these areas of cognition over the year after fracture. The influence of biomarkers measured in cerebrospinal fluid at baseline, medication exposure, perioperative hypotension or hypoxia could be examined. Elderly medical patients with delirium could be followed over the same timecourse as this study to see if the cognitive trajectory is the same, and to examine the HPA axis in a different cohort, using salivary cortisol measurement which is generally well accepted. Other elective surgical cohorts with a high risk of postoperative delirium such as cardiac or vascular surgery could be studied, with a detailed preoperative cognitive battery, perioperative delirium assessment, and postoperative assessment for persistent delirium and cognitive change. This could be combined with neuroimaging to explore the influence of regional or global cerebral atrophy or white matter change.

In summary, this study has shown that cortisol levels remain elevated up to three months post-hip fracture in those with persistent delirium features but without syndromal delirium, and suggests there may be ongoing loss of diurnal rhythm at three months. Persistent features of delirium are common after hip fracture, and these patients show impairments of working memory, verbal learning and memory, visual memory and executive dysfunction. After

delirium resolution, patients show impairment of verbal learning and memory. The overall cognitive trajectory demonstrated in this study was of improvement, which may be due to attrition of frailer and more cognitively impaired participants, recovery from delirium and a learning effect of the cognitive tests. Baseline cortisol measures and delirium status did not influence the cognitive trajectory in the subset of the sample with full prospective data.

10 General Discussion

The studies described in this thesis aimed to explore the relationships between the HPA axis and inflammation with delirium, persistent delirium and post-delirium cognitive change in patients aged over 60 with acute hip fracture. Specifically, I aimed to determine whether cortisol levels are elevated acutely, with loss of diurnal rhythm during delirium after hip fracture, whether this HPA axis dysfunction is sustained, and whether it predicts persistent delirium and deterioration in cognition over the year after hip fracture. This thesis also investigated whether CSF levels of cortisol, the CNS damage marker S100B, the Alzheimer's dementia marker tau and pro-inflammatory cytokines are elevated in delirium, and whether anti-inflammatory cytokines are reduced in delirium after hip fracture.

Since this was a cohort with acute hip fracture, there was consequent evidence of a stress and inflammatory response in the whole cohort, but this response was generally greater in those who developed delirium. This thesis has described evidence of HPA axis dysfunction in delirium, with elevated CSF cortisol in active delirium, elevated morning serum cortisol in the early postoperative period which was predictive of delirium, and loss of cortisol diurnal rhythm in the acute phase. Where delirium persisted, there was evidence of sustained HPA axis activity with elevated morning serum cortisol three months after hip fracture and a trend towards persistent loss of diurnal rhythm. There was little evidence of any change in CNS inflammatory response in delirium. However, in the periphery there was an acute shift towards a pro-inflammatory state in delirium, with persistent elevation of pro-inflammatory cytokines IL-6 and TNF-α and the chemokine IL-8 for several months after hip fracture in those who had developed delirium. There was also evidence of acute CNS damage and astrogliosis in delirium with elevated S100B in CSF, although this was not related to cortisol levels.

Since these studies were observational, causation cannot be inferred. It may be the case that those participants who were more unwell with greater trauma responses and less physiological reserve to withstand the insults of fracture and operation, had more dysfunctional HPA axis responses, loss of cortisol diurnal rhythm and a shift to a pro-inflammatory state, and developed delirium because they were more acutely unwell with

greater underlying co-morbidity. Where possible I adjusted for confounding factors such as age, illness severity, comorbidity and prior dementia, and cortisol remained independently predictive of delirium during the perioperative period. Cortisol and several of the inflammatory cytokines also correlated with delirium severity, suggesting that the more severe the delirium the more deranged the biology.The association between persistent delirium and persistent elevation of cortisol, and the persistence of a pro-inflammatory state with raised IL-6, IL-8 and TNF-α in those who had had delirium during the perioperative period also suggest that it is not just the acute insults that lead to these stress responses and also lead to delirium, but rather that HPA axis dysfunction and a pro-inflammatory state are among the underlying mechanisms of delirium. Indeed, several of the findings are biologically plausible mechanisms for delirium pathogenesis; within the CNS, elevation of cortisol and S100B may both lead to neurotoxicity with disruption of important cognitive attentional pathways. Disruption of cortisol diurnal rhythm and elevation in IL-6 could both plausibly contribute to sleep-wake cycle disturbance.

The relationship between delirium and post-delirium cognitive trajectory has been the most challenging to study and led to some unexpected results. Although at first glance the group who had had delirium perioperatively appeared to have performed worse on most cognitive tests, when the effect of persistent features of delirium was taken into account, those whose delirium had resolved only performed worse on tests of verbal and visual memory. The latter is a consistent finding in post-hip fracture delirium (Witlox, Slor et al. 2013), and both findings merit further investigation. The overall cognitive trajectory in this cohort was of cognitive improvement during the year after hip fracture, which was the opposite response to that hypothesised; I suspect that this was due to a combination of attrition of frailer and more cognitively impaired participants and recovery from delirium or early postoperative cognitive dysfunction. Those participants who did not develop syndromal delirium perioperatively were still exposed to the same insults which precipitated delirium in their peers, and may have been recovering cognitively in the same way that they were recovering physically and functionally during the year after fracture.

The studies described in this thesis have several important limitations which must be acknowledged. This was an observational study and causation cannot be inferred. Since I was the sole investigator for many aspects of the study, I could only recruit participants in an opportunistic manner. Since I performed all the delirium assessments during the perioperative period and it was not possible for me to do this every day, I may have missed some brief

episodes of delirium. I also performed many of the biological assays and may have introduced bias. The small numbers for some of the substudies mean that these must be viewed as exploratory and hypothesis-generating. The hypotheses in relation to cognitive trajectory following delirium were in particular difficult to test, due to attrition and missing data.

