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TNF inhibitors for the prevention of Alzheimer’s disease

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I would like to dedicate this thesis to my Nanny Emma who passed from Alzheimer’s disease in July 2014 and to my Nanny Rachel who also had Alzheimer’s disease and passed in June 2019.
Abstract

Background
As the ageing population continues to rise, so too do the number of cases of age-related diseases. Alzheimer's disease (AD) is the most common cause of dementia and is one of the leading causes of death among older adults. There has been little success with interventions that target the amyloid cascade in AD, highlighting the need for new therapeutic targets. In recent years, genome-wide association studies (GWAS) have identified polymorphisms in several genes which implicate the role of inflammation in AD progression and development. This is of particular relevance in rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease, as these patients have higher levels of systemic inflammation than the general population. If AD is driven by inflammation, then RA may be the ideal model in which to observe the accelerated effects of systemic inflammation on cognitive decline for future interventional trials. Moreover, there have been several inflammatory biomarkers which have been found to be peripherally elevated in both diseases. A raised level of serum tumour necrosis factor-alpha (TNFα) observed in patients with AD has been associated with an increased rate of neurodegeneration and risk of conversion from MCI to AD dementia. This led to the hypothesis that reduction in peripheral TNFα via TNF inhibitors (TNFi) may slow or prevent cognitive decline associated with AD. TNFi, originally developed as a treatment for RA, have been successfully repurposed for other chronic inflammatory conditions though large observational studies are required to inform the decision to conduct clinical trials before recommending any TNFi as a viable preventative intervention for AD.

Aim
The aim of this thesis was to test the hypothesis that TNFi slow the rate of cognitive decline in a population of adults with RA and MCI compared to conventional synthetic anti-rheumatic disease modifying drugs (csDMARDs).

Methods
This thesis is based on results from the rheumatoid arthritis medication and memory study (RESIST), an 18-month observational study which aims to compare the rate of cognitive decline between TNFi and conventional anti-rheumatic treatment in patients with both rheumatoid arthritis (RA) and MCI. Participants were recruited from rheumatology clinics in Northern Ireland and Southampton and underwent a longitudinal assessment of cognitive change using the Free and Cued Selective Reminding Test (FCSRT) and Montreal Cognitive Assessment (MoCA) with additional assessments of mood, pain and disease activity. This thesis involved i) a preliminary analysis of all participants who were screened for RESIST
in order to investigate the prevalence of MCI - according to a cut-off score of ≤27 in the Montreal Cognitive Assessment (MoCA) - and identify predictors of cognitive impairment in a UK population of adults with RA; ii) a longitudinal analysis of TNFi treatment on cognitive performance (as measured by the FCSRT and MoCA) with adjustments made for baseline scores and other confounders among RESIST 6-month and 12-month cohorts.

**Results**

In analysis of RESIST screening data (n=716), 72% of participants showed indication of cognitive impairment. Regression analysis identified age, educational attainment, rheumatoid factor status and disease activity as predictors of cognitive impairment in this population. In longitudinal analysis of the RESIST follow-up data (6-month cohort, n=130; 12-month cohort, n=69) there was no evidence of a difference between TNFi and csDMARD treatment in cognitive outcomes measured by FCSRT (6-month cohort (mean difference 0.58, 95% CI -1.40, 2.56, p = 0.565); 12-month cohort (mean difference -1.09, 95% CI -3.57, 1.38, p = 0.381)) or MoCA (6-month cohort (mean difference -1.09, 95% CI -3.57, 1.38, p = 0.381); 12-month cohort (mean difference -0.04, 95% CI -1.06, 0.98, p = 0.940)) after adjustment for baseline; nor was there a difference in cognitive outcomes between treatment groups after adjustment for mood, disease severity and other confounding variables.

