PERIPHERAL AND CENTRAL MARKERS
OF INFLAMMATION IN MILD COGNITIVE
IMPAIRMENT

A thesis submitted to the University of Manchester for the degree of MD
in the Faculty of Medical and Human Sciences

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Abstract

The University of Manchester

Name: Dr Salman Karim

Degree: MD in the Faculty of Medical and Human Sciences

Title: Peripheral and central markers of inflammation in mild cognitive impairment.

There has been accumulating scientific evidence, over the last three decades, of the role of inflammatory processes in the development of Alzheimer’s disease (AD). Population based studies suggest that plasma levels of inflammatory markers are raised in peripheral blood of people with AD. People on long term use of non-steroidal anti-inflammatory drugs have a lower prevalence of AD. Moreover, both animal and human histopathology studies have reported localization of inflammation in brain areas primarily affected by AD pathology. Areas of increased inflammation can be visualized in vivo by Positron Emission Tomography (PET) scans using the PK11195 ligand that binds with the benzodiazepine receptor sites of activated microglial cells. Cognitive decline in AD has been shown to correlate with levels of microglial activation using PK11195 PET scans. People with amnestic mild cognitive impairment (MCI) are known to be at high risk of developing AD. We aimed to investigate the association between peripheral and central markers of inflammation and cognitive decline in a group of people with amnestic MCI.

MCI subjects (n=70) underwent cognitive testing, IL-6 and CRP in peripheral blood were measured and repeated after 1 year. A subgroup (n=15) was followed up for another year and central brain microglial activation was measured by PET using PK11195 along with cognitive and peripheral inflammatory marker measurement.

The mean CRP and IL-6 levels of the cohort increased over one year but the rise was only significant for CRP. No association was detected between inflammatory markers levels and cognition as measured by a battery of cognitive instruments. Group comparisons of the PET cohort with healthy controls (n=5) showed increased PK11195 binding (mean binding potential) in frontal lobe, temporal lobe, parietal lobe, putamen, occipital lobes and significantly increased binding in posterior cingulate gyrus.

This study, to our knowledge, is unique in studying makers of inflammation in amnestic MCI participants both in peripheral blood and brain. The results of this study, in the light of current literature, add to the importance of recognition of inflammatory processes in people at risk of developing AD. The results suggest that CRP levels rise significantly over time and are detectable in peripheral blood by using practically simple laboratory techniques. The results also suggest that activated microglia in amnestic MCI patients can be visualized in vivo by using PK11195 PET scans and show higher levels of activation as compared to healthy controls. These finding could be useful in identifying people with malactivated (pro-inflammatory) microglia as potential targets for prevention/early treatment strategies. Further studies with larger samples sizes and long term follow-up are needed to investigate whether these peripheral and central inflammatory markers could shed light on the aetiology of AD and be useful in monitoring disease progression.
**Declaration**

The data presented in this thesis form part of a collaborative clinical study of peripheral and central inflammation in people with mild cognitive impairment funded by the Medical Research Council (MRC). Prof. Alistair Burns (AB) was the principal investigator and Pippa Tyrrell (PT), Steve Hopkins (SH), Nitin Purandari (NP) and Salman Karim (SK) were co-applicants. The study was conducted in conjunction with a research nurse, Jackie Crowther (JC). The author of this thesis (SK) contributed to the study design and protocol.

He wrote the demographic information proforma, patient/GP information letters and corresponded with the local ethics committee to get ethical approval for the study. SK corresponded with the MRC to submit annual reports and gaining extensions for the study. SK identified and recruited the patients for the study at two sites (Wythenshawe Hospital and Stepping Hill Hospital) where he was working as a clinical lecturer and specialist registrar. SK performed the initial assessments, blood tests and follow-up assessments with JC. SK collaborated with Prof. Alan Jackson (Neuro-radiologist) for writing up the MR protocol. SK collaborated with colleagues at the Wolfson Molecular Imaging Centre (WMIC), Rainer Heinz (RH) and Karl Hereholz (KH) for writing the PET scan protocol. The inflammatory marker essays were conducted by SH and his colleagues at Hope hospital. SK analysed the demographic, blood and neuropsychological with the help of Andy Vail (AV), Julie Morris (JM) and SH. SK and JC organized the PET and MR scans. The PET data was analysed by Zhangjie Su and Alex Gerhard at the WMIC and SK coordinated the results of the clinical, lab and imaging assessments.

No portion of the work referred to in the thesis has been submitted in support of application for another degree or qualification of this or any other university or other institute of learning.
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I would like to thank Professor Alistair Burns, Dr Pippa Tyrrell and Dr Stephen Hopkins for their support and guidance through all stages of this project. I would also like to thank and acknowledge the valuable contribution of Mrs Jackie Crowther as a research nurse in the recruitment, assessments and organization of this project. I am grateful to Dr Zhangjie Su and Dr Alex Gerhard for their PET data analysis. I am also grateful to Mr Andy Vail and Miss Julie Morris for their advice with the statistical analysis. I would like to acknowledge the help offered by the ‘Memory Clinic’ team at the Wythenshawe Hospital (Manchester Mental Health and Social Care Trust) and Dr Steven Bradshaw and his team at the Old Age Psychiatry department in Stepping Hill Hospital (Pennine Care NHS Foundation Trust) for recruitment for this study.

Finally I would like to thank all the participants and their families who agreed to take part in this study.
Dedication

I would like to dedicate this thesis to my wife for her love and support, to my parents who instilled in me the love of learning and to my children who make my life worthwhile.
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<td>AD</td>
<td>Alzheimer’s disease</td>
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<td>APP</td>
<td>Amyloid precursor protein</td>
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<td>BMI</td>
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<td>CAMCOG-R</td>
<td>Cambridge Cognitive Examination –Revised</td>
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<td>COX</td>
<td>Cyclo-oxygenase</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CSF</td>
<td>cerebro-spinal fluid</td>
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<td>DLB</td>
<td>Dementia Lewy Body</td>
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<td>ELISA</td>
<td>Enzyme linked immuno-sorbent assay</td>
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<td>FDG</td>
<td>Fluro-deoxyglucose</td>
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<td>IL-6</td>
<td>interleukin – 6</td>
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<td>IL-I</td>
<td>interleukin - 1</td>
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<td>LPS</td>
<td>Lipopolysaccharides</td>
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<td>LREC</td>
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<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<td>MRC</td>
<td>Medical Research Committee</td>
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<td>NART</td>
<td>National Adult Reading Test</td>
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<td>Non-steroidal anti-inflammatory drugs</td>
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<td>PBM'C</td>
<td>Peripheral blood mononuclear cells</td>
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<td>PET</td>
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<td>RAGE</td>
<td>Recepter for Advanced Glycation End</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SPECT</td>
<td>Single photon emission tomography</td>
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<td>Acronym</td>
<td>Description</td>
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<td>SPM</td>
<td>Statistical Parametric Mapping</td>
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<td>TIA</td>
<td>Transient ischemic attack</td>
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<td>TNF-α</td>
<td>Tumour necrosis factor - alpha</td>
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<td>VaD</td>
<td>Vascular dementia</td>
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<td>WMIC</td>
<td>Wolfson Molecular Imaging Centre</td>
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Chapter 1: Introduction
Introduction

The importance of inflammatory processes in the aetiology of Alzheimer’s disease (AD) and other neurodegenerative disorders is increasingly recognised. The concept that neuro-inflammation is associated with psychiatric illness is not new and was first suggested by Wagner-Jauregg in 1887 (Edward Shorter, 1997). He hypothesised that infection inducing agents could be used for treating psychiatric illnesses and studied the possible role of infection with malarial parasite as a therapy for dementia paralytica. He was subsequently awarded the first Nobel Prize for psychiatry in 1927 for introducing the unique concept of the role of inflammation in the causation and treatment of psychiatric illness. Lack of availability of sophisticated histo-pathological and neuro-chemical technology probably thwarted further developments at that time.

The last decade has, however, seen a resurgence of interest in the role of neuro-inflammatory processes in the aetiology of neurodegenerative disorders in general and AD in particular. Evidence from experimental studies, both animal and human, suggests that inflammation plays a fundamental role in the pathogenesis of AD. (Eikelenboom et al 2002; McGeer et al, 2007; Tan and Seshadri, 2010).

Search strategy for the literature review

I searched PubMed, Google scholar, Medline and the Cochrane Library for all relevant articles using the terms ‘Alzheimer’s disease’, ‘mild cognitive impairment’, ‘inflammation’, ‘Non steroidal anti-inflammatory agents’ and ‘PK11195 PET scans’. The search outcome was then divided into review and original research articles and recorded on two spread sheets. The review articles were then classified on the basis of subject, authors/research groups, year and journal of publication. The original research articles were classified on similar criteria along with sample size and outcome (both positive and negative outcome studies were included).
Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) is a term used to describe a syndrome where an individual presents with cognitive decline greater than expected from age and educational level but which does not interfere with every day activities (Gauthier et al, 2006). Clinically, the person presents with cognitive problems, corroborated by an informant, with an objective evidence of cognitive impairment but no evidence of dementia, in the absence of any other illness likely to be the cause of the symptoms. Epidemiological studies of MCI, based on this broad definition, have reported prevalence figures, in the elderly population, ranging from 3 to 19% and a risk of developing dementia of 11 to 33% over 2 years (Ritchie et al, 2004). A study done at the Mayo clinic on a cohort of older people (mean age 79 years) showed a 12% conversion rate to dementia per year in those with MCI as compared to 1-2% per year for the rest of the cohort (Petersen et al, 2004). Nearly 80% of the MCI cohort converted to dementia when followed up for 6 years. These studies suggest that MCI is a pathological condition and not part of normal ageing. However, other population based studies have reported that up to 44% of people with MCI become normal after a year (Ritchie et al, 2004; Ganguli et al, 2004) yet others remain stable, highlighting the fact that reversible factors such as depression and use of anticholinergic drugs could be affecting the cognitive performance of older people presenting with MCI (figure1.1; figure 1.2).
Figure 1.1: Progression of people from normal functioning to Alzheimer’s Disease
(Modified from W.B. Saunders).
Clinically MCI can be classified into amnestic, multiple domain and single non-memory domain types depending on the area of cognitive deficits with which the patient presents (Petersen, 2004). Amnestic MCI is characterized by memory complaints corroborated by an informant, memory impairment relative to age and education, general cognitive decline, largely intact activities of daily living and no evidence of dementia. Non-amnestic type MCI presents with cognitive problems in other cognitive domains such as visuo-spatial deficits (figure 1.3). Studies on amnestic MCI have reported the highest rates of progression to AD as compared to the general elderly population. In a 3 year multicenter randomized trial, Petersen et al, (2005) reported a conversion rate of 16 % per year and this was similar to other studies conducted previously (Grundman et al, 2004). One study (Geslani et al, 2005) reported a conversion rate of 41% after 1 year and 64% after 2 years.

Figure 1.2: Conversion of people having a mild cognitive impairment to dementia over 6 years. Approximately 80% convert to dementia during this time. (Modified from Petersen et al, 2004).
Figure 1.3: Progression of the sub-types of mild cognitive impairments to dementia (Modified from Petersen et al, 2001).

Amnestic MCI, can be considered to represent the transitional period between normal ageing and very early AD, in other words, a pre AD stage (Burns and Zaudig, 2002). Studies on this high risk group provide the opportunity to develop early diagnosis and prevention strategies for AD.

**Inflammation**

Inflammation is defined as a reaction of living tissue to injury (Robins et al 1981). The injurious agent can be foreign, like a virus, or part of self, for example a necrotic cell. Inflammation encompasses the spectrum of patho-physiological responses generated by the immune system as a response to tissue injury. The inflammatory response is a series of coordinated and regulated chemical and cellular events culminating in repair and resolution. Sometimes, however, inflammation becomes excessive and persistent resulting in acute
and chronic tissue injury and this process is known to play an important role in the pathophysiology of a number of diseases (Nathan, 2002).