Through undertaking the studies in this thesis, my wider reading, discussion with other investigators in the delirium field, and my clinical practice, I have come to increasingly believe that delirium is a heterogeneous syndrome, and it seems unlikely that there is a single final common pathway in its pathogenesis. I believe that some episodes of delirium involve significant dysfunction of the HPA axis and some episodes involve a significant inflammatory response, which can be very persistent and damaging. Some episodes of delirium may have a specific trigger such as medication which can be reversed relatively quickly. Importantly, the clinical management of frail, older patients varies substantially, and so the balance of preventable risks for delirium such as dehydration, uncontrolled pain, etc., is different from patient to patient. This means that the relative contribution of HPA axis dysfunction and/or inflammatory pathways to a given patient's delirium varies according to the contribution of other risk factors. Additionally, the response of patients to similar insults is also heterogeneous, depending perhaps on their degree of frailty, polypharmacy (well-tolerated drugs when people are well which significantly exacerbate the effects of physiological insults when people become unwell), cognitive resilience and reserve or other unknown CNS factors. This may explain the inconsistent results particularly in recent CSF studies in delirium including the studies described in this thesis.

In the context of the wider literature, this thesis describes cohesively one of the most detailed longitudinal studies of the HPA axis and inflammation in delirium, and it is the largest study to have explored cortisol diurnal rhythm in delirium. One of the study's strengths has been that it has investigated both central and peripheral cortisol and inflammatory responses in delirium. The studies reported here will add significantly to the previously small literature on CSF biomarkers in delirium (Hall, Shenkin et al. 2011). It adds further detail to other studies which have found a pro-inflammatory milieu in delirium (van Munster, Korevaar et al. 2008, van den Boogaard, Kox et al. 2011), and that cortisol is predictive of delirium (Mu, Wang et

al. 2010, Nguyen, Huyghens et al. 2014). It also expands our knowledge of the possible cognitive trajectories after delirium, with one of the most detailed assessments of the cognitive deficits in persistent and resolved delirium which has potentially important implications for rehabilitation, follow-up and decision-making following delirium. It has several implications for future studies and clinical practice, which will be discussed in Section 10.1.

10.1 Implications for clinical practice and future work

The results described in this thesis have several important implications for clinical practice, and new avenues for future studies have also been revealed, both in delirium pathophysiology and potential new treatments.

10.1.1 Implications for clinical practice

In terms of clinical practice, the demonstration of raised CSF cortisol and S100B in active delirium is important, given that both mechanisms are potentially damaging to the CNS in the longer term if sustained, and reinforce the idea that delirium itself may be dangerous, or a marker of a dangerous process which should be prevented, mitigated and managed urgently. In the future, it might be possible to incorporate collection of a small CSF sample during routine care for high risk procedures under spinal anaesthetic, with analysis of biomarkers to predict those at high risk of delirium, persistent delirium or incipient dementia.

Both prevalent and incident delirium were associated in this study with longer waiting time for operation, highlighting the importance of avoiding long waits for surgery in patients with acute hip fracture and other settings where elderly patients undergo urgent surgery. Measurement of cortisol diurnal rhythm using blood or saliva samples would be low risk and generally well tolerated in delirium, and might give very useful information on those patients who have particular disruption of the HPA axis. Indeed, the findings of this study suggest that those with delirium, particularly where it persists, may have a form of catabolic state with elevated cortisol and a sustained pro-inflammatory milieu. Therefore, these patients are likely to benefit clinically from attention to good sleep hygiene, nutrition, skin integrity, wound healing and physiotherapy. It would also be prudent to avoid the prescription of steroids unless absolutely necessary.

The demonstration of executive dysfunction, and deficits in verbal and visual learning and memory in those with persistent features of delirium has potentially very important implications for clinicians, nursing staff and allied health professionals caring for these patients and those involved in making decisions about their future care. Attention should be paid to the way information is presented, minimising distraction and maintaining clarity,

information and instructions should be repeated where necessary, and patients should be given both adequate time to respond and also in more general terms, adequate time to recover. In particular, the persistence of executive dysfunction even after syndromal delirium has resolved means that patients may have difficulty participating in complex rehab tasks and in making life-changing decisions about their future care. Given the trajectory of cognitive improvement demonstrated here for some patients, these deficits may improve over several months, meaning that decisions should not be taken too early or should be kept under review. Patients recovering from delirium should have follow-up assessment of their cognition, with particular attention paid to the deficits highlighted here, not only to seek evidence of underlying dementia but also to seek signs of improvement.