**Conclusion**

This research found little evidence of a relationship between TNFi and better cognitive performance, therefore suggesting that TNFi may not be clinically beneficial in the prevention of AD. Nevertheless, the RESIST study is a crucial step towards determining the potential of TNFi as a preventative treatment for AD and is a valuable contribution in a topic that is largely under researched.
Oral Presentations


Poster Presentations


Awards
**Publications**


Abbreviations:

AAIC  Alzheimer's Association International Conference
ACE  Addenbrooke's Cognitive Examination
Ach  Acetylcholine
AChE  Acetylcholinesterase
ACR  American College of Rheumatology
AD  Alzheimer's Disease
ADAPT  Alzheimer's Disease Anti-inflammatory Prevention Trial
ADAs  Antidrug antibodies
ADAS-Cog  Alzheimer’s Disease Assessment Scale – Cognitive Subscale
ADL  Activities of Daily Living
aMCI  amnestic Mild Cognitive Impairment
AMCs  Amoeboid microglial cells
ANCOVA  Analysis of Covariance
Anti-CCP  Anti-cyclic citrullinated peptide
APC  Antigen Presenting Cell
ApoE  Apolipoprotein E
APP  Amyloid Precursor Protein
ARUK  Alzheimer's Research UK
Aβ  Amyloid-beta
bDMARD  biologic DMARD
BDNF  Brain-derived neurotrophic factor
BHPR  British Health Professionals in Rheumatology
BHSCt  British Health and Social Care Trust
BMcD  Bethany McDowell (PhD student, Centre for Public Health, QUB)
BMcG  Dr Bernadette McGuinness (Centre for Public Health, QUB)
BPSD  Behavioural and psychological symptoms of dementia
BSR  British Society of Rheumatology
BSRBR  British Society of Rheumatology Biologics Registry
Ca²⁺  Calcium ions
CCL5  C-C Motif Chemokine Ligand 5
CH  Professor Clive Holmes (University of Southampton)
ChAT  Choline acetyltransferase
ChEIs  Cholinesterase inhibitors
CI  Confidence interval
Cl⁻  Chloride ions
CNS  Central Nervous System
COVID-19  Coronavirus Disease
COX  Cyclooxygenase
CRP  C-reactive protein
csDMARDs  conventional synthetic DMARDs
CSF  Cerebrospinal fluid
CT  Computerised Tomography
CXCL10  C-X-C Motif Chemokine Ligand 10
DAMPs  Damage-associated molecular patterns
DAS28  Disease Activity Score Calculator for Rheumatoid Arthritis
DLB  Dementia with Lewy Bodies
DMARDs  Disease modifying anti-rheumatic drugs
EDTA  Ethylenediaminetetraacetic acid
EMA  European Medical Agency
PHF  Paired helical fragments
PNS  Peripheral Nervous System
PPE  Personal Protective Equipment
PRRs  Pattern recognition receptors
PS1  Presenilin 1 gene
PS2  Presenilin 2 gene
PsA  Psoriatic arthritis
p-tau  hyperphosphorylated tau
QUB  Queen's University Belfast
RA  Rheumatoid arthritis
RCT  Randomised controlled trial
REC  Research Ethics Committee
RESIST  Rheumatoid arthritis medication and memory study
RF  Rheumatoid factor
RMCs  Ramified microglial cells
ROS  Reactive Oxygen Species
SAEs  Serious Adverse Events
SD  Standard deviation
SIB  Severe Impairment Battery
SIEs  Systemic inflammatory events
SLZ  Sulfasalazine
sTNFα  soluble TNFα
TACE  TNFα converting enzyme
Tfh  T follicular helper cell
TGF  Tumour Growth Factor
Th  T helper cell
tmTNFα  transmembrane TNFα
TNFi  Tumour Necrosis Factor Inhibitor
TNFR1  TNFα receptor type 1
TNFR2  TNFα receptor type 2
TNFα  Tumour necrosis factor-alpha
Tph  T peripheral helper cell
Treg  Regulatory T cell
tsDMARDs  targeted synthetic DMARDs
UHS  University Hospital Southampton NHS Foundation Trust
UoS  University of Southampton
VaD  Vascular dementia
VAS  Visual Analogue Scale
α  Alpha
β  Beta
γ  Gama
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Chapter 1: Literature Review

1.1 Introduction

The population of adults aged 65 and over is the fastest growing age group in the world.¹ This is likely due to advances in the medical field resulting in a longer life expectancy. However, given that age is the biggest risk factor for neurodegenerative diseases we are now also experiencing a global rise in the number of cases of Alzheimer’s disease (AD) with numbers projected to triple by the year 2050, stressing the need for effective preventative measures.² Understanding the mechanisms that underlie disease pathology and how they differ to those involved in normal ageing are crucial in the search for a preventative treatment for AD.