Inflammation has been traditionally divided into acute and chronic types and involves innate and adaptive immune responses. Innate immune response is characterized by activation of macrophages, natural killer cells, the complement system and numerous cytokines and chemokines (Suffredini et al, 1999, Akiyama et al, 2000). The adaptive immune response uses T and B lymphocytes and specific antibodies.

The process of inflammation can be described as a complex series of interactions between cells responding to the insult/injury. These interactions are coordinated by numerous inflammatory mediators including acute phase proteins, cytokines, antibodies, complement proteins, kinins, histamine, nitric oxide, clotting proteins and lipid mediators including leukotrienes, prostaglandins and platelet activating factor.

Acute phase proteins are released in the acute phase of the inflammation and although a number of them have been described, C-reactive protein (CRP) is the most commonly studied. CRP is regarded as a sensitive marker of the acute phase of inflammation and its plasma concentration can be easily measured.

Cytokines are a large group of regulatory peptides of inflammation. They are up regulated by mono-nuclear cells or lymphocytes in response to inflammatory stimuli. They exert their effect on target cells by interacting with specific receptors and inducing gene expression (Arend, 2002). There are three main groups/families of cytokines including the interleukin- 6 family (IL-6), interleukin-1 family (IL-1) and tumour necrosis factor (TNF- \( \alpha \)). These play a key role as early mediators of inflammation and are also involved in regulating multiple steps in regulating the inflammatory response. IL-1 and TNF-\( \alpha \) act locally but IL-6 is released in the peripheral circulation to act at distant sites. Plasma levels of IL-6 can be measured quite accurately by using immunoassays such as enzyme-linked
immunosorbent assay (ELISA) but IL-1 and TNF-α cannot be detected consistently (Hopkins, 1995).

Cytokines are of particular relevance in clinical research as they have been implicated in a number of neuro-degenerative disorders including AD (Hirsch et al, 2003; Bermajo et al, 2008).

**Neuro-inflammation**

Inflammatory reactions in the brain are different from those in the periphery. This is because of to the brain’s unique innate defence system comprising of glial cells (microglia, astrocytes) and its isolation from the rest of the body’s immune system due to the blood brain barrier. Inflammation in the brain is characterised by activation of microglia and astrocytes, and as in the periphery, is rapidly followed by release of a host of inflammatory mediators (Lucas et al, 2006).

The term neuro-inflammation is generally used to denote the inflammatory reaction characterized by the innate immune response involving activated microglia and astrocytes in the brain. Neuro-inflammation results in chronic and sustained cycles of injury and response by microglia and inflammatory mediators, for example, complement proteins, cytokines and chemokines. Emerging evidence in the field of neurobiology suggests that the cumulative effect of chronic activation of glial cells, particularly microglia, results in the maintenance and worsening of the disease process (Streit et al 2004).

Neuro-inflammatory mechanisms are implicated in the aetiology and pathogeneses of AD and other neurodegenerative diseases, for example multiple sclerosis, Parkinson’s and Huntington’s disease (Akiyama et al 2000, Lue et al, 2001, Streit et al 2004).
The amyloidal cascade/neuro-inflammation hypothesis for Alzheimer’s disease

Amyloid deposition has been suggested as the catalyst for a cascade of damage starting from the deposition of extracellular deposits of beta amyloid (Aβ) leading to neurofibrillary tangle (TNF-α) formation and ultimately resulting in neuronal death (Hardy and Allsop 1991; Selkoe 1996). This concept has become more refined with the growing evidence of the role of neuroinflammation and it is suggested that the Aβ deposits lead to activation of microglial cells which produce neurotoxic substances such as reactive oxygen and nitrogen species, proinflammatory cytokines, complement proteins and other inflammatory mediators that bring about neurodegenerative changes (Akiyama et al 2000, Eikelenboom et al 2002, Streit et al 2004, Magaki et al, 2007).

The clinical importance of amyloid hypothesis is twofold. First, it opens a wide range of avenues to be explored for developing pharmacological treatments (in contrast to the current limited approach of targeting neurotransmitters) ranging from developing vaccines for Aβ to the use of anti-inflammatory drugs (Akiyama et al 2000). Second, there is potential for early diagnosis in people at risk (MCI) or in the very early stage of the disease with laboratory or imaging techniques to detect and localise early signs of neuroinflammation. Moreover as Aβ deposition is also seen in normal ageing and other dementias such as Dementia with Lewy bodies (DLB), an understanding of the role of neuro-inflammation could lead to the development of new pharmacological interventions in these conditions (Figure 1.4).

The evidence for the role of neuroinflammation in AD comes from a wide range of research areas including post-mortem histo-pathological studies, animal model studies, genetic and gene expression studies, epidemiological studies on use of anti-inflammatory agents, studies on the biological markers of inflammation and their association with AD, and more recently neuro-imaging studies aiming at studying evidence of inflammation in brains of living subjects.
Neuro-inflammation in Alzheimer’s disease

The extra-cellular deposition of Aβ in the form of amyloid plaques in AD triggers a chronic inflammatory reaction (Akiyama et al, 2000, Wyss-Coray and Mucke, 2002; Lucas et al, 2006). The bulk of evidence comes from a number of in vitro studies showing that Aβ aggregates can activate a variety of inflammatory pathways. For example, Aβ deposition is well known to trigger microglial activation (Sheng et al, 1998) by binding to the Receptor for Advanced Glycation End products (RAGE), scavenger receptors and signalling receptors such as CD40 (Yan et al, 1999; Paresce et al, 1996; El Khouray et al,
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1996; Tan et al, 1999). Aβ fibrils can also bind to RAGE on neurons resulting in the release of inflammatory factors (Yan et al, 1999). Aβ deposits have been shown to activate the complement system in vitro through the classical pathway by binding C1q and through the alternative pathway by binding C3b (Rogers et al, 1992; Webster et al, 1997; Bradt et al, 1998). Shen et al (2001) showed that neuro fibrillary tangle preparations from human AD brains can also activate the complement pathway. The role of different inflammatory pathways and their importance as biological markers will be discussed in the next section.

Cytokine Pathways

Cytokines are proteins which act as chemical messengers between the immune cells stimulating or inhibiting their growth and activity both in brain and periphery. Their persistently increased levels in plasma can be an indication of a chronic inflammatory process including AD. Most commonly studied cytokines includes interleukin -1 (IL-1), interleukin- 6 (IL-6) and tissue necrosis factor alpha (TNF-α).

IL-1 has been shown to be over expressed in plaque formation areas of AD brains (Griffin et al 1995). The over expression of IL-1 occurs even in the early stages of plaque formation (Griffin et al 1998) and also promotes the production of amyloid precursor protein (APP) thus suggesting its possible role in initiating the cycle of excessive amyloid deposition leading to plaque formation that in turn activates microglia to further IL-1 formation (Goldgaber et al 1989; Barger et al 1997).

IL-6 is another important mediator of immune responses in the central nervous system. It is barely detectable in normal brains but is strongly over expressed by microglia, astrocytes and neurons in pathological conditions (Valieres et al 1997; Van Wagoner et al 1999). A number of pathological studies on AD brains have shown increased IL-6 immunoactivity in areas with localised diffused (early) plaque formation in AD brains) and less immunoactivity in classical (established) suggesting its possible role in early plaque
formation (Bauer et al 1991; Hull et al 1996). It is also known to increase Aβ production influencing the amyloid precursor protein expression (figure 1.5).

TNF-α is a messenger protein that plays a key role in promoting inflammation both in brain and the periphery. It controls the production of other proinflammatory molecules and also helps in cellular healing and repair. A number of studies have shown elevated levels of TNF-α in peripheral blood and cerebral cortex of AD patients (Fillit et al 1991; Darkowski et al 1999). It has shown to increase the expression of the amyloid precursor protein and Aβ (Lucas et al, 2006), kill cells, and modulate neuro-transmission (Stellwagen and Malenka, 2006) (figure 1.5).

**Figure 1.5:** Accumulation of amyloid plaques and associated inflammation response resulting in release of pro-inflammatory cytokines such as IL-1beta, IL-6, and TNF-alpha (Google images, 2011).
In summary, the combination of increased cytokine production and Aβ aggregation appears to play a causal role in neuronal degeneration (Licastro et al 2003 and Reale et al 2004). The levels of IL-6 and TNF-α can be measured accurately in peripheral blood and cerebrospinal fluid and can potentially be used as markers of disease progression.

**Complement Pathway**

The complement pathway is divided into classical and alternate types. The classical pathway is made of about twenty proteases that can be activated one after the other as an amplifying cascade. The final result of its activation is the opening of transmembrane channels at the surface of the neurons resulting in free movement of ions and small molecules in and out of the cell causing disruption of cellular homeostasis and ultimately cell death.

Beta amyloid and neurofibrillary tangles can activate both, the classical and alternative complement pathways (Rogers et al 1992 and 1998). The complement proteins from both these pathways have been shown to interact with beta amyloid to form dissociation resistant complexes in senile plaques (Bradt et al, 1998). Complement proteins attract inflammatory cells (microglia and astrocytes) which lead to an aggregation of these cells in areas of plaque formation (McGeer et al 1989; Shen et al 1997). Microglia and astrocytes can also produce all the complement proteins when activated and their production has shown to be up regulated in AD brains (Lue et al, 1997).

In summary, the evidence in AD brains of a cycle of increased beta amyloid production, activation and increased production of complement proteins and aggregation of microglia and astrocytes is suggestive of a chronic state of inflammation (Akiyama 2000; Wilson et al 2002; Robert and Griffin, 2005).
**Chemokine System**

Chemokines induce movement of inflammatory cells towards the site of inflammation and constitute a large family of polypeptides (more than 50 have been identified so far). Increased expression of chemokines and their receptors have been reported in a wide range of CNS illnesses for example multiple sclerosis, infection, trauma and stroke (Glabinski et al, 1999) but more recent studies on AD brains have shown evidence of increased levels of some chemokines (monocyte chemo-attractant protein-1 and IP-10) and their receptors close to areas of senile plaque formation (Strohmeyer and Rogers 2001).

**Cyclo-oxygenase**

Cyclo-oxygenase (COX) is an enzyme that plays a crucial role in the synthesis of prostaglandins, which play a key role in the regulation of inflammatory processes. NSAIDs are known to exert their inhibitory effect on inflammation through inhibition of COX. Some studies have suggested that pro-inflammatory mediators such as IL-1 and TNF-α regulate COX expression to amplify the inflammatory reaction. Indirect evidence of the importance of COX’s pivotal role in regulating the inflammatory processes come from the epidemiological studies on NSAIDs showing their protective effect and the histochemical studies reporting their increased expression by neurons and microglia in AD brains (Kitamura et al 1999; Yermakova et al 1999).

**Other Factors**

A number of other mediators of inflammation (blood coagulation factors, fibrinolysins, acute inflammatory phase proteins and free radicals) have also been reported to be overactive in AD brains (Akiyama et al, 2000; Tuppo et al, 2005).
Importance of Biological Markers

The importance of biological markers of inflammation in AD from a clinical point of view rests on the fact that they could potentially act as markers of disease presence or severity. As a result, the concept of the “ideal bio-marker” for AD has been postulated (Nancy and Ronald Reagan Research Institute of the Alzheimer’s Association (1998). An ideal biomarker would be:

(a) Directed at the fundamental CNS patho-physiology of AD.
(b) Validated in neuropathologically confirmed cases
(c) Would mark more than increased risk of disease
(d) Would mark the presence of the disease itself
(e) Would track disease severity at early or pre clinical stages
(f) Should have a diagnostic sensitivity of more than 80% for detecting AD
(g) Should have a specificity of more than 80% for distinguishing other dementias
(h) Should be reliable, reproducible, non-invasive, simple to perform and inexpensive.