10.1.2 Future studies

Cerebrospinal fluid studies in delirium are costly and difficult to perform, with several practical and ethical challenges. I approached almost three times as many patients as I recruited, and samples were collected from 60% of the final cohort, with several hours of researcher time for each successful recruit. Due to the time pressures of hip fracture repair, there were huge challenges in gaining informed consent, performing preoperative delirium assessments and arranging for sample collection when the time window was often only a few hours, and the urgent and unpredictable nature of the trauma theatre list was an added challenge. The collaborative nature of the recent studies of cerebrospinal fluid biomarkers in delirium, including those described in this thesis, between centres in Scotland, The Netherlands, Norway and Australia, has greatly increased our understanding of delirium pathophysiology, but there is still much more work on CSF needed. An international collaborative biobank could maximise the potential of these difficult to obtain and precious samples. In future it might also be possible to operationalise collection of a small sample of cerebrospinal fluid during routine clinical care where spinal anaesthetic is performed for high risk surgical procedures, with consent built-in to the main surgical consent form. These samples could also be biobanked and linked with diagnostic data from ISD (Information Services Division) on postoperative delirium and dementia

There are several other lines of enquiry which could be followed in CSF biomarker research in delirium. Amino acids such as the serotonin precursor tryptophan, the dopamine and

norepinephrine precursors tyrosine and phenylalanine and glutamate could be investigated, to test the neurotransmitter hypothesis of delirium (Flacker and Lipsitz 1999). Prostaglandins, which are small inflammatory molecules whose synthesis is induced by the action of pro-inflammatory cytokines such as IL-1β at the blood brain barrier would also be interesting to investigate as an important branch of the inflammatory cascade. Given that there is preliminary evidence that incident dementia that follows delirium may have a different mechanism to "standard" dementia neuropathology (Davis, Muniz Terrera et al. 2012), further investigation is warranted into potential mechanisms for this and CNS injury during and after delirium. For example, nucleosomes are strands of cell-free DNA, and their presence is thought to represent a pro-inflammatory response and cell death. After hip fracture, there is a rise in nucleosomes in serum, with different levels before, during and after delirium (de Rooij, Zeerleder et al. 2014); it could be hypothesised that levels would also rise in CSF in delirium.

Studies of delirium pathophysiology sometimes produce contradictory or counterintuitive results. Future studies investigating delirium mechanisms should include detailed aetiological characterisation of the predisposing and precipitating factors in the delirium episode. A standardised risk factor model for assigning a delirium episode to one or more aetiological classes would be useful, which could be used across delirium studies. This might mean that more firm conclusions can be drawn on the mechanisms of different aetiological classes of delirium, and limit the contradictory results found in several areas of delirium pathophysiological research. Performing research studies in units whose baseline practice involves good delirium prevention and management will help to reduce the influence of simple direct causes of delirium such as opioid toxicity, therefore improving the signal-noise ratio when examining particular biomarkers.

Hospitalisation, particularly in the frail elderly and even more so following trauma such as hip fracture, is a stressful experience, with patients in unfamiliar surroundings, in pain and fearful of the need for surgery. Delirium compounds this stressful and distressing experience with disorientation, delusions and hallucinations (Partridge, Martin et al. 2012). Psychological stress for example the Trier Social Stress Test leads to cortisol elevation, with higher levels in older women and more elevation in salivary cortisol in older men (Kudielka,

Buske-Kirschbaum et al. 2004). This psychological distress might therefore contribute to the stress response, leading to more severe or more prolonged delirium. A study of how to improve patient experience in conditions such as hip fracture, with quantifcation of cortisol diurnal rhythm and delirium, would be very interesting.

The evidence for dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in delirium and persistent delirium has been further strengthened by the results of the studies described here. A randomised placebo-controlled clinical trial of an 11β-hydroxysteroid dehydrogenase inhibitor, to limit the effects of the dysregulated HPA axis response, could be undertaken. Given the pro-inflammatory milieu in delirium, there might also be a role for anti-inflammatory medication, which would address not only potential mechanisms in delirium but also the acute injury and trauma response, which may be more exaggerated and damaging in the frail elderly. These drugs could either be trialled prophylactically in high-risk groups such as patients with hip fracture or critical illness, or could be started in confirmed delirium to limit its duration. Outcome measures could include delirium duration and severity, with parallel assessment of cortisol diurnal rhythm and inflammatory profile.

The studies described in this thesis therefore add significantly to the literature on the role of the HPA axis and inflammation in delirium, in particular within the CNS, providing evidence of potential CNS damage, elevated CSF and peripheral cortisol during delirium and a persistent HPA axis and inflammatory response that follows delirium. This thesis also describes cognitive deficits in persistent and resolved delirium which have the potential for particular clinical importance and should be further investigated. New avenues for research and potential therapeutic targets have been identified which may be of great benefit in this common and potentially devastating syndrome.

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11.1 Appendix 1 Schedule of assessments CDHIP cohort

Timepoint	Preoperative visit	Perioperative visits days 1, 2, 3, 4, 7, 10-14	Follow-up visits 3, 6, 12 months
Delirium assessment	Delirium Rating Scale-revised '98 Confusion Assessment Method Edinburgh Delirium Test Box-1 Observational Scale of Level of Alertness Richmond Agitation Sedation Scale	Delirium Rating Scale-revised '98 Confusion Assessment Method Edinburgh Delirium Test Box-1 Observational Scale of Level of Alertness Richmond Agitation Sedation Scale	Delirium Rating Scale-revised '98 Confusion Assessment Method Edinburgh Delirium Test Box-1 Observational Scale of Level of Alertness Richmond Agitation Sedation Scale
Cognitive assessment	Mini-Mental State Examination	Mini-Mental State Examination Informant Questionnaire of COgnitive Decline in the Elderly National Adult Reading Test	Mini-Mental State Examination Verbal Fluency Digit span forwards and backwards HVLT-R list learning Visual reproduction Victoria Stroop Digit Symbol Substitution Test
Assessment of function and other domains	Katz personal activities of daily living scale Lawton Instrumental activities of daily living scale Acute Physiology and Chronic Health Evaluation II Charlson Co-morbidity Index Rockwood Brief Frailty Score		Katz personal activities of daily living scale Lawton Instrumental activities of daily living scale Geriatric Depression Score EQ-5D Apathy Evaluation Scale
Biological samples	AM and PM saliva Serum at anaesthetic induction CSF at anaesthetic induction	AM and PM saliva days 4, 7, 10-14 Serum on days 4 and 10-14	AM and PM saliva 3, 6 and 12 months Serum at 3, 6 and 12 months