1.2 An overview of the nervous system

The nervous system is the most complex system in the human body. It receives information about our environment and elicits responses to help us adapt. The nervous system is split into central and peripheral divisions.³ The central nervous system (CNS) is often referred to as the ‘control centre’ of the body. It is responsible for all aspects of cognition and control of body movement.⁴ The CNS comprises the brain, the brainstem and the spinal cord which are protected by meningeal membranes – the dura mater (outermost), arachnoid mater (middle) and pia mater (innermost). The dura mater contains the blood vessels which supplies the brain with blood. The space between the arachnoid mater and pia mater contains cerebrospinal fluid (CSF); a colourless fluid predominantly made of water.⁵ CSF is constantly produced by tissue called the choroid plexus in the four interconnected ventricles of the brain.⁶ The main functions of the CSF are to protect the brain by acting as a shock absorbing layer, supply the brain with nutrients, remove metabolic waste products and maintain cranial pressure. CSF can often be used in the diagnosis of neurological diseases.⁷ The peripheral nervous system (PNS) refers to nervous tissue which arises from the spinal cord and connects the CNS to receptors and effectors throughout the body.⁸ Both the CNS and PNS are made up of neurons and glial cells. Neurons are the main functional unit of the nervous system while glial cells are supporting cells which surround the neurons and provide insulation.³
1.2.1 Structure of a neuron

The CNS contains an estimated 85-100 billion neurons which are arranged in layers (laminae) on the outermost surfaces of the brain; the cerebral cortex & cerebellar cortex.\(^9\) Neurons are highly specialised cells which rapidly receive, process and transmit electrical signals called action potentials to other neurons, muscles or glands to bring about a response e.g. movement or secretion. Neurons have a high metabolic demand and therefore require a constant and stable supply of oxygen and glucose. Their metabolic demand and inability to regenerate after injury or death makes neurons highly vulnerable to conditions such as hypoxia and hypoglycaemia.\(^{10, 11}\)

Neurons can vary in size, shape and type but most will consist of three key structures: a cell body, dendrites and an axon: (i) The cell body (also known as a soma or perikaryon) contains the nucleus and surrounding cytoplasm filled with organelles, particularly ribosomes for protein synthesis.\(^{12}\) (ii) Dendrites are long, highly branched processes that extend from the cell body. They receive input signals from the environment and other cells and relay those signals to the cell body. Dendrites can vary in both number and size with an estimated 5-7 dendrites per neuron.\(^{13}\) Neurons can be pseudounipolar, bipolar or multipolar depending on the location and number of both the dendrites and axon hillock. Multipolar neurons are the most common and have several dendrites, bipolar neurons have a single dendrite and pseudounipolar neurons have sensory endings in place of dendrites.\(^{14}\) The branches (dendritic spines) form synaptic junctions for cell-cell communication. Reduced numbers of dendritic spines have been observed in various diseases including dementia and may be associated with impaired cognitive function.\(^{15-17}\) (iii) The axon is a long process that extends from the cell body at a site called the axon hillock. Each neuron has a single axon which can vary in size and complexity; many neurons in larger vertebrates are surrounded by an insulating membrane called a myelin sheath and are thus categorised as myelinated neurons.\(^{18}\) Myelin is formed by Schwann cells in the PNS and oligodendrocytes in the CNS. The myelin sheath does not cover the entire axon, instead leaving small gaps (nodes of Ranvier) which allow for rapid saltatory conduction of a signal along the length of the axon.\(^{19}\) The axon initial segment in the mammalian CNS is a short region of unmyelinated axon that originates from the axon hillock. It contains a high density of voltage-gated channels and is believed to be the site of action potential generation.\(^{20}\) The axon terminal branches off into fine fibres (terminal arbors) with a bulbous end (terminal bouton), they lie close to the surface membrane of an adjacent cell, usually the dendrite or cell body of an adjacent neuron or effector cell, forming specialised junctions called synapses.\(^{21}\)
1.2.2 Synaptic Transmission