No such “ideal biomarker” is known to exist. Plasma and cerebrospinal fluid (CSF) levels of inflammatory markers, such as IL-6, may provide some information about peripheral and central inflammation but do not fulfil these criteria.

Current evidence for biomarkers of inflammation in Alzheimer’s disease

Based on the above mentioned evidence of increased inflammatory activity in AD it is logical to look for evidence of increase in the levels of inflammatory markers such as cytokines both in the centre and periphery. Most studies have looked for evidence of raised cytokine levels in the cerebro-spinal fluid (CSF) and peripheral blood. Others have investigated the cytokines released by the peripheral blood mono-nuclear cells (PMBCs).
CSF markers of inflammation in Alzheimer’s disease and Mild Cognitive Impairment

Although the CSF cytokine levels are more likely to be closely correlated with brain levels but they are less practical and difficult to conduct prospectively. The initial studies such as Lanzerin et al (1998) and Engleborghs et al (1999) were small in sample size and did not report any differences in inflammatory marker levels between AD patients and controls. Tarkowski et al (1999) compared AD, vascular dementia (VaD) and controls. They reported a 25 fold increase of TNF-α in AD group. In a more recent study (Tarkowski et al, 2003) compared 56 mild cognitive impairment (MCI) patients with 25 controls and reported increased TNF-α in the MCI group. Martinez et al (2000) reported an increase in the IL-6 levels in CSF of AD patients and Tarkowski et al (1999) reported a correlation between the IL-6 levels and Tau protein levels in CSF of AD patients.

Blood markers of inflammation in Alzheimer’s disease and Mild Cognitive Impairment

Peripheral Blood

A number of population based studies have reported an association of IL-6 and CRP levels in peripheral blood with cognitive decline. Schmidt et al (2002) in the ‘Honolulu-Asia Aging’ study reported a three fold increase in relative risk of developing dementia with increasing serum CRP levels. Yaffe et al (2003) in a longitudinal prospective study of a population based sample reported that base baseline IL-6 and CRP levels were associated with poor cognitive performance both at baseline and follow-up. A similar study by Engelhart et al (2004) reported increased risk of dementia with increase in baseline levels of CRP and IL-6.

Studies investigating peripheral blood levels are easier and less invasive than CSF studies. There is however a debate in the scientific community about the link between the serum cytokines levels and central inflammation although some research evidence exists that
shows links between central inflammation and serum cytokine levels. Song et al (2001) reported that after intra-cerebral administration of Aβ 1-42, IL-6 gene expression increases not only in brain but also in many peripheral organs.

Studies investigating blood levels, like CSF studies, have reported mixed results with some showing no difference between AD patients and controls (Lombardi et al, 1999) and others (Kalman et al, 1997; Licastro et al, 2000; Reale et al, 2004) showing raised IL-1, IL-6 and TNF-α in AD patients. However, there may be issues such as sampling time (e.g. diurnal variation), concurrent infection or medication that may affect cytokine concentrations in serum and CSF, and the relationship between plasma and CSF levels remains unclear.

**Peripheral Blood Mononuclear Cells (PMBCs) studies:**

PMBCs consist of lymphocytes (85%) and monocytes (15%) and can be stimulated to release cytokines in response to mitogens such as lipopolysaccharides (LPS) and phytohemagglutinin (PHA). They have been used for modelling CNS microglial responses in other illnesses such as multiple sclerosis. Research in this area has again shown mixed results but more recent studies have shown an increased release of cytokines both in AD and MCI patients (Guerreiro et al, 2007; Magaki et al, 2007). Two studies (Reale et al, 2004 and Gambi et al, 2004) have reported a decrease in cytokine levels after treatment with Donepezil in AD patients and an increase in IL-4 which is believed to be ‘anti-inflammatory’ or an antagonist to the pro-inflammatory cytokines.

In summary, there is some evidence from the current literature that cytokine levels in serum or CSF may have a role as biomarkers of MCI or AD but there have been few large systematic studies.
The role of anti-inflammatory drugs in the development and progression of Mild Cognitive Impairment and Alzheimer’s disease

The discovery of activated microglia in the close vicinity of the senile plaques and tangles in a number of histo-pathological studies lead to the hypothesis that people on long term use of anti-inflammatory agents may be protected from the chronic brain inflammation in AD. This lead to a number of epidemiological studies looking at case records of patients suffering from various types of arthritis (arthritis, osteoarthritis and rheumatoid arthritis) who were on long term treatment with anti-inflammatory medication. A number of case control and clinical series indicated that regular use of anti-inflammatory agents was associated with an estimated six fold sparing of AD as compared to age matched people in general population (1996, 2006). As non-steroidal anti-inflammatory agents (NSAIDs) were the most commonly used anti-inflammatory agents further studies were focused on their use.

NSAIDs and Alzheimer’s disease pathology

NSAIDs are known to exert their anti-inflammatory effects by decreasing the production of the key inducers of inflammation - prostaglandins and thromboxanes. Their direct target is cyclooxygenase which has two common forms COX-1 and COX-2 and they vary in their ability to inhibit these two forms (Kaufman et al, 1997). Traditional NSAIDs like aspirin inhibit COX-1 and have a variable action on COX-2. They may also inhibit neuroinflammation by target \( \gamma \)-secretase, an enzyme required for the generation of \( \text{A} \beta \) peptides, and decrease the production of the more toxic and fibrillogenic \( \text{A} \beta_{42} \). Aside from these effects, neuroinflammation and disease progression may also be influenced by NSAIDs acting on peripheral cells or immune cells, thereby altering the production of growth factors and cytokines, which can act across the blood-brain barrier (Tony Wyss-Coray, 2005) (figure 1.6).
Newer drugs like celecoxib have a selective action on COX-2 and have fewer gastric side effects. There has been a debate in literature about whether conventional COX-1 inhibiting NSAIDs are more relevant to inhibition of inflammation in AD as compared to the newer COX-2 inhibitors. COX-1 has been shown to be up regulated in reactive microglia (Hoozemans et al. 2002) but COX-2 has not yet been detected in AD microglia (Hoozemans et al. 2003). This may suggest that NSAIDs with COX-1 inhibitory properties are more likely to reduce brain inflammation selectively (McGeer, 2006).

**Figure 1.6:** NSAIDs and neuroinflammation in Alzheimer’s Disease. Modified from Tony Wyss-Coray (2005).
A post-mortem study (Mackenzie, 1998 & 2001) reported that arthritis patients using conventional NSAIDs showed a three times reduction in the number of activated microglia as compared to normal controls without arthritis and long-term NSAIDs use. It was also noted that NSAID use did not alter the number of amyloid plaques and tangles in both groups.

**NSAIDs and animal models of Alzheimer’s disease**

Transgenic mice are the most commonly used animal model for AD. Although all such studies have the inherent disadvantage of applying an animal model to human disease, they also have a number of advantages like controlling the type, dose and duration of NSAID used.

Lim et al (2001) showed that, in APP transgenic mice, 6 months treatment with ibuprofen lead to reduction in amyloid plaque numbers and microglial activation around plaques and the expression of inflammatory markers interleukin-1β (IL-1β). Other studies using ibuprofen and indomethacin showed similar results (Jantzen et al, 2002; Yan et al, 2003; Quinn et al, 2003). Out of two studies with the newer COX-2 inhibiting NSAID (celecoxib), one did not show any benefit (Jantzen et al, 2002) and the other showed a substantial increase in amyloid deposition (Kukar et al, 2005). This disparity between the outcomes of the use of COX-1 and COX-2 inhibiting NSAIDs probably suggest that COX-2 inhibition is the wrong target for reducing inflammation in AD brains or NSAIDs like ibuprofen act through other pathways to reduce amyloid production (Weggen et al, 2001; Yyss-Coray et al, 2006; Sastre et al, 2006).

**Disease modifying role of NSAIDs**

McGeer et al (1996) reviewed 17 epidemiological studies and reported an overall odd ratio of 0.475 for NSAID use and AD. Two other studies conducted in the early 90’s, one,
comparing NSAID users and non-users AD patients showed significantly slower disease progression in users (Rich et al 1995) and the other a double blind placebo controlled trial of indomethacin in AD patients over six months showed significantly less cognitive decline in users as compared to the placebo group (Rogers et al 1993). A prospective study conducted in Baltimore comparing users and non-users of NSAIDs reported a relative risk of 0.50 among users (Stewart et al 1997).

In contrast to the above, 2 subsequent major studies showed no evidence of benefit from use of NSAIDS in AD. Beard and colleagues (1998) in a case control study of around 300 new onset AD patients with 300 controls found no association between the disease and NSAIDs use in the 2 years prior to onset. The Rotterdam study group (in ’t Veld et al, 1998) in a three year prospective study of 74 AD patients and 232 matched controls found no association between NSAID use and risk of incident AD. An explanation for these apparently conflicting results from different studies can be inferred from a large long term prospective study (N=6989) (Velt et al, 2001). The risk of AD was estimated in relation to the use of NSAIDS for short term (less than a month) intermediate term (1-24 months) and long term (24 months or more). Results showed that the relative risk of AD was 0.20 in those with long term use but no major difference between short and intermediate term use was found. This suggests that short term NSAID use may not be protective against AD. This also suggests that the apparent protective affect of NSAIDs against AD is limited to their use 2 or more years before the onset of dementia (Brightener and Zandi 2001).

The dose of NSAIDS or aspirin does not appear to be crucial and these medications taken in low doses could be equally effective (Broe et al 2000; Anthony et al 2000). Englehart, et al (2003) in a systematic review of 9 studies reported a relative risk of 0.27 amongst people who are users of NSAIDS for more than 2 years. A more recent study comparing a traditional non selective NSAID (naproxen) with a selective COX 2 inhibitor (Rofecoxib) in patients with mild to moderate AD over a period of one year showed no evidence for
the slowing of cognitive decline in AD patients compared with placebo suggesting that NSAIDS may be more beneficial in prevention rather than in established disease.

**Other Anti-inflammatory Agents**

Steroids or synthetic glucocorticoids are known to be important anti-inflammatory agents but their action on the central nervous system has been shown to have different short term and long term consequences. In short term, treatment with glucocorticoids has been shown to attenuate post traumatic and post ischemic neuronal damage (Cacabelos et al 1994) but long term exposures can prevent recovery from brain injury (Morse et al 1992 and Woods et al 1999). A randomised placebo control trial of use of low doses of prednisone or placebo in a group of 138 subjects with AD did not show any difference in the rate of decline (Aisen et al 2000).

Use of other anti-inflammatory drugs such as hydroxychloroquine, vitamin E and selegilene did not show benefit on the progression of AD (Van Gool et al 2001; Sano et al 1997).

**Use of neuro-imaging in the diagnosis and assessment of Mild Cognitive Impairment and Alzheimer’s disease:**

**Structural neuro-imaging**

The use of neuro-imaging as a diagnostic and assessment tool in AD has evolved with the advancements in neuro-imaging techniques over the last few decades. A number of studies using Cat-axial Tomography (CT) scans and Magnetic Resonance (MR) imaging have shown promising results in helping with the diagnosis and progression of established AD. Moreover MR techniques using hippocampal volume measures have been shown to have a high correlation with cognitive decline in people with AD as compared to general population (Mueller and Dickerson, 2008). However the structural decline in hippocampal
volume does not appear to be linear during the progression from MCI to AD. In MCI the volumetric measures by MRI have a less predictive accuracy for progression to AD and functional neuro-imaging holds promise in detecting and diagnosing very early functional changes that precede the full blown clinical picture in AD.