11.2 Appendix 2. Search strategy for systematic literature review of CSF biomarkers in delirium (chapter 3)

EMBASE and EMBASE classic search: 1564 abstracts on 5th January 2015

Database: 1947 to 2014 December 30

1. delir$.tw OR Delirium/ OR "acute confusional state".tw OR (acute adj3 conf$).tw OR "acute brain failure".tw OR Acute Confusion/ OR Confusion/
2. delirium tremens.ti
3. 1 NOT 2
4. cerebrospinal fluid/ OR cerebrospinal fluid.tw OR CSF.tw OR spinal puncture.mp OR lumbar puncture/ OR lumbar puncture.tw
5. 3 AND 4

Medline search: 347 abstracts on 5th January 2015

Database: 1946 to 5th January 2015

1. delir$.tw OR "acute confusional state".tw OR (acute adj3 conf$).tw OR "acute brain failure".tw OR exp Confusion/ (includes Delirium/)
2. delirium tremens.ti
3. 1 NOT 2
4. exp Cerebrospinal Fluid/ OR cerebrospinal fluid.mp OR csf.tw OR Spinal Puncture/ OR lumbar puncture.tw OR spinal puncture.tw
5. 3 AND 4

Psychinfo search: 75 abstracts on 5th January 2015

Database: 1806 to December week 5 2014

1. delir$.tw OR delirium/ OR mental confusion/ OR "acute confusional state".tw OR (acute adj3 conf$).tw OR "acute brain failure".tw
2. delirium tremens.ti
3. 1 NOT 2

4. csf.tw OR cerebrospinal fluid/ OR cerebrospinal fluid.tw OR lumbar puncture.tw OR spinal puncture.tw
5. 3 AND 4

Cochrane Library: 52 results using Pubmed search strategy on 5th January 2015, none relevant.

Web of science (including conference papers) 1864 – 2015-01-05: 1287 abstracts

Advanced search:

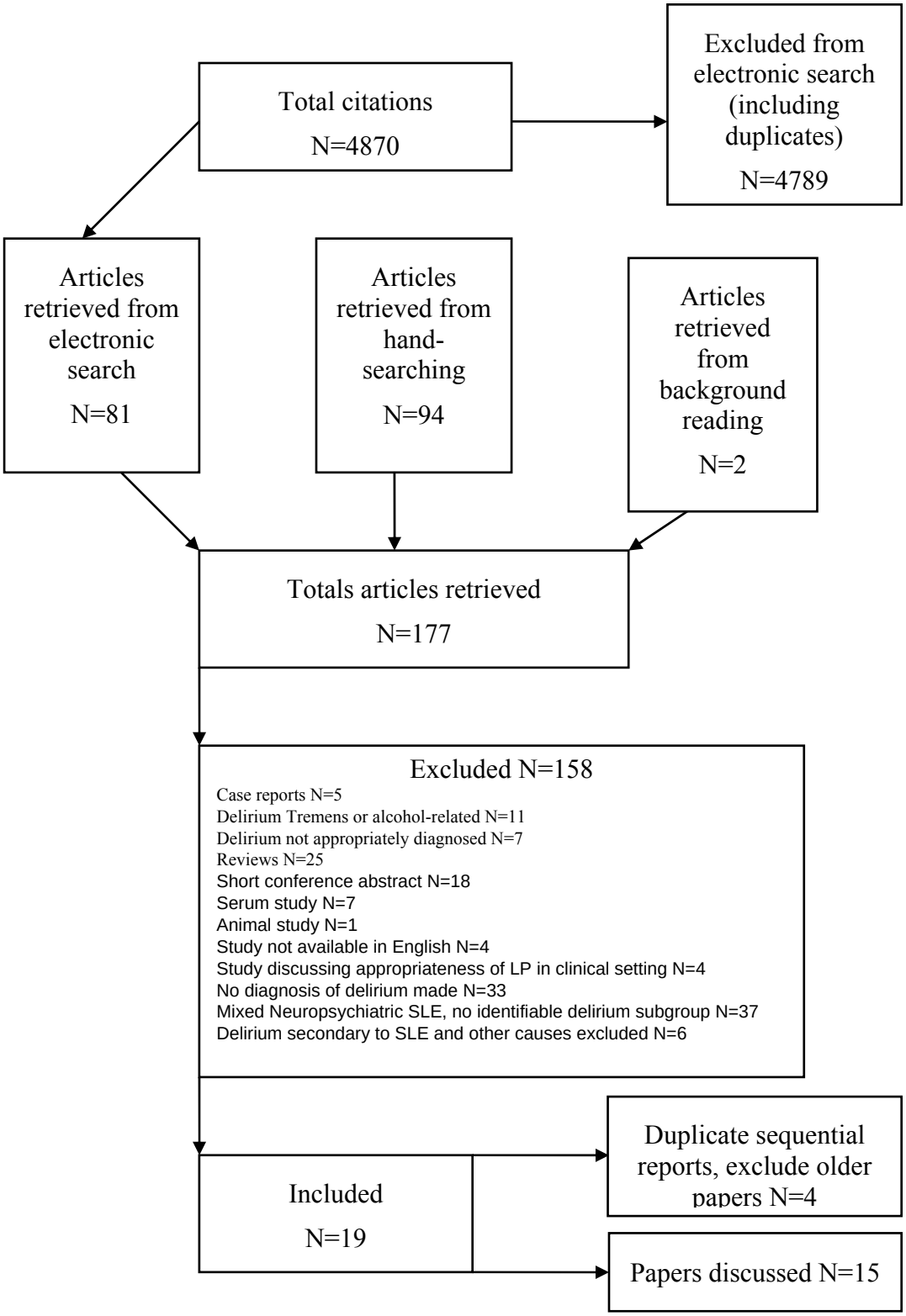
TS=(delirium OR "acute confusional state" OR confusion OR "acute brain failure" OR "acute confusion") AND TS=(CSF OR "cerebrospinal fluid" OR "spinal puncture" OR "lumbar puncture")