The synapse is a specialised intercellular junction which enables neuronal communication; it refers to both pre- and post-synaptic terminals along with the space between them known as the synaptic cleft. Synapses can be categorised as chemical or electrical. Chemical synapses rely on diffusion of a chemical neurotransmitter across the synaptic cleft (Figure 1.1) while an electrical synapse is a type of gap junction in which paired channel proteins align to form a pore between two cells, enabling passive flow of an ionic current. Most synapses in the CNS are chemical. An action potential, generated in the axon initial segment, is conducted along the length of the axon toward to presynaptic terminal which contains synaptic vesicles loaded with neurotransmitter. Arrival of the action potential at the presynaptic terminal depolarises the presynaptic membrane and enables a rapid influx of calcium ions (Ca\(^{2+}\)) through voltage gated Ca\(^{2+}\) channels. The increase in Ca\(^{2+}\) concentration triggers the synaptic vesicles to fuse with the presynaptic membrane and release their neurotransmitter contents into the synaptic cleft. The neurotransmitter diffuses across the cleft and binds to receptors on the postsynaptic membrane.

Figure 1.1: Illustration of synaptic transmission across a chemical synapse.\(^{24}\)
Chemical synapses can be inhibitory or excitatory depending on the action of the neurotransmitter that is released from the presynaptic membrane and whether or not it induces generation of an action potential. Glutamate is an example of an excitatory neurotransmitter that causes membrane depolarisation and an influx of sodium ions (Na⁺) in the postsynaptic cell thus generating a new action potential. If an inhibitory neurotransmitter is released from the presynaptic cell e.g. gamma-aminobutyric acid (GABA) the post-synaptic cell membrane does not undergo depolarisation, often becoming hyperpolarised due to an influx of chloride ions (Cl⁻) or efflux of potassium ions (K⁺), and so no action potential is generated.

For the signal to be terminated the neurotransmitter must be removed from the synapse in one of three ways. (i) Small molecule neurotransmitters can be taken up by the presynaptic cell or glial cells where they can be recycled. (ii) The neurotransmitter can be degraded in the synaptic cleft by an enzyme e.g. monoamine oxidase. (iii) The neurotransmitter can simply diffuse away from the synaptic cleft where it enters the blood stream.

Synaptic plasticity describes the ability of a neuron to change the efficacy of its synapse. These changes can vary in duration and mechanism. One of the best-known examples of synaptic plasticity is long-term potentiation i.e. the mechanism thought to underlie memory and learning in mammalian brains which involves increases in both strength and number of synapses. Several studies have documented changes in synaptic plasticity during ageing in both animal and human models, including deterioration and loss which may contribute to age-related memory decline.

1.3 The brain

The brain is one of the largest and most complex organs in the body, it is responsible for controlling essential body functions such as breathing and heart rate and is also responsible for movement, speech, memory and sensory perception. The brain is comprised of billions of neurons and glial cells that are organised into defined areas. Neurons can be clustered together to form a nucleus or arranged in laminae. Neurons in the outermost layers of the brain are arranged in lamina whereas most neurons in the deeply located midbrain and hindbrain are arranged in nuclei. Due to advances in brain imaging it is possible to observe the brain in both healthy and diseased states and to monitor the brain’s response to different treatments in a way that is non-invasive for the patient, e.g. computerised tomography (CT) and positron emission tomography (PET) are methods of observing the anatomy and functional activity of
1.3.1 Organisation of the brain