**Functional neuro-imaging**

Positron Emission Tomography (PET) is a technique in which the uptake of radio-labelled tracer molecules into the brain can be quantitatively measured to obtain regional measurements. PET provides the opportunity to study brain function in vivo and the most commonly used tracer is Fluoro-deoxyglucose (FDG) (Herholz, 2003; Foster et al, 2007). Its uptake by the brain reflects glucose consumption which is closely related to neuronal function as glucose is the energy source for neurons to maintain ionic gradient and synthesize neurotransmitters. PET FDG studies suggest that it can provide a better prediction of conversion from MCI to AD as compared to structural imaging (Herholz, 2003) which could be useful for clinical use in future. It however lacks the specificity with regards to the molecular mechanisms of functional decline in AD. The development of newer radiolabelled tracers that bind specifically to receptor subtypes allows the quantitation of receptor numbers and occupancy (Banati, 2002).

**Imaging inflammation in the Brain:**

Measuring inflammatory processes in life with imaging has become possible recently due to the development of PET tracer molecules that bind specifically to activated microglia. As microglial activation is closely linked and anatomically confined to the sites of inflammation they can be used as a target for clinical imaging (Banati, 2002). The process of activation of microglia from their normal resting state is associated with an increased expression of certain receptors cites which can act as binding cites for radio-active ligands.
Two different types of receptors sites have been described in activated microglia including the benzodiazepine (Gavish et al, 1999) and cannabinoid receptors (Cabral and Cabral, 2005).

The benzodiazepine binding cite has a strong affinity for the radio labelled ligand PK11195. PET scanning with PK11195 provides the opportunity to detect areas of increased microglial activity in patients with a variety of disorders.

Acute and long term activation of microglia using PK11195 has been shown in herpes encephalitis (Cagnin 2001), peripheral nerve injuries (Banati et al 1997), ischemic stroke (Pappata et al 2000), vasculitis (Goerres et al 2001), and multiple sclerosis (Banati et al 2000). The data on AD and MCI is limited and summarised in table 1.1.
### Table 1.1: PK11195 studies in Alzheimer’s disease and Mild Cognitive Impairment

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (N)</th>
<th>Controls (N)</th>
<th>Design</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groom et al, 1995</td>
<td>8 AD* Patients</td>
<td>1</td>
<td>Cross sectional</td>
<td>No difference in PK11195 binding with control.</td>
<td>First study with PK11195 in AD patients.</td>
</tr>
<tr>
<td>Cagnin et al, 2001</td>
<td>8 AD patients</td>
<td>15</td>
<td>Cross sectional</td>
<td>Increased cortical PK binding in AD patients.</td>
<td>Only 1 MCI subject.</td>
</tr>
<tr>
<td></td>
<td>1 MCI** patient</td>
<td></td>
<td></td>
<td>Intermediate PK binding in MCI subject.</td>
<td>Potential for further studies in MCI.</td>
</tr>
<tr>
<td>Versijpt et al, 2003</td>
<td>10 AD patients</td>
<td>9</td>
<td>Cross sectional</td>
<td>Increased cortical PK binding in AD patients.</td>
<td>Usefulness of PK in measuring microglial activation.</td>
</tr>
<tr>
<td>Edison et al, 2008</td>
<td>13 AD patients</td>
<td>10</td>
<td>Cross sectional</td>
<td>Increased cortical PK binding in AD patients.</td>
<td>PK binding correlated with MMSE scores.</td>
</tr>
<tr>
<td>Okello et al, 2009</td>
<td>13 MCI patients</td>
<td>No controls</td>
<td>Cross sectional</td>
<td>Increased PK binding in 5 out of 13 MCI patients.</td>
<td>Used broader criteria for MCI.</td>
</tr>
<tr>
<td>Clayton et al, 2009</td>
<td>6 AD patients</td>
<td>5</td>
<td>Cross sectional</td>
<td>No difference in PK binding between MCI and control subjects.</td>
<td>Used broader criteria for MCI.</td>
</tr>
<tr>
<td></td>
<td>6 MCI patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Alzheimer’s Disease **Mild Cognitive Impairment
In an initial small study of AD Groom et al (1995) did not find an increase in binding of PK11195 in mild to moderate AD cases but subsequent studies using more refined techniques showed more promising results.

Cagnin et al (2001) studied 8 AD patients, 1 MCI patient and 15 healthy controls from ages 30-80 years. They reported increased uptake in widespread brain regions including temporal lobe, left parahippocampal gyrus left amygdala, left posterior cingulate gyrus, fusiform gyrus and inferior parietal lobe. The regional distribution of PK11195 uptake was correlated with the regional volume loss over 12 to 24 months seen on MR imaging and also with decreased blood flow on PET FDG. In the single MCI patient the uptake was intermediate between controls and AD.

In a comparable study Versipjt et al (2003) used PK 11195 as a ligand for single photon emission tomography (SPECT) and reported increased uptake in AD but in a different regional distribution with frontal lobes more involved. They also reported a significant relationship between regional increased uptake of PK11195 and cognitive deficits on neuropsychological testing.

Two studies have been reported in the literature on MCI subjects with PK11195 and have drawn somewhat conflicting conclusions. Okello et al (2009), in 13 subjects with MCI, reported that microglial activation could be detected in MCI subjects and 5 subjects showed a higher uptake of PK11195 as compared to healthy controls. A similar but smaller study by Clayton et al (2009), in 6 subjects with MCI, did not find any difference in PK11195 uptake compared with healthy controls and AD subjects.

The limited evidence available suggests that microglial activation might play a role in all the stages of AD including MCI and PET using PK11195 is able to provide in vivo imaging evidence of microglial activation. However larger studies including all stages of AD (from mild cognitive impairment to established dementia) are required to determine
whether PET with PK11195 can be used as a diagnostic tool in the early detection and diagnosis of the disease, and to identify those at high risk.

**Conclusion:**

There is good evidence to suggest that inflammatory processes play an important role in the pathogenesis of AD. Raised inflammatory markers in peripheral blood and activated neuroglia in vivo using PET have been studied in established AD and provide an opportunity to study people with MCI who are at a high risk of developing the disease. Further studies are needed to study the peripheral makers of inflammation and microglia activation in MCI and their possible use in early diagnosis and progression of AD.

**Aims**

This study aimed to investigate the peripheral and central inflammatory markers in a group of participants with amnestic MCI.

**Hypothesis**

1. Cognitive decline in people with amnestic mild cognitive impairment is associated with a chronic inflammatory process.
2. IL-6 and CRP levels in peripheral blood and PK11195 binding measured by PET can be used as markers of the inflammatory process.
Chapter 2: Methods
Ethics

Ethics approval was obtained from the South Manchester and Stockport Local research ethics committees (LREC) for recruitment of patients. Permission to administer PK11195 at the WMIC was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) of the United Kingdom.

Methods

Study population (figure 2.1)

The participants were recruited from the Memory Clinic at Wythenshawe Hospital in South Manchester (Manchester Mental Health and Social Care Trust) and the Old Age Psychiatry services at Stepping Hill Hospital (Pennine Care NHS Foundation Trust) in Stockport (figure 2.1). They were invited to participate in the study via an information letter sent by post (appendix 1). They were also provided an opportunity to see SK or JC along with their family members to clarify any details of the project. Written informed consent (appendix 3) for participation and genetic testing was obtained by SK and JC at the time of recruitment. General Practitioners (GPs) were sent information letters about their patient’s participation in the study (appendix 2). Personal and nominated consultees assent forms were also designed in line with the Mental Capacity Act (appendix 4 &5).

All participants underwent a comprehensive assessment process by a multidisciplinary team including a senior old age psychiatrist and psychologist. The initial assessment involved detailed history taking, neurological examination, cognitive assessment, routine blood tests and CT brain scans. A diagnosis of amnestic mild cognitive impairment was established using Petersen’s criteria for MCI (Petersen et al 2005).
Inclusion Criteria

- Memory complaint corroborated by an Informant
- Memory impairment relative to age-matched and education-matched healthy people
- Typical general cognitive function
- Largely intact activities of daily living
- Not clinically demented

Exclusion Criteria

- Established AD or other neurodegenerative disorders
- Vascular dementia
- History of chronic/recurrent inflammatory disease
- History of auto-immune disease
- Depression
- Severe mental illness

Cerebro-vascular pathology

All potential participants were assessed for the possibility of cerebro-vascular disease’s contribution to the cognitive decline in the multi-disciplinary assessment. People with vascular dementia were excluded along with those where cerebro-vascular disease was considered to be the main cause of cognitive decline. People with vascular factors were however not excluded as they are recognized risk factors for AD.
Figure 2.1: Recruitment

120 Patients approached

81 Agreed to participate

70 Patients Consented/recruited

8 Dropped out (2 died, 3 moved out of area, 3 withdrew consent)

62 Patients reviewed after 12 months

30 Patients agreed for scans (PET and MR)

16 dropped out (10 withdrew consent, 2 dropped out, 1 DNAed, 3 scans failed due to low PK yield)

14 Patients scanned
Study entry assessment

The baseline assessment included collection of demographic and clinical information, brief physical examination, administration of infection screening questionnaire, venepuncture for blood samples and neuropsychological assessments.

Demographic/Clinical information

The demographic information included age, sex, ethnicity and address. The clinical information was obtained both from the participants and their case notes and included the following:

- Vascular risk factors: smoking, hypertension, diabetes mellitus, dyslipidemia, coronary artery disease, atrial fibrillation, previous transient ischemic attack (TIA)/stroke.
- History of any infection or serious health problem in the past 6 weeks.
- Current list of medication

Physical examination

The physical examination included a record of height, weight, temperature, pulse rate and rhythm, blood pressure and body mass index (BMI).

Venous blood sampling

Venous blood samples (15 mls) were taken for the plasma measurement of IL-6 (measured using a sandwich ELISA) and CRP (using a high sensitivity competitive ELISA) and Apolipoprotein E status (measured by PCR). A small amount of the blood sample was stored, with appropriate consents, for possible use in future research. A standard protocol (appendix 7) was observed for all the venous blood sample/processing (Collection time 9 a.m. to 12:30 p.m).
Measures taken to reduce the possibility of false positive results:

- To avoid measurement of inflammatory markers that might be attributable to acute events the participants were encouraged to report any acute physical health problem/infection at the time of organizing the appointment for both baseline and follow-up assessment. The assessment was delayed for 2 weeks if an acute event such as infection was reported.

- The patients were administered an infection screen questionnaire (appendix 6) to all the participants, on the day of assessment, both at baseline and follow-up. This questionnaire is a check list of possible infections over the last 6 weeks along with a record of the details of infection including type, duration and treatment. The blood sample taking was delayed for 2 weeks if the participants scored positive on the infection screen.

- On baseline and follow-up assessments, if the CRP or IL-6 concentrations were raised above 3mg/l, a second sample was taken after an interval of at least 6 weeks. The lower of the two readings was considered for the data analysis. For both CRP and IL-6 reading a total of 16 samples were repeated both at baseline and follow-up.

Neuropsychological assessments

A battery of neuropsychological tests including the following instruments was administered:

1. Mini Mental State Examination (MMSE), Folstein et al (1975): It is a brief 30-point questionnaire assessing various cognitive domains including orientation in time and place, concentration, arithmetic, recall, language use and comprehension, visuo-spatial tasks and
basic motor skills. It is internationally validated for use both as a screening instrument for
dementia and to estimate the severity of cognitive impairment at a given point in time.

2. Cambridge Cognitive Examination-Revised (CAMCOG-R), Roth et al (1999). It is a
comprehensive neuro-psychological test which forms part of the Cambridge Examination
for Mental Disorders of the Elderly (CAMDEX), Roth et al, 1986. It has 105-points and
allows the assessment and comparison of eight ‘domains’ of cognitive function including
orientation, comprehension, expression, memory, attention, calculation, praxis, abstract
thinking and perception. A score of 80/105 is suggestive of cognitive impairment. One of
its major advantages is the ability to detect mild forms of cognitive impairment.