PubMed search: 1545 abstracts on 5th January 2015

(((delirium or "acute confusional state" or "acute brain failure" or "acute confusion") AND (csf or "cerebrospinal fluid" or "spinal fluid")) NOT ("case report" or "delirium tremens"))

11.3 Appendix 3 Flow diagram for systematic literature review of CSF biomarkers in delirium (chapter 3)

This flow diagram demonstrates the results of the electronic database searches, the bibliography searches and the study selection process.



11.4 Appendix 4 Patient Information Sheet and Consent Form

Version #6

Date 26th November 2009

PATIENT INFORMATION SHEET PART 1

Study title: Cortisol and delirium in patients with hip fracture

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and talk to others if you wish.

Part 1 tells you the purpose of the study and what will happen if you take part

Part 2 gives you more detailed information about the conduct of the study

Ask us if there is anything that is not clear or if you would like to know more. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

People who have had a hip fracture sometimes have short-term problems with thinking and memory after having surgery. This study is trying to find out more about what types of changes in thinking and memory happen after surgery. This is also an educational study contributing towards a PhD for Dr Roanna Hall.

Why have I been chosen?

We are asking you to take part simply because you have had a hip fracture and are over the age of 60.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this Part 1 and Part 2 of the information sheet to keep and be asked to sign a consent form. You can decide to stop your involvement at any time. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

What do I have to do?

You will be asked to do some standard short tests of thinking and memory. These tests will last around 15 minutes. We will also take specimens of saliva to measure the stress hormone cortisol. This involves chewing on a small cotton swab and placing it in a sterile plastic container. We will also examine your medical notes and speak to the ward nurses

and doctors about your case. With your consent we would also like to contact your GP to inform them that you are taking part in the study.

At the time of your operation when the spinal anaesthetic is administered, we will take a small sample of the fluid which bathes the brain and spinal cord, to measure levels of cortisol and markers of inflammation and damage. A blood sample will also be taken at that time.

We will also visit you after your surgery each day until the fourth day, and after one week and after ten days to two weeks to repeat the tests of thinking and memory. On the fourth day and after one week and after ten days to two weeks we will take further saliva samples. We will also take two blood samples to measure markers of inflammation.

After your discharge we will either invite you to come to Hospital for further tests of thinking and memory, or offer to visit you where you are living. With your permission we will contact your GP prior to arranging these meetings to check on your health at the time. We will assess your memory and thinking. We will also take saliva samples and a blood sample.

The blood and CSF samples will be stored anonymously for 5 years for future analysis. This is because with new scientific advances in the field of thinking and memory research, we will be keen to do new tests on the samples in the coming years, which will hopefully give us more information on the causes of thinking and memory problems. This will not include tests on DNA.

What are the possible disadvantages and risks of taking part?

There are minimal risks involved in the study, because we are not testing new treatments.

However, you may find doing memory tests upsetting or irritating if you are not sure of the answers.

What are the possible benefits of taking part?

With your permission, we will inform the medical and nursing team responsible for your care about the results of your tests. This may add more detailed information than they have already, and it may help you for them to know this.

What happens when the research study stops?

No further involvement is required.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or possible harm you might suffer will be addressed.

Will my taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept strictly confidential. The details are included in Part 2.

Contact Details

Dr. Roanna Hall, Clinical Research Fellow, Geriatric Medicine, Room S1642, New Royal Infirmary of Edinburgh. Tel. 0131 242 6481.

That completes Part 1 of the Information Sheet. You will be given a copy of Parts 1 and 2 of the Information Sheet and a signed consent form to keep.

Version #6

Date 26th November 2009

PATIENT INFORMATION SHEET PART 2

What if there is a problem?

Complaints:

If you have a concern about any aspect of the study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the complaints procedure in the hospital. Details can be obtained from the hospital.

Harm:

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal hospital complaints mechanisms will be available to you.

What will happen to the results of the research study?

The results will be published in international journals to help doctors and nurses across the world have a better idea of the causes of memory and thinking problems in patients who have had hip fracture. The results will also contribute towards the PhD thesis of Dr Roanna Hall.

Who is organising and funding the research?

Research into Ageing, the research department of Help the Aged is funding this study. The study is being organised by a team of doctors and researchers working in the Royal Infirmary of Edinburgh and the University of Edinburgh.

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. The information will be stored securely and any identifying information will be removed. The information will be retained for ten years and then disposed of securely.

Who has reviewed the study?

The study has been approved by the Scotland A Research Ethics Committee.