The brain can be divided into three distinct areas: the cerebrum, the cerebellum and the brainstem. The cerebrum is the largest and most anterior part of the brain that is split into left and right hemispheres. The two hemispheres remain connected through a bundle of white matter fibres called the corpus callosum which allows information to pass from one side to the other in such a way that the left hemisphere controls the right side of the body and the right hemisphere controls the left side of the body.\textsuperscript{38} On its external surface the cerebrum has a highly folded or ‘wrinkly’ appearance. This is due to the cerebral cortex which consists of 6 layers of grey matter folded into hills (gyri) and valleys (sulci).\textsuperscript{39} Despite being the largest part of the brain the cerebrum only contains around 20\% of the brain’s neurons; the cerebellum, which makes up only 10\% of brain mass, contains an estimated 80\% of neurons in the brain.\textsuperscript{40} The cerebellum, or ‘little brain’, sits at the base of the brain (above the brainstem) and is mostly involved in controlling balance, coordinating movement and learning new motor tasks.\textsuperscript{41}

The brainstem connects the brain to the spinal cord and consists of the midbrain, medulla oblongata and the pons. It is responsible for controlling important autonomic functions including breathing, blood pressure and heart rate.\textsuperscript{42}

1.3.2 The four major lobes

The cerebral cortex of each hemisphere is divided into four functionally distinct lobes: frontal, parietal, temporal and occipital.

(i) The frontal lobe is the largest of the lobes, comprising approximately 40\% of the cerebral cortex.\textsuperscript{43} The frontal lobe in humans is much larger than in other mammals due to its role in a range of executive functions and higher order cognitive processes e.g. planning, emotional control and working memory.\textsuperscript{44} Contained within the frontal lobe is the primary motor cortex for the control and coordination of skeletal muscle and Broca’s area for the production of speech.\textsuperscript{45}

(ii) The temporal lobe is located on the side of the brain where it sits below the temporal bone. It can be subdivided into inferior, middle and superior temporal lobes which are each responsible for different functions.\textsuperscript{46} The superior temporal lobe contains the auditory association cortex for processing sound and Wernicke’s area for language comprehension. The inferior temporal lobe receives input from the occipital lobe and is involved in visual recognition and has also been implicated in some aspects of short-term memory. The medial temporal lobe contains the hippocampus and several related structures which have a crucial role in long-term declarative memory.\textsuperscript{47-49}
(iii) The parietal lobe is located at the top of the brain, between the frontal and occipital lobes. It contains the somatosensory cortex which is responsible for processing, interpreting and integrating sensory information e.g. touch, pressure, temperature.\textsuperscript{50} The parietal lobe is involved in the perception of location and movement of the body (proprioception) and has also been implicated in the comprehension of speech and motor control of writing.\textsuperscript{51-53}

(iv) The occipital lobe is located at the back of the cerebral hemispheres, superior to the cerebellum, and is the smallest of the four lobes. It contains the primary visual cortex and visual association cortices which are involved in processing information for the interpretation and recognition of objects including perception of colour, depth and motion.\textsuperscript{54}

1.3.3 The limbic system

The limbic system refers to a group of interconnected cortical and subcortical structures that have roles in memory, behaviour, emotion and olfaction. Some of the key structures within the limbic system are the cingulate gyrus, hippocampus, amygdala, thalamus and hypothalamus along with anatomically related areas i.e. entorhinal, perirhinal and parahippocampal cortices.\textsuperscript{55}

In 1878, Paul Broca coined the term ‘le grand lobe limbique’ (the great limbic lobe) to collectively describe the hippocampus, cingulate gyrus and anterior olfactory region. He noted that the limbic lobe structures received many projections from the olfactory system and therefore believed it to be primarily involved in processing and interpreting smells.\textsuperscript{56,57}

The role of the limbic structures was further elaborated by Papez (1937) and MacLean (1949) who documented the involvement of the limbic system, particularly the amygdala and cingulate gyrus, in social behaviour and emotional responses e.g. fear, anxiety, arousal.\textsuperscript{58-60} The hippocampus, located deep in the temporal lobe, was the location in which long term potentiation was first discovered and was therefore thought to be crucial for learning and memory.\textsuperscript{61} The hippocampus works in coordination with the amygdala for the integration of emotion and memory, enabling us to store memories of our emotions so that they can be recognised when experienced again. Integration of emotion and memory is also important in emotion-based learning; motivation and stress have both been shown to affect learning and memory performance.\textsuperscript{62-65}