3. National Adult Reading. Test (NART), Blair & Spreen (1989). It is a widely
used instrument for estimating pre-morbid level of intellectual ability in people with
brain damage and dementia. The test requires subjects to read out loud a set of 50 words
which are irregular in terms of their grapheme- phoneme correspondences (Coltheart et al,
1987). The responses are individually scored as correct or incorrect, according to their
pronunciation. This score can then be used to derive a pre-morbid IQ estimate.

been widely used to identify prevalent dementia, predict future dementia, identify those
patients with MCI destined to develop AD and distinguish AD from nonAD dementias
(Grober et al, 2011). Unlike most other memory tests, the FCSR begins with a study phase
designed to control attention and cognitive processing to identify memory impairment that
is not secondary to other cognitive deficits. Patients identify pictured items (e.g., grapes,
vest) in response to category cues (fruit, clothing). In the test phase, subjects are asked to
recall the items they learned (free recall). The category cues are used to prompt recall of
items not retrieved by free recall to generate a score termed cued recall. The sum of free and cued recall is termed total recall. These procedures are based on the theoretical concept that controlled learning remediates the mild retrieval deficits that occur in many healthy elderly individuals but has only modest benefits in patients with dementia, thus enhancing the FCSRT's discriminative validity for the diagnosis of dementia in comparison to tests that do not control the conditions of learning.

5. Verbal fluency test (FAS), Spreen and Strauss (1998). It is a widely used test of frontal lobe/executive function where the participants are asked to tell as many word as possible starting with letters F, A and S. A total score of 30 is considered normal.

6. Cognitive Estimation Test (CET), Axelrod and Millis (1994). This instrument measures cognitive estimation/executive skills. The participants are asked questions that require individuals to make approximate judgments using deductive reasoning and problem-solving skills. The scoring is based on the degree to which an individual’s response deviates from the mean standardized responses. Possible scores for the CET range from 0 to 20 points, with higher scores representing greater deviation from the responses given by the normative sample.

**Follow-up assessments**

Eight participants dropped out and 62 were re-assessed after an interval of about twelve months. The assessment included clinical information, a brief physical examination, and administration of infection screening questionnaire, venous blood sample and neuropsychological assessments.
Brain scans
At the time of recruitment for the study we planned and identified 30 participants for scanning. Unfortunately there was a delay of more than 24 months in starting the PET scans at the WMIC. This resulted in a number of methodological and practical difficulties in carrying out the scans. A number of patients became unavailable for brain scans. Ten participants withdrew consent, 2 dropped out, 1 did not attend, 3 scans failed due to low PK11195 yield on the day of scanning. We were able to do 14 successful scans.
On the day of scanning the participants had a physical examination, an infection screen and blood test for inflammatory markers on the day of scanning and blood was collected on a subsequent occasion (>6 wk later) for CRP and IL-6 measurement if the initial values were higher than 3mg/l. All the participants gave written informed consent prior to the scanning. The MR scans were done within 2 weeks of PET scans for structure-function co-registration and excluding structural lesions.

Power calculation and statistical analysis
We used linear regression to establish that 70 participants would provide an 80% chance of identifying a correlation of at least 0.33 between inflammatory markers and cognitive decline. Thirty participants would be sufficient for the microglial activation studies to provide an 80% chance of identifying a correlation of at least 0.50 between either PK11195 binding and inflammatory markers or between PK11195 binding and cognitive decline.
In order to analyse the data for an association between inflammatory marker levels and cognitive decline we encountered the problem of having two inflammatory marker scores (CRP and IL-6) which are strongly correlated. We calculated a ‘combined inflammatory score’ that would provide a combined measure of the inflammatory burden by the following statistical methods:
**Step 1.** 'Normalise' values for each inflammatory marker by taking natural logarithms of the value.

**Step 2.** 'Standardise' each inflammatory marker by subtracting the mean and dividing by the SD. The standardised value, or "Z-score", measures how many standard deviations above or below the mean each individual's value is.

**Step 3.** Average these z-scores to give a combined measure of inflammatory burden.

The data was analysed using SPSS to look at demographics, changes in inflammatory marker levels and neuropsychological tests scores over the follow-up period. We used regression analyses to look at the relationship between inflammatory markers levels and cognition and controlled for the confounding role of demographic/baseline features.

**PET data analysis:**

From each subject’s raw imaging PET data, comprising of 18 frames in 60 minutes, a summative image was obtained using Linux windows PET data processing programme. The summed PET images and MR scans of each subject were co-registered by using software package Vinci (Max-Planck Institute for Neurological Research, Cologne, Germany).

Statistical parametric mapping (SPM5, Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, University College London, UK) was used for segmentation of the co-registered images into grey matter and white matter images. Object maps of these images were created by using the Hammersmith Brain Atlas maximum probability map (Hammers, et al. 2003) and ‘Analyze’ software (Analyze AVW, Mayo Clinic, Rochester, US). This process created an individualized anatomic brain atlas with 83 regions for each subject which is later used to sample $BP_{ND}$ values from the parametric images of binding potential (BP). $BP_{ND}$ represents the ratio of specifically bound radioligand over non-displaceable ones in tissues at equilibrium. In reference tissue models
it compares the concentration of radio-ligand in receptor-rich and receptor-free regions.

Parametric images of $BP_{ND}$ were generated using a simplified reference tissue model (SRTM), using the software ‘RPM the basis function implementation of SRTM’ written in Matlab (The Mathworks Inc., Natick, MA). $BP_{ND}$ values were estimated using the SRTM with a supervised clustering algorithm to extract a reference tissue input function. We chose the appropriate reference region by fitting the time-activity curve of each pixel with a database of tissue kinetics (normal gray and white matter, blood pool, muscle, skull, and pathologic tissue with high benzodiazepine receptor density) and defined a reference grey matter tissue devoid of specific benzodiazepine receptor binding (Turkheimer et al. 2007).

We applied the whole brain and grey matter object map to extract region of interest (ROI) data from the $BP_{ND}$ parametric images. The groups mean $BP_{ND}$ values were sampled using the ‘Analyze’ software in the following regions of interest: temporal lobe, parietal lobe and association cortices, frontal lobe, occipital lobe, posterior cingulate gyrus and putamen. Statistical comparison of $BP_{ND}$ values in ROIs between MCI subjects and healthy controls was performed using Wilcoxon rank sum test in Matlab.

To compare binding of PK11195 in all the MCI patients with controls the individual parametric images of $BP_{ND}$ were normalized and spatially smoothed. Statistical comparisons of $BP_{ND}$ parametric images between the MCI patients and controls were made using a two sample t-test in SPM with a voxel threshold of $p<0.01$ and an extent threshold of 50 voxels. All clusters with $p<0.01$ were considered to be significant.
Chapter 3: Results
Patient characteristics

The majority (62) of patients were recruited from the ‘memory clinic’ at Wythenshawe hospital in South Manchester. Eight patients were recruited from the old age psychiatric services at the ‘Stepping Hill Hospital’ in Stockport. The baseline characteristics at entry and follow up are presented in tables 3.1, 3.2 & 3.3.

Sixty percent were males and 40% were females. The mean age of the cohort was 70 years and majority of the participants were white (84%). The percentage of patients with one or more vascular risk factor was high with more than half having a history of dyslipidemia and 21% having a history of cerebrovascular event in the past. The smoking rate was also high with 50% ex and 17% current smokers. More than half of the group were APoE 4 positive.

The mean height was 1.67 meters and mean weight 75.2 kg. The mean body mass index was 26.8 with a range of 26 to 28 and the mean systolic blood pressure was 141 mm Hg. Thirty eight participants were on anti-inflammatory drugs and 47% were on more than one drug (table 3.3).
Table 3.1: Demographics of MCI patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42 (60)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (40)</td>
</tr>
<tr>
<td>Ethnicity (white)</td>
<td>59 (84)</td>
</tr>
<tr>
<td>Ethnicity (other)</td>
<td>11 (16)</td>
</tr>
<tr>
<td>Smokers (current)</td>
<td>12 (17)</td>
</tr>
<tr>
<td>Smokers (ex)</td>
<td>35 (50)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (39)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>7 (10)</td>
</tr>
<tr>
<td>Stroke</td>
<td>15 (21)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>39 (56)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>13 (19)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>17 (24)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>8 (11)</td>
</tr>
<tr>
<td>APoE4* positive</td>
<td>37 (53)</td>
</tr>
</tbody>
</table>

*APoE4* = Apolipoprotien E 4
Table 3.2: Demographics MCI patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (95% CI*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69.8 (68 – 72)</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>1.67 (1.64 – 1.69)</td>
</tr>
<tr>
<td>Weight (kilograms)</td>
<td>75.2 (71 – 79)</td>
</tr>
<tr>
<td>Waist (inches)</td>
<td>37.2 (36 – 38.5)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.8 (26 – 28)</td>
</tr>
<tr>
<td>Systolic BP** (mm of mercury)</td>
<td>141 (136 – 148)</td>
</tr>
</tbody>
</table>

* Confidence interval; ** blood pressure

Table 3.3: Anti-inflammatory drug use in MCI patients

<table>
<thead>
<tr>
<th>Group</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>38 (54)</td>
</tr>
<tr>
<td>NSAIDs*</td>
<td>26 (37)</td>
</tr>
<tr>
<td>Statins</td>
<td>38 (54)</td>
</tr>
<tr>
<td>Combination</td>
<td>34 (48)</td>
</tr>
</tbody>
</table>

* Non steroidal anti-inflammatory drugs
**Blood Counts**

The results of the full blood count showed that the mean values of haemoglobin levels, white cell and platelet count were with normal range (table 3.4).

**Table 3.4: Blood test results MCI patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>14.1 (11.7 – 16.8)</td>
</tr>
<tr>
<td>White cell count</td>
<td>6.12 (4.0 – 10.0)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>4.69 (4.0 – 6.0)</td>
</tr>
</tbody>
</table>

**Inflammatory marker levels**

The inflammatory marker levels were done at base line and repeated after one year. The mean baseline levels were within the normal range. The CRP levels increased significantly but the change in IL-6 levels was insignificant over the follow up period (table 3.5).

**Table 3.5: Inflammatory marker levels of MCI patients over 2 years**

<table>
<thead>
<tr>
<th></th>
<th>Geometric mean values (range)</th>
<th>Ratio 1 year/baseline (95% CI)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 year</td>
<td></td>
</tr>
<tr>
<td>IL-6 (n=61)</td>
<td>2.25 (1, 21)</td>
<td>2.36 (0, 25)</td>
<td>1.05 (0.85,1.28)</td>
</tr>
<tr>
<td>CRP (n=61)</td>
<td>1.35 (0, 10)</td>
<td>1.69 (0, 20)</td>
<td>1.25 (1.06, 1.48)</td>
</tr>
</tbody>
</table>
Cognitive tests:

The cognitive test results are shown in table 3.6. There was no significant change in the scores apart from the NART over the follow-up period.