Version #6

Date 26th November 2009

Participant Identification Number for this study:

CONSENT FORM

Title of Project: Cortisol and delirium in patients with hip fracture

Name of Researcher:

Please tick box

1. I confirm that I have read and understand the information sheet dated ..... (version .....) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsibleindividuals from the University of Edinburgh or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records and to contact my GP to inform them of my participation in the study and to enquire about my health status prior to arranging follow-up visits in the future. ☐

4. I agree to the samples of blood and CSF that are taken being stored anonymously and analysed in future studies of thinking and memory (except DNA). ☐

5. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

11.5 Appendix 5 Katz and Lawton ADL scales

KATZ BASIC ACTIVITIES OF DAILY LIVING (ADL) SCALE		
	Independent	
	YES	NO
1. Bathing (sponge bath, tub bath, or shower) Receives either no assistance or assistance in bathing only one part of body		
2. Dressing - Gets clothes and dresses without any assistance except for tying shoes.		
3. Toileting - Goes to toilet room, uses toilet, arranges clothes, and returns without any assistance (may use cane or walker for support and may use bedpan/urinal at night.		
4. Transferring - Moves in and out of bed and chair without assistance (may use can or walker).		
5. Continence - Controls bowel and bladder completely by self (without occasional "accidents").		
6. Feeding - Feeds self without assistance (except for help with cutting meat or buttering bread).		

LAWTON - BRODY INSTRUMENTAL ACTIVITIES OF DAILY LIVING SCALE (I.A.D.L.)			
A. Ability to Use Telephone		**E. Laundry**	
1. Operates telephone on own initiative-looks up and dials numbers, etc.	1	1. Does personal laundry completely	1
2. Dials a few well-known numbers	1	2. Launders small items-rinses stockings, etc.	1
3. Answers telephone but does not dial	1	3. All laundry must be done by others	0
4. Does not use telephone at all	0		
B. Shopping		**F. Mode of Transportation**	
1. Takes care of all shopping needs independently	1	1. Travels independently on public transportation or drives own car	1
2. Shops independently for small purchases	0	2. Arranges own travel via taxi, but does not otherwise use public transportation	1
3. Needs to be accompanied on any shopping trip	0	3. Travels on public transportation when accompanied by another	1
4. Completely unable to shop	0	4. Travel limited to taxi or automobile with assistance of another	0
		5. Does not travel at all	0
C. Food Preparation		**G. Responsibility for Own Medications**	
1. Plans, prepares and serves adequate meals independently	1	1. Is responsible for taking medication in correct dosages at correct time	1
2. Prepares adequate meals if supplied with ingredients	0	2. Takes responsibility if medication is prepared in advance in separate dosage	0
3. Heats, serves and prepares meals, or prepares meals, or prepares meals but does not maintain adequate diet	0	3. Is not capable of dispensing own medication	0
4. Needs to have meals prepared and served	0		
D. Housekeeping		**H. Ability to Handle Finances**	
1. Maintains house alone or with occasional assistance (e.g. "heavy work domestic help")	1	1. Manages financial matters independently (budgets, writes checks, pays rent, bills, goes to bank), collects and keeps track of income	1
2. Performs light daily tasks such as dish washing, bed making	1	2. Manages day-to-day purchases, but needs help with banking, major purchases, etc.	1
3. Performs light daily tasks but cannot maintain acceptable level of cleanliness	1	3. Incapable of handling money	0
4. Needs help with all home maintenance tasks	1 0		
5. Does not participate in any housekeeping tasks			

11.6 Appendix 6 Geriatric Depression Score

Geriatric Depression Score

Choose the best answer for how you have felt over the past week:

1. Are you basically satisfied with your life? YES / **NO**
2. Have you dropped many of your activities and interests? **YES** / NO
3. Do you feel that your life is empty? **YES** / NO
4. Do you often get bored? **YES** / NO
5. Are you in good spirits most of the time? YES / **NO**
6. Are you afraid that something bad is going to happen to you? **YES** / NO
7. Do you feel happy most of the time? YES / **NO**
8. Do you often feel helpless? **YES** / NO
9. Do you prefer to stay at home, rather than going out and doing new things? **YES** / NO
10. Do you feel you have more problems with memory than most? **YES** / NO
11. Do you think it is wonderful to be alive now? YES / **NO**
12. Do you feel pretty worthless the way you are now? **YES** / NO
13. Do you feel full of energy? YES / **NO**
14. Do you feel that your situation is hopeless? **YES** / NO
15. Do you think that most people are better off than you are? **YES** / NO

Answers in bold indicate depression. Score 1 point for each bolded answer.

A score > 5 points is suggestive of depression.

A score >10 points is almost always indicative of depression.

Score _____/15

11.7 Appendix 7 EQ-5D

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

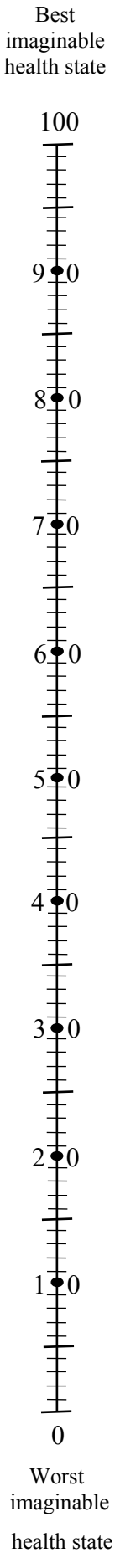
I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to Whichever point on the scale indicates how good or bad your health state is today.

Your own health state



11.8 Appendix 8 Permission to use "A systematic literature review of cerebrospinal fluid (CSF) biomarkers in delirium

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Cc: **Stalder-Frech, Franziska** f.stalder@karger.com, **Meier, Silvia** s.meier@karger.com

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Hopefully, I have been of assistance to you with the above.