**Table 3.6: Cognitive test results of MCI patients over 2 years**

<table>
<thead>
<tr>
<th></th>
<th>Mean (sd) values</th>
<th>Difference (95% CI)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 year</td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>26.9 (2.3)</td>
<td>26.3 (3.3)</td>
<td>-0.5 (-1.2, 0.1)</td>
</tr>
<tr>
<td>FAS</td>
<td>37.3 (11.0)</td>
<td>38.8 (12.4)</td>
<td>1.4 (-0.8, 3.7)</td>
</tr>
<tr>
<td>CAM Cog</td>
<td>90.4 (7.4)</td>
<td>89.0 (11.1)</td>
<td>-1.3 (-3.0, 0.3)</td>
</tr>
<tr>
<td>Cognitive Estimates</td>
<td>6.3 (4.1)</td>
<td>7.0 (4.4)</td>
<td>0.8 (-0.2, 1.7)</td>
</tr>
<tr>
<td>NART</td>
<td>115.8 (8.7)</td>
<td>117.3 (8.3)</td>
<td>1.4 (0.6, 2.3)</td>
</tr>
<tr>
<td>BUSCHKE</td>
<td>79.7 (22.4)</td>
<td>80.2 (24.1)</td>
<td>0.5 (-3.9, 4.9)</td>
</tr>
</tbody>
</table>
**Inflammatory maker levels and cognitive change**

We looked at the association between IL-6 and CRP and cognitive change over the follow-up period. There was no significant association between changes in IL-6 and CRP levels and changes in any of the neuropsychological instruments.

A multiple regression analysis showed that the findings were not confounded by demographic/baseline features including age, sex, smoking status, BMI, systolic blood pressure, history of stroke and diabetes.

Average of the z-scores were calculated for log IL-6 and log CRP at baseline (z-inflam-baseline) and for the change between baseline and follow-up (z-inflam-change) and found no significant correlation with z-inflam-change with change in any of the cognitive measures.

**Comparison of APOE +ve and –ve subjects:**

The cohort was divided into APOE positive and negative groups and the possible differences in inflammatory marker levels at baseline and change over 12 months were looked into. No significant differences were detected between the two groups for both the inflammatory markers.

The possible differences between the two groups on the scores of cognitive instruments were also looked into. No significant differences were detected in changes of cognitive scores over 12 months.

**Comparison between users and non NSAIDs users:**

The cohort was divided into the above mentioned groups to investigate possible differences in scores of cognitive measures. The analysis did not reveal statistically significant differences between the two groups both in cognition and inflammatory marker levels.
**PET scan cohort**

The PET cohort (14 subjects) had 79% males with a mean age of 65.4 years (table 3.7). Half were APoE positive and around 60% were using non steroidal anti-inflammatory drugs (NSAIDs) or statins. The inflammatory marker concentrations (table 3.8) did not show a significant change over two year’s period. The average scores of cognitive instruments, as a group, did not change significantly, apart from the NART, measuring pre-morbid IQ (table 3.9 and 3.10). However 2 patients had declined significantly over 24 months to fulfil the criteria for AD.

**Healthy controls:**

PET data from 4 age matched healthy controls was compared with the PET cohort data. The healthy controls included 3 males and 1 female with a mean age of 64 years. The mean CRP levels at baseline screening were 0.76 (range: 0.55–1.00) and IL-6 levels were 2.46 (range: 1.10–3.61). At the time of PET scans, the mean CRP were 1.56 (range: 1.18–2.05) and the mean IL-6 levels were 4.79 (range: 1.00–8.08).

The visual inspection of $BP_{\text{ND}}$ parametric images of scans of MCI subjects revealed increased $^{11}\text{C-(R) PK11195}$ binding as compared to controls. Figure 3.1 shows $BP_{\text{ND}}$ parametric images of one subject and one control. Figure 3.2 shows transaxial, coronary and sagittal projections of statistical parametric maps of areas with significantly increased ($p<0.01$, uncorrected) $^{11}\text{C-(R) PK11195}$ binding of $^{11}\text{C-(R) PK11195}$ in the MCI group compared with controls. Figure 3.3 (upper row) shows transaxial, coronary and sagittal projections of statistical parametric maps of areas of significantly increased binding ($p<0.01$, uncorrected) of $^{11}\text{C-(R) PK11195}$ in APoE +ve MCI patients compared with APoE –ve patients and with controls. These differences however did not remain significant after correction for multiple comparisons.

We then performed group comparison of $^{11}\text{C-(R) PK11195}$ binding in different regions of brain of the MCI group and controls. The results revealed significantly increased binding
the occipital lobe, temporal lobe, frontal lobe, parietal lobe, putamen, and the posterior part of cingulate gyrus in the MCI group. After corrections for multiple comparisons, PK11195 uptake in the posterior cingulate gyrus of MCI group remained significantly raised as compared to healthy controls (Table 3.11). No significant correlation was detected between the MMSE scores of MCI subjects and $^{11}$C-(R) PK11195 binding in any of the regions.

Table 3.7: PET cohort Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>11(79%) Male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>11(79%) White</td>
</tr>
<tr>
<td>APoE +ve</td>
<td>7(50%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>3(21%)</td>
</tr>
<tr>
<td>NSAID* use</td>
<td>9(64%)</td>
</tr>
<tr>
<td>Statin use</td>
<td>8(57%)</td>
</tr>
</tbody>
</table>

* Non steroidal anti-inflammatory drugs
### Table 3.8: Changes in inflammatory marker concentrations (PET cohort)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>1st year</th>
<th>2nd year</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP*</td>
<td>1.29</td>
<td>1.48</td>
<td>1.37</td>
<td>P=0.70***</td>
</tr>
<tr>
<td></td>
<td>(0.24 to 6.70)</td>
<td>(0.43 to 6.59)</td>
<td>(0.64 to 5.64)</td>
<td></td>
</tr>
<tr>
<td>IL6**</td>
<td>1.7</td>
<td>1.7</td>
<td>1.2</td>
<td>P=0.13****</td>
</tr>
<tr>
<td></td>
<td>(0.7 to 21.3)</td>
<td>(0.7 to 18.3)</td>
<td>(0.5 to 8.2)</td>
<td></td>
</tr>
</tbody>
</table>

* CRP Geometric mean (range)  
** IL6 Median (range)  
*** Repeated measures ANOVA  
**** Friedman test

### Table 3.9 Cognitive tests results over 3 years (PET cohort)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Geometric mean (range)</th>
<th>Repeated measures ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>F-UP (1)</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.6</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>(25 to 30)</td>
<td>(23 to 30)</td>
</tr>
<tr>
<td>FAS</td>
<td>34.1</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>(12 to 54)</td>
<td>(17 to 54)</td>
</tr>
</tbody>
</table>
Table 3.10 Cognitive test results over 3 years (PET cohort)

<table>
<thead>
<tr>
<th>Cognitive Test</th>
<th>Median (range)</th>
<th>Friedman test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>F-UP (1)</td>
</tr>
<tr>
<td>CAM Cog</td>
<td>90.5 (85 to 102)</td>
<td>94.0 (75 to 102)</td>
</tr>
<tr>
<td>Cognitive</td>
<td>5.0 (1 to 23)</td>
<td>6.0 (1 to 20)</td>
</tr>
<tr>
<td>NART</td>
<td>116 (95 to 127)</td>
<td>121 (98 to 129)</td>
</tr>
</tbody>
</table>

Figure 3.1: Transaxial, coronary and sagittal projections of $\text{BP}_{\text{ND}}$ parametric images of one MCI patient (upper row) and one healthy volunteer (lower row).*

*The colour bar indicates $\text{BP}_{\text{ND}}$ values.
Figure 3.2: Areas of significantly increased $^{11}$C-(R)PK11195 binding in MCI group compared with control group.*

*The colour bar indicates t values.

Figure 3.3:
Upper row: Areas of significantly increased $^{11}$C-(R)PK11195 binding in APoE +ve compared with APoE-ve MCI subjects.
Lower row: Areas of significantly increased $^{11}$C-(R)PK11195 binding in APoE +ve MCI compared with controls.*

* The colour bar indicates t values.
Table 3.11: Mean $^{11}$C-(R)PK11195 BP$_{ND}$ values in different brain regions in MCI patients compared with health controls

<table>
<thead>
<tr>
<th>Region</th>
<th>MCI patients (mean ± SD, n=12)</th>
<th>Healthy volunteers (mean ± SD, n=4)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>0.030 ± 0.036</td>
<td>-0.038 ± 0.057</td>
<td>0.025</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>0.101 ± 0.060</td>
<td>0.008 ± 0.068</td>
<td>0.018</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>-0.014 ± 0.064</td>
<td>-0.100 ± 0.070</td>
<td>0.025</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>0.086 ± 0.031</td>
<td>0.008 ± 0.073</td>
<td>0.021</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>0.081 ± 0.048</td>
<td>-0.002 ± 0.053</td>
<td>0.004</td>
</tr>
<tr>
<td>Posterior cingulate (R)</td>
<td>0.138 ± 0.063</td>
<td>-0.012 ± 0.070</td>
<td>0.001*</td>
</tr>
<tr>
<td>Posterior cingulate (L)</td>
<td>0.111 ± 0.060</td>
<td>-0.012 ± 0.062</td>
<td>0.001*</td>
</tr>
<tr>
<td>Putamen (R)</td>
<td>0.106 ± 0.074</td>
<td>-0.012 ± 0.070</td>
<td>0.009</td>
</tr>
<tr>
<td>Putamen (L)</td>
<td>0.078 ± 0.070</td>
<td>-0.038 ± 0.080</td>
<td>0.018</td>
</tr>
<tr>
<td>Pallidum (L)</td>
<td>0.123 ± 0.085</td>
<td>-0.036 ± 0.080</td>
<td>0.007</td>
</tr>
<tr>
<td>Insula (R)</td>
<td>0.024 ± 0.056</td>
<td>-0.056 ± 0.077</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*remains significant after correction for multiple comparisons
Chapter 4: Discussion
The aim of this study was to investigate the association between peripheral and central markers of inflammation and cognitive decline in people with amnestic MCI. A group of MCI subjects were recruited; their cognitive functions and inflammatory marker levels (IL-6 and CRP) in peripheral blood were measured at baseline and over 1 year follow up period. A sub group of participants was followed up for another year and central brain microglial activation was measured by PET using PK11195 along with cognitive and peripheral inflammatory marker measurement. The main findings were:

1. There were a relatively high proportion of APoE4 positive participants in the cohort.
2. The mean CRP and IL-6 levels of the cohort increased over one year but the rise was only significant for CRP.
3. No association was detected between change in IL-6 and CRP levels and changes in cognitive test.
4. There was no significant correlation between the combined inflammatory score (Z score) of IL-6 and CRP and change in any of the cognitive tests over 1 year.
5. Group comparisons of the PET cohort (14 participants) with healthy controls (4 participants) showed increased PK11195 binding (mean binding potential) in frontal lobe, temporal lobe, parietal lobe, putamen, occipital lobes and significantly increased binding in posterior cingulate gyri.

This section discusses the general methodology of the study, results and their interpretation, future direction of research and draws conclusions at the end.
Demographics of the cohort

The memory clinic at Wythenshawe hospital in south Manchester is an established assessment, teaching and research center. It provides memory assessments and treatment to local residents along with assessment and diagnostic service to the whole of North West region. A multi-disciplinary team, of which SK was a part, is involved in the assessment, diagnosis and treatment process. Stepping Hill hospital is the district general hospital for Stockport and has an established old age psychiatric service providing memory assessment and treatment to the local population. The patients recruited for the study from these two services underwent a rigorous assessment process as described in the methods section. No major difficulties were encountered in the recruitment of participants due to the large number of patients referred to these services. The study incorporated well established measures of cognitive function and decline.