Kind regards,
David Schaub

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11.9 Appendix 9 Permission to use "Levels of cerebrospinal fluid S100B in hip fracture patients with delirium versus controls"

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11.10 Appendix 10 Correlations among serum inflammatory markers

Table 8.10 Spearman's Rho correlations among cytokines and chemokines in serum at baseline, N=84

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	**rho=0.319 p=0.003**	**rho=0.254 p=0.020**	rho=0.205 p=0.061	**rho=0.350 p=0.001**	**rho=0.345 p=0.001**	**rho= -0.240 p=0.028**	rho=0.062 p=0.575	rho=0.079 p=0.477	**rho=0.304 p=0.005**
IL-1ra		**rho=0.329 p=0.002**	**rho=0.386 p<0.001**	**rho=0.571 p<0.001**	**rho=0.395 p<0.001**	rho= -0.205 p=0.061	**rho=0.518 p<0.001**	**rho=0.469 p<0.001**	**rho=0.525 p<0.001**
IL-5			**rho=0.234 p=0.032**	**rho=0.369 p=0.001**	**rho=0.341 p=0.001**	rho= -0.201 p=0.066	**rho=0.513 p<0.001**	**rho=0.437 p<0.001**	**rho=0.307 p=0.004**
IL-6				**rho=0.553 p<0.001**	**rho=0.578 p<0.001**	rho= -0.061 p=0.580	**rho=0.307 p=0.005**	**rho=0.323 p=0.003**	**rho=0.395 p<0.001**
IL-8					**rho=0.505 p<0.001**	rho= -0.183 p=0.095	**rho=0.514 p<0.001**	**rho=0.579 p<0.001**	**rho=0.536 p<0.001**
IL-10						rho= -0.044 p=0.692	**rho=0.478 p<0.001**	**rho=0.288 p=0.008**	**rho=0.603 p<0.001**
M1P-1α							rho= -0.114 p=0.302	rho= -0.161 p=0.143	rho= -0.047 p=0.673
MIP-1β								**rho=0.469 p<0.001**	**rho=0.451 p<0.001**
MCP-1									**rho=0.459 p<0.001**

Table 8.11 Spearman's Rho correlations among cytokines and chemokines in serum on postoperative day 4, N=83

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	rho=0.181 p=0.101	**rho=0.487** **p<0.001**	**rho=0.219** **p=0.046**	rho=0.215 p=0.051	**rho=0.385** **p<0.001**	**rho= -0.319** **p=0.003**	rho=0.062 p=0.578	rho= -0.018 p=0.872	rho=0.194 p=0.079
IL-1ra		**rho=0.590** **p<0.001**	**rho=0.329** **p=0.002**	**rho=0.393** **p<0.001**	**rho=0.318** **p=0.003**	**rho= -0.327** **p=0.003**	**rho=0.467** **p<0.001**	**rho=0.498** **p<0.001**	**rho=0.544** **p<0.001**
IL-5			**rho=0.382** **p<0.001**	**rho=0.411** **p<0.001**	**rho=0.508** **p<0.001**	**rho= -0.442** **p<0.001**	**rho=0.441** **p<0.001**	**rho=0.450** **p<0.001**	**rho=0.518** **p<0.001**
IL-6				**rho=0.399** **p<0.001**	**rho=0.455** **p<0.001**	**rho= -0.233** **p=0.034**	**rho=0.258** **p=0.018**	**rho=0.361** **p=0.001**	**rho=0.543** **p<0.001**
IL-8					**rho=0.463** **p<0.001**	rho= -0.158 p=0.155	**rho=0.538** **p<0.001**	**rho=0.711** **p<0.001**	**rho=0.640** **p<0.001**
IL-10						rho= -0.189 p=0.088	**rho=0.347** **p=0.001**	**rho=0.253** **p=0.021**	**rho=0.551** **p<0.001**
M1P-1α							rho= -0.092 p=0.407	**rho= -0.234** **p=0.033**	rho= -0.084 p=0.453
MIP-1β								**rho=0.540** **p<0.001**	**rho=0.541** **p<0.001**
MCP-1									**rho=0.591** **p<0.001**

Table 8.12 Spearman's Rho correlations among cytokines and chemokines in serum on postoperative day 10-14, N=34

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	rho=0.222 p=0.207	**rho=0.547 p=0.001**	**rho=0.339 p=0.050**	**rho=0.434 p=0.010**	rho=0.289 p=0.097	rho= -0.258 p=0.141	rho=0.186 p=0.292	**rho=0.343 p=0.047**	rho=0245 p=0.162
IL-1ra		rho=0.248 p=0.157	**rho=0.636 p<0.001**	**rho=0.469 p=0.005**	rho=0.297 p=0.088	rho= -0.243 p=0.167	**rho=0.530 p=0.001**	**rho=0.699 p<0.001**	**rho=0.565 p<0.001**
IL-5			**rho=0.356 p=0.039**	**rho=0.403 p=0.018**	**rho=0.457 p=0.007**	rho= -0.308 p=0.076	**rho=0.445 p=0.008**	**rho=0.343 p=0.047**	rho=0.094 p=0.595
IL-6				**rho=0.673 p<0.001**	**rho=0.373 p=0.030**	rho= -0.193 p=0.274	**rho=0.444 p=0.008**	**rho=0.509 p=0.002**	**rho=0.467 p=0.005**
IL-8					**rho=0.486 p=0.004**	rho= -0.232 p=0.187	**rho=0.564 p=0.001**	**rho=0.673 p<0.001**	**rho=0.403 p=0.018**
IL-10						rho= -0.084 p=0.638	**rho=0.453 p=0.007**	rho=0.192 p=0.278	**rho=0.404 p=0.018**
M1P-1α							rho= -0.249 p=0.156	rho= -0.259 p=0.139	rho= -0.180 p=0.308
MIP-1β								**rho=0.465 p=0.006**	**rho=0.377 p=0.028**
MCP-1									rho=0.336 p=0.052

Table 8.13 Spearman's Rho correlations among cytokines and chemokines in serum at 3 months after hip fracture N=80

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	rho=0.122 p=0.279	**rho=0.683 p<0.001**	rho=0.106 p=0.349	rho=0.166 p=0.142	**rho= -0.266 p=0.017**	**rho=0.307 p=0.006**	**rho=0.278 p=0.013**	rho= -0.123 p=0.276	**rho=0.426 p<0.001**
IL-1ra		rho=0.045 p=0.694	**rho=0.450 p<0.001**	**rho=0.313 p=0.005**	rho=0.175 p=0.120	**rho=0.235 p=0.036**	rho=0.110 p=0.331	rho= -0.116 p=0.307	rho=0.182 p=0.105
IL-5			rho= -0.110 p=0.333	rho= -0.026 p=0.181	**rho= -0.473 p<0.001**	rho=0.086 p=0.448	**rho=0.410 p<0.001**	**rho= -0.249 p=0.026**	**rho=0.354 p=0.001**
IL-6				**rho=0.373 p=0.001**	**rho=0.388 p<0.001**	**rho=0.310 p=0.005**	rho= -0.008 p=0.947	rho=0.026 p=0.817	**rho=0.303 p=0.006**
IL-8					rho=0.182 p=0.107	**rho=0.401 p<0.001**	rho=0.173 p=0.124	**rho=0.249 p=0.026**	**rho=0.418 p<0.001**
IL-10						**rho=0.258 p=0.021**	rho= -0.208 p=0.064	rho=0.166 p=0.142	rho=0.089 p=0.431
M1P-1α							rho=0.205 p=0.068	rho=0.039 p=0.731	**rho=0.440 p<0.001**
MIP-1β								rho= -0.072 p=0.524	**rho=0.236 p=0.035**
MCP-1									rho=0.043 p=0.704

Table 8.14 Spearman's Rho correlations among cytokines and chemokines in serum at 6 months after hip fracture, N=73

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	**rho=0.259 p=0.027**	**rho=0.306 p=0.008**	**rho=0.519 p<0.001**	**rho=0.285 p=0.014**	**rho=0.444 p<0.001**	**rho=0.324 p=0.005**	rho=0.145 p=0.221	rho=0.127 p=0.284	**rho=0.446 p<0.001**
IL-1ra		rho=0.077 p=0.518	**rho=0.502 p<0.001**	rho=0.139 p=0.241	**rho=0.315 p=0.007**	**rho=0.278 p=0.017**	rho=0.045 p=0.706	rho= -0.067 p=0.575	**rho=0.308 p=0.008**
IL-5			**rho=0.473 p<0.001**	**rho=0.347 p=0.003**	rho= -0.014 p=0.905	rho=0.031 p=0.795	rho= -0.120 p=0.312	rho=0.087 p=0.465	**rho=0.335 p=0.004**
IL-6				**rho=0.343 p=0.003**	**rho=0.270 p=0.021**	**rho=0.356 p=0.002**	rho=0.011 p=0.923	rho=0.094 p=0.431	**rho=0.511 p<0.001**
IL-8					rho=0.109 p=0.359	rho=0.119 p=0.315	rho=0.024 p=0.842	rho=0.167 p=0.157	**rho=0.420 p<0.001**
IL-10						**rho=0.232 p=0.049**	rho= -0.034 p=0.776	rho=0.125 p=0.291	**rho=0.530 p<0.001**
M1P-1α							rho=0.083 p=0.488	**rho=0.241 p=0.040**	**rho=0.325 p=0.005**
MIP-1β								rho=0.168 p=0.155	rho=0.016 p=0.892
MCP-1									rho=0.128 p=0.280

Table 8.15 Spearman's Rho correlations among cytokines and chemokines in serum at 12 months after hip fracture, N=68. Interleukin-5 was detected in small amounts, and correlations were not possible.

	IL-1ra	IL-5	IL-6	IL-8	IL-10	M1P-1α	MIP-1β	MCP-1	TNF-α
IL-1β	rho=0.111 p=0.367		rho=0.218 p=0.074	**rho=0.302 p=0.012**	**rho=0.249 p=0.040**	rho=0.168 p=0.171	rho=0.095 p=0.439	rho=0.218 p=0.074	rho=0.237 p=0.052
IL-1ra			**rho=0.445 p<0.001**	**rho=0.254 p=0.037**	rho=0.034 p=0.785	rho=0.048 p=0.695	rho=0.076 p=0.537	rho=0.002 p=0.985	rho=0.225 p=0.065
IL-5									
IL-6				**rho=0.399 p=0.001**	rho=0.163 p=0.185	rho=0.113 p=0.359	rho= -0.019 p=0.879	**rho=0.262 p=0.031**	**rho=0.300 p=0.013**
IL-8					rho= -0.039 p=0.751	rho=0.086 p=0.486	rho=0.183 p=0.135	**rho=0.310 p=0.010**	**rho=0.387 p=0.001**
IL-10						rho= -0.030 p=0.806	rho= -0.218 p=0.075	rho= -0.158 p=0.197	rho=0.238 p=0.051
M1P-1α							**rho=0.262 p=0.031**	rho=0.159 p=0.197	rho=0.057 p=0.646
MIP-1β								rho=0.189 p=0.122	rho=0.119 p=0.334
MCP-1									rho=0.144 p=0.241