The demographic profile of the participants (table 1, 2 & 3) was generally representative of the memory services in South Manchester and Stockport. An audit of the memory clinic in South Manchester (2005) looking at the profiles of 405 referrals reported that mean age was 69.7 years and 59.5% of them were females. 37% (151) of the referrals were diagnosed with mild cognitive impairment and 43% (175) with dementia. The study cohort had a similar mean age of 69.8 but had more males (60%). The percentage of current smokers (12%) is similar to the national figure of 13% for people above the age of 60 (Office of National Statistics, 2010). The percentage of ex-smokers (35%) was higher than the national average of 30% which could be explained on the basis that the North West has been reported to have highest smoking rates amongst all the regions of UK and a larger percentage of men are smokers (Office of National Statistics, 2010). The percentage of participants with vascular risk factors including hypertension, coronary heart disease, diabetes and dyslipidemia was higher than the national average (Majeed and Moser, 2005; Office of National statistics, 2010) but similar to population studies investigating vascular
risk factors in MCI (Lopez et al, 2003). Around half of the study patients were on statins and aspirin and the mean systolic blood pressure was 141 mm of Hg indicating that the risk factors were being treated in primary care. The higher prevalence of vascular risk factors in this cohort could also be explained on the basis of unhealthy life style linked with of high levels of deprivation (Shohaimi et al 2003) in the North West in general and Wythenshawe area in particular (Indices of Deprivation, 2000).

Fifty three percent of participants were found to be ApoE4 positive which has been reported as a risk factor both for AD (Aggarwal et al, 2005) and MCI (Boyle et al, 2010). The higher percentage of ApoE4 positive participants is significant for the outcome of this study as it has been associated with lower levels of inflammatory markers (Eiriksdottir et al, 2006; Roberts et al, 2009). A population based study by Lopez et al (2003) looking at risk factors for MCI reported that 26% of a sample of 577 MCI patients were ApoE4 positive. Another hospital based study (Wang et al, 2010) reported that out of a sample of 304 MCI patients, 26.6% were ApoE4 positive. Schuitemaker et al (2009) enrolled 67 MCI participants in a tertiary referral center for memory disorders to investigate the relationship of inflammatory markers with cognitive decline in MCI and AD patients. They reported that 52% of their cohort was ApoE4 positive. The relatively high proportion of ApoE4 positive people in our cohort is probably indicative of them being recruited from a selected population of people referred to the memory assessment services.

**Cognitive testing**

The scores of cognitive tests did not change significantly over 1 year apart from the increase in NART scores. Four patients (6%) were clinically diagnosed to have converted to AD over the one year period. Although people with amnestic MCI are known to have a high risk of conversion to AD and some studies have reported annual conversion rates of up to 15% (Petersen et al, 2004), it is recognized to be a heterogeneous condition and up to
44% of people are likely to remain stable or even improve over time (Palmer et al, 2008). Moreover the diagnosis of conversion to AD from MCI is based on clinical criteria and not exclusively on the change in cognitive scores. The individual scores of converters are expected to change but the mean scores of the cohort might not change significantly. Dik et al (2005), investigating cognitive decline and inflammatory markers, followed up a population based sample of more than one thousand older people over 3 years. They reported that in the cohort, median MMSE score of 28 did not show a significant change over the follow-up period.

The significant increase in NART scores is unexpected as it is a test of vocabulary and provides a measure of pre-morbid intelligence Blair & Spreen (1989). NART is known to have a relatively high degree of inter-rater reliability when used by experienced psychologist (O’Carroll, 1987). Both assessors for the study, the author and the research nurse, were not experienced psychologists and the unexpected result has probably resulted due to poor inter-rater reliability.

**Inflammatory markers in peripheral blood**

The mean baseline levels of both inflammatory markers (IL-6 and CRP) were within normal range. One of the strengths of this study was that measures were adopted to avoid higher measurements of inflammatory markers that might be attributable to acute events. The participants were encouraged to report any acute physical health problem/infection at the time of organizing the appointment for the baseline assessment. A second sample was taken with an interval of 2 weeks if the CRP or IL-6 concentrations were raised above 3mg/l on the first blood test result and the lower of the two readings was considered for the data analysis. There was a rise in the mean levels, at one year follow-up, of both CRP and IL-6 but the change was only significant for CRP. This indicates a rise in peripheral inflammation over time despite using rigorous protocols to avoid false positive readings.
We compared the users and non-users of NSAIDs but no significant differences were observed.

We then looked at the association between IL-6 and CRP change and cognitive change over the follow up period. There was no significant association in both IL-6 and CRP levels with any of the neuropsychological measures. We looked at possible confounding factors including NSAIDs use. Multiple regression analysis showed that this finding was not confounded by factors including NSAIDs use and other demographic factors including age, sex and vascular risk factors including smoking status, systolic blood pressure, history of stroke and diabetes. As IL-6 and CRP are strongly correlated with each other, we calculated a combined inflammatory score (Z score) that would provide a combined measure of the inflammatory burden (see chapter 2 for details). Change in the Z scores over the follow up period did not show a significant correlation with change in any of the cognitive measures. We also looked at the possible differences in people with or without NSAIDs use and other demographic variables by multiple regressions analysis and they did not reveal any significant differences.

In summary the results of our study suggest that although there was a rise in both inflammatory maker levels over 1 year, it was only significant for the CRP and the change in the total inflammatory burden was not significantly correlated with change in cognitive measures. These findings suggest that although the mean inflammatory maker levels, of the cohort, rose over 1 year but the mean scores of the cognitive measures did not change significantly in this time period and no significant correlation between the rise in inflammatory marker levels and cognitive decline was observed.

Other studies, on general population, AD and MCI samples, exploring the link between inflammatory markers and cognitive decline have reported conflicting results. Yaffe et al (2003) looked at a population based sample of about three thousand people, followed them up for 2 years and found that those with high levels of CRP and IL-6 in
peripheral blood had poor cognitive performance at baseline and were at a higher risk of
decline at follow-up. They however did not do extensive neuropsychological tests and
used modified MMSE scores only. Similar results were reported by Weaver et al (2002) in
a 7 year follow-up study of a cohort of 776 elderly participants showing that people with
highest levels of IL-6 were more likely to decline over time. Other population based
studies have also reported significant association between IL-6 and cognitive decline
(Ozturk et al, 2007; Alley et al, 2008).
Yet other population based studies have reported negative results. Dik et al (2005) enrolled
a population based sample of more than a thousand people from general population and
followed them up for 3 years and found no relationship between IL-6 and CRP levels with
cognitive decline. Similarly Weuve et al (2006) investigated a population sample of about
four thousand women who were participating in a women’s health study. They looked at
the CRP levels taken over a gap of 4-6 years and performance on extensive cognitive tests
and found no evidence of a link between raised CRP and cognitive decline.
Komulainen et al (2007) investigated the relationship between peripheral blood CRP
concentration at baseline and cognitive function at 12 year follow-up in a population based
sample. MMSE scores were done at baseline but extensive tests including MMSE, Word
Recall Test and Cognitive speed tests were performed at follow-up. They found that higher
baseline CRP was associated (longitudinal association) with poor performance on Word
Recall Test on 12 year follow-up but there was no association between CRP levels at
baseline and MMSE scores or cognitive speed at follow-up. Similarly there was a weak
association (cross-sectional association) between CRP levels and Word Recall Test but
there was no association between CRP levels at follow up and MMSE scores or cognitive
speed tests.
In summary, the majority of population based studies have shown an association between cognitive decline and inflammatory markers but not one specific marker or cognitive test. Yet others have reported negative results.

Studies comparing MCI, AD and controls to investigate the link between cognitive decline and inflammation have also reported conflicting results but there have been differences in methodology. The main difference between these studies is the methods of measurement of inflammatory markers in peripheral blood and secretion of inflammatory markers by peripheral blood mononuclear cells (PBMCs).

Studies investigating secretion of a variety of inflammatory makers including IL-6 by PBMCs monocytes have reported increased cytokine production including IL-6 in MCI and AD patients as compared with healthy controls (Guerreiro et al, 2007, Magaki et al, 2007). By contrast, more recent studies of inflammatory marker levels in peripheral blood have not reported raised IL-6 levels in MCI patients. Bermejo et al (2008) compared MCI, AD and healthy controls and reported raised IL-6 levels in AD but not in MCI suggesting the possibility of a difference in the pattern of inflammatory markers in AD and MCI subjects. Similar findings were reported by Schuitemaker et al (2009) from two groups of people with MCI (n=67) and AD (n=145) and measured inflammatory markers in peripheral blood and cerebro-spinal fluid (CSF). They reported significantly high levels of CRP both in peripheral blood and CSF in MCI subjects as compared to AD but no difference was detected for IL-6 between the two groups. Roberts et al (2009), in a large population based sample of about five thousand people, identified those with normal cognition, MCI and dementia. MCI subjects were further divided into amnestic and non-amnestic MCI. They looked at the association between CRP, IL-6 and TNF –alpha levels in peripheral blood and cognitive performance using an extensive battery of tests. They reported a significant overall association between CRP levels and the total MCI cohort but not with IL-6 and TNF –alpha. The significance of association of CRP varied with the
MCI subtype. The amnestic MCI subtype did not show a significant association with CRP and it was only significant with non-amnestic MCI. They suggested that the lack of association of CRP levels in the amnestic MCI cohort could be due to a higher proportion of APOE4 positive subjects (31.5%) as compared to non-amnestic MCI cohort as it is has been associated with lower inflammatory marker levels.

The findings, in this study, of a significant rise of CRP but not of IL-6 in peripheral blood over the 1 year period are consistent with the more recent studies on MCI subjects discussed above. They suggest that in the MCI cohort, secretion of IL-6 by the PMBC might be a better alternative to measurements in the peripheral blood. It is however likely to make it practically less useful if inflammatory marker levels were to be used in clinical setting. The lack of overall association between CRP and change in cognitive measures is also consistent with the Robert et al (2009) study as our cohort had amnestic MCI and more than half were APOE4 positive. It is possible that a longer follow-up period, as in most studies discussed above, could have revealed significant changes in cognitive scores and provided an opportunity to explore their association with changes in inflammatory marker levels. Other possible explanation of our results could be firstly that circulating cytokines in peripheral blood in MCI might not reflect the tissue levels, especially so in the brain because of the blood brain barrier. Secondly, the rise in the CRP levels over the follow-up period could be unrelated to neurodegenerative processes and linked to systemic factors such as vascular disease and ageing (Tan et al, 2007).

**PET cohort**

The PET cohort had the inflammatory marker levels measured at baseline, 1st and 2nd years. Both IL-6 and CRP levels did not change significantly over this period. The PET scans showed increased PK11185 binding (table 11) in all the 4 lobes of cerebral cortex along with significantly increased binding in posterior cingulate gyrus as compared to the
controls. No correlation was detected between the cognitive scores and the regions of interest showing increased binding of PK11195.

There have been a number of PET studies with PK11195 in established AD. A small initial study by Groom et al (1995) did not report increased binding of PK11195. More recent studies however reported increased binding in a number of brain regions including frontal, temporal, parietal, occipital and cingulate cortices (Versipjt et al, 2003; Edison et al, 2008) indicating widespread microglial activation in cerebral cortex. Edison et al (2008) also reported that microglial activation correlated with MMSE scores in AD subjects. Only two studies, to our knowledge, have been conducted on MCI subjects with PK11195 and have drawn somewhat conflicting conclusions. Okello et al (2009), in 13 subjects with amnestic MCI, reported that in 5 subjects there was an increased binding of PK11195 as compared to healthy controls. A similar but smaller study by Clayton et al (2009), in 6 subjects with MCI, did not find any difference in PK11195 binding compared with healthy controls and AD subjects. They however used a broader criterion for MCI and did not recruit amnestic MCI subjects specifically.

Our results are consistent with Edison et al (2008) on AD patients as they have shown similar pattern of microglial activation in the cerebral cortex. Our cohort of amnestic MCI is also considered to be closest to AD in terms of having the highest risk of developing the disease (Petersen et al, 2008). We however did not detect a correlation between cognitive scores and microglial activation which could be explained on the basis lower levels of inflammation in MCI as compared to established AD. A bigger cohort with a longer follow-up period and repeat of PK11195 PET scans might be able to detect the increase in levels of inflammation over time along with its correlation with cognitive decline. We also did not detect any significant rise/change in the inflammatory makers in peripheral blood in this cohort despite the microglial activation over large areas of cerebral cortex. This finding raises the possibility, as discussed in the previous section, of the inflammation in
MCI being more central and detectable by PK11195 PET scans and not in peripheral blood. It also suggests that both in AD and MCI the inflammation appears to be a more diffuse phenomenon and not confined to a particular part of cortex such as hippocampus.

**Conclusion**

This study, to our knowledge, is unique in studying makers of inflammation in a high risk group of AD participants (amnestic MCI) both in peripheral blood and brain. The results of this study, in the light of current literature, add to the importance of recognition of inflammatory processes in people at risk of developing AD. The results suggest that CRP levels rise significantly over time and are detectable in peripheral blood by using practically simple laboratory techniques. The results also suggest that activated microglia in amnestic MCI patients can be visualized in vivo by using PK11195 PET scans and show higher levels of activation as compare to healthy controls. These finding could be useful in identifying people with malactivated (pro-inflammatory) microglia as potential targets for prevention/early treatment strategies. Further studies with larger samples sizes and long term follow-up are needed to investigate whether these peripheral and central inflammatory markers could shed light on the aetiology of AD and be useful in monitoring disease progression.
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Appendix 1

INFORMATION SHEET FOR PATIENT

MEASURES OF INFLAMMATION IN PEOPLE WITH MILD COGNITIVE IMPAIRMENT

Dear

I am writing to ask if you would be prepared to take part in a research study. This information sheet provides details of the project. Please take your time to read it carefully and discuss it with your family, friends, and/or family doctor. Thank you for your time.

What is the study about?

We are investigating the reasons why people develop memory loss, why that memory loss gets worse, and why people develop diseases like Alzheimer’s disease. One theory is that there is an inflammation in the body, particularly in the brain, which sets up a reaction which, after many years, can affect the way the brain works and eventually cause symptoms of memory loss, emotional changes, and sometimes diseases like Alzheimer’s disease. We are trying to discover whether we can detect any changes in the blood or in the brain that may shed light on the reason for this inflammation. If we understand this better, then this potentially may help in trying to discover new drugs which can lessen the symptoms of memory loss and perhaps even lead on to something that might prevent diseases such as Alzheimer’s disease. We would like to take a blood sample and do some tests on memory and attention with about 70 patients. On about 30 of these people, chosen at random from the 70, we will be asking whether they wish to take part further in the study involving more tests of memory and brain scans.
**Why have I been chosen?**

You have been chosen because you have symptoms of memory loss and have asked for help in discovering the reasons for this. By examining people who complain of memory loss but who do not have diseases such as Alzheimer’s disease, we feel we will learn more about the role inflammation has in causing these symptoms.

**What will the study involve?**

We would like to take a blood sample at the start of the study and after one year to measure proteins in the blood that might be associated with inflammation. We would also like to perform tests aimed at discovering the genetic basis of memory problems and store the blood sample for future research in these areas. We may ask for a second sample to check that your levels of blood protein have not altered since the first sample was taken.

The memory tests will consist of paper and pencil tests which can be carried out at the hospital or in your own home. These are similar to the tests you may have had before and will test your memory and attention and will take about 1 hour to complete. The memory tests will also be carried out when you enter the study and 12 months later.

**Can I refuse to take part?**

Most definitely:- Whether you take part in this research project or not will not affect in any way whatsoever the care you receive in the hospital or from your GP (who will be told if you do agree to take part in the study). Even if you agree to take part, you can withdraw at any time without giving a reason.
What will happen to the information in the trial?

The results of your tests will be stored anonymously on a computer for analysis at the end of the study.

Who is funding the study?

The Medical Research Council in the UK is funding the study.

What happens if I come to any harm?

You are protected by the NHS Complaints Procedure. Full details are available from: Consumers for Ethics in Research (CERES) publish a leaflet entitled ‘Medical Research and you’. This leaflet gives more information about medical research and looks at some questions that you may want to ask. A copy may be obtained from CERES, PO Box 1365, London N16 0BW.

If you have any questions about this study, please contact:

Dr Salman Karim/Professor Alistair Burns
Appendix 2

GP INFORMATION LETTER

Dear Dr

Re: Your Patient:

We have approached the above named patient of yours to consider taking part in a study we are carrying out in Manchester. We are trying to find whether levels of inflammatory markers in blood or increased inflammatory activity in the brain seen on PET scans can shed light on inflammatory aetiology of neurodegenerative diseases like Alzheimer’s disease.

We are aiming to recruit 70 people diagnosed as mild cognitive impairment. Initially, we will do neuropsychological testing, and blood samples will be taken from the participants to measure the levels of inflammatory markers and genetic studies. Thirty patients from this group will undergo MRI and PET scanning to look for increased inflammatory activity in the brain. After a gap of one year, the process of taking blood samples and brain scanning will be repeated.

The above mentioned investigations will be conducted at The Hope Hospital, The Wellcome Clinical Research Facility, and The Woolfson Molecular Imaging Centre.

The study is funded by the Medical Research Council and an approval from the local ethics committee has been obtained. Please do not hesitate to contact us if you have got any concerns about this patient’s participation in the above mentioned project.

Yours sincerely
Appendix 3

Consent form for Patient: Blood sample and memory tests

CONSENT FORM

MEASURES OF INFLAMMATION IN PEOPLE WITH MILD COGNITIVE IMPAIRMENT

I ………………………………………. have read and understood the Information Sheet about this study.

I confirm that:

(delete as necessary)

I have read and understood the Information Sheet  Yes  No

I have had the opportunity to ask questions and discuss the study  Yes  No

I agree to having tests on my memory and attention  Yes  No

I agree to a blood sample being taken and a second sample if necessary at the start and after 1 year of the study  Yes  No

I agree to my GP being informed about the study  Yes  No

I agree that my blood sample be included in genetic research  Yes  No

I agree to a blood sample being stored for future research  Yes  No
APPENDIX-4

PERSONAL-CONSULTEE ASSESSMENT FORM

MEASURES OF INFLAMMATION IN PEOPLE WITH MILD COGNITIVE IMPAIRMENT

The researcher has given me my own copy of the project information sheets which I have read and understand. The sheet explains the nature of the research and what my relative would be asked to do as a volunteer.

I confirm that:

(delete as necessary)

(1) I have read and understood the Information Sheets,  Yes  No

(version 6, march 2007 brain scans and blood samples  version 1 July 2007, tests on memory and attention)

(2) I have had the opportunity to ask questions and discuss this part of the study with the researcher.

(3) I agree to support Mr./Mrs./Ms./Miss…………………………………  Yes  No

in their participation in the study including brain scans, blood samples and tests on their memory and attention.

(4) I understand that participation of the person for whom I care is voluntary, and that they may be withdrawn or I can withdraw them from the study at any time without giving a reason and that their medical care or legal rights will not be affected.
(5) I understand that data collected during the study and relevant sections of the medical notes of the person for whom I care may be looked at by responsible individuals in the research team. I give permission for these individuals to have access to the appropriate records.

Yes  No

6) I agree to the G.P. of the person for whom I care and other relevant health care staff being informed of their participation.

Yes  No

7) I agree to the person for whom I care continuing to take part in the study and have no reason to believe that they would not have wished to participate if they were able to make that decision.

Yes  No

Name of Participant ……………………………………………………..

Name of Personal Consultee…………………………………………………………

Signed ………………………………………………………………………..

Date ………………………………………………………………………..

Name of Researcher ……………………………………………………..

Signed ………………………………………………………………………..

Date ………………………………………………………………………..
Appendix 5

NOMINATED CONSULTEE ASSESSMENT FORM

MEASURES OF INFLAMMATION IN PEOPLE WITH MILD COGNITIVE IMPAIRMENT

The researcher has given me copies of the project information sheets which I have read and understand. The sheets explain the nature of the research and what Mr./Mrs./Ms./Miss……………… would be asked to do as a volunteer.

I confirm that:

(delete as necessary)

(1) I have read and understood the Information Sheets, Yes No

(version 7, Jan 2008 brain scans and blood samples, version 1 July 2007, tests on memory and attention)

(2) I have had the opportunity to ask questions and discuss this part of the study with the researcher. Yes No

(3) I agree to support Mr./Mrs./Ms./Miss…………………………… Yes No

in their participation in the study including brain scans, blood samples and tests on their memory and attention.

(4) I understand that participation of Mr./Mrs./Ms./Miss……………… Yes No

is voluntary, and that they may be withdrawn or that I can withdraw them from the study at any time without giving a reason and that their medical care or legal rights will not be affected.
(5) I understand that data collected during the study and relevant sections of the medical notes of Mr./Mrs./Ms./Miss…………………………. may be looked at by responsible individuals in the research team. I give permission for these individuals to have access to the appropriate records.

Yes No

(6) I agree to provide information required during the study to monitor any changes or progress of Mr./Mrs./Ms./Miss…………………………. Yes No

(7) I agree to Mr./Mrs./Ms./Miss G.P. being informed they are participating in this study.

Name of Participant …………………………………………………

Name of Nominated Consultee…………………………………………

Signed …………………………………………………………………

Date …………………………………………………………………

Name of Researcher …………………………………………………

Signed …………………………………………………………………

Date …………………………………………………………………
APPENDIX 6

INFECTION SCREEN

A cold or cold-like symptoms? □
Cough? □
Cough productive of sputum? □
Sore throat? □
Episodes of high temperature? □
Episodes of shivering? □
Discharge from the ears? □
Discharge from eyes? □
Stomach upset such as vomiting and/or diarrhoea? □
Discomfort or burning when passing urine? □
Dental/oral infection? □
Skin rashes/boils/blisters/abscesses/wounds? □
Any other infection that you are aware of? □
Appendix 7

Protocol for collection of blood (MCI Study)

Subjects: Patients with mild cognitive impairment
Collection time: 9 a.m. to 12:30 p.m.

1) Turn on centrifuge (in B326) to cool to 4°C.
2) Collect red-capped polypropylene tubes, each containing 100u pyrogen-free heparin (10u/ml final after blood collection), from the rack in the cold room and place in polystyrene racks of a designated cool box. Add a large freezer block from the freezer.
3) Collect 15ml blood into a 20ml syringe.
4) Remove needle and transfer to the 10 ml to a polypropylene tube (Red cap) containing 100u pyrogen-free heparin (10u/ml). Add remaining blood to f.b.c. tube to be sent to haematology.
5) Mix heparinised blood tube thoroughly by gentle inversion x 10 and label tube with subject name.
6) Record Patient name, collection time, subject No. and hospital No. on label
7) Replace heparin tube(s) in the cool box rack and place this in the sample collection box.
8) Transfer samples to the laboratory.
9) Remove 4 x 50µl aliquots of blood from heparin tube and place in 4 clear, screw
topped 0.5ml freezer vials. Label each tube with the following information -
Subject initials, Subject Code Number, Date sample was taken and the Lab. Code
Number. Write Lab. Code Number on vial lid.

10) Remaining blood in heparin tube to be balanced with like tubes, or with pre-
prepared similar balance tubes, in the centrifuge and centrifuged for 15 min at
2,000 x g.

11) Transfer approx. 4 - 5ml plasma from each heparin tube into to a labelled, red-
topped ‘decant’ tube, using a sterile transfer pipette and taking great care not to
disturb cell layer.

12) Dispense 6 x ~0.75ml aliquots of the heparin ‘decant’ tube plasma to 6 clear, 2ml
screw-topped freezer vials, using a sterile transfer pipette. Label each tube with the
following information - Subject initials, Subject Code number, Date sample was
taken and the Lab. code number. Write Lab. Code Number on vial lid.

13) Switch off centrifuge.

14) Wipe working surfaces with (whatever local safety rules require) and switch off fan
and light.

15) Place all racked sample tubes in the MCI storage tray in the designated -70°C study
freezer.