Degeneration of the limbic system structures, particularly the hippocampus, is commonly seen in amnesia and neurodegenerative diseases like Alzheimer’s disease in which memory loss is often the primary symptom. Hippocampal abnormalities have also been implicated in epilepsy, depression and psychiatric disorders e.g. schizophrenia.\textsuperscript{61,66}
1.4 Memory

Memory is an essential part of cognition, without which we would not be able to accomplish day-to-day activities. It is a term used to describe the structures and processes involved in encoding, storing and retrieving information.\(^{67}\)

Despite the earlier works of Ebbinghaus (1885) and James (1890), a distinction between long term and short-term memory was not widely accepted until the 1970s when a patient with damage to parietal and temporal lobes showed an impaired ability to remember recent events but could still recall childhood memories.\(^{68,69}\) This was perhaps aided by the discovery of long-term potentiation in the hippocampus.\(^{70}\)

By the end of the 80s both long-term and short-term memory had been further subdivided.\(^{71-73}\)

1.4.1 Short-term memory

Short-term memory is a type of immediate memory which reflects our ability to hold a limited amount of information (e.g. a list of numbers or words) for a short period of time, typically several seconds.\(^{74}\) Miller (1956) proposed that the capacity of short-term memory was 7 (+/-2) items.\(^{75}\) A large amount of information in the short-term memory store will be forgotten and cannot be recovered, however some may undergo a process called memory consolidation in which information is transferred to long-term memory stores. Which memories are chosen to be consolidated to long-term stores depends on level of attention, personal significance and repetition of the information in the short-term store.\(^{76}\)

Sensory memory is a type of automatic short-term memory in which sensory information e.g. sight, hearing or touch is received, processed and encoded (i.e. as visual, auditory or haptic) before being relayed to the short-term memory store. Sensory memory lasts only milliseconds with less than one-hundredth of the sensory information we receive reaching our short-term memory stores.\(^{77,78}\)

Working memory was a term first introduced by Miller \textit{et al.} (1960) to describe a type of short-term memory that is associated with more complex activities e.g. mental arithmetic, planning and execution of tasks.\(^{79,80}\) The working memory model proposed by Baddeley and Hitch (1974) proposes that working memory is a multicomponent system that consists of dedicated stores for certain types of information. This model (\textbf{Figure 1.2}) includes the phonological loop and visuospatial sketchpad for the processing and temporary storage of verbal and visual information and the central executive for assigning information to these areas.\(^{81,82}\) The model was updated by Baddeley in 2000 to include the episodic buffer for integration of information between the components of the working memory model in order to create a cohesive memory.\(^{83}\)
1.4.2 Long-term memory

Long-term memory differs from short-term memory in both capacity and duration with the ability to hold large amounts of information for days, weeks or even years. Long-term memory was subdivided into explicit and implicit in 1985.Implicit or procedural memory is primarily responsible for performing tasks without conscious recollection e.g. driving a car, tying a shoelace.

Implicit memory draws on previous experiences in order to facilitate a relatively automatic action and can be established through different mechanisms of learning e.g. priming, conditioning, habit learning, motor and cognitive skill learning. Implicit memory does not require the hippocampus for formation or storage and therefore typically remains intact in patients with amnesia.

Explicit or declarative memory is information that can be consciously recalled e.g. names and dates. The medial temporal lobe, including the hippocampus, are essential in the formation, maintenance and retrieval of declarative memory and therefore will be impaired in amnesia. Declarative memory can be further categorised as either episodic (events) or semantic (facts and general knowledge).

Episodic memory is closely related to autobiographical memory, a uniquely human system that stores memories of personal experiences and knowledge about our own lives. Some studies have proposed that there is dissociation between episodic and semantic memory systems as it was observed that damage to the medial temporal lobe, particularly in the hippocampus, can affect episodic memory while leaving semantic memory largely intact - this suggests that episodic memory may more heavily rely on the hippocampus. Others have...
suggested that episodic and semantic memory are interdependent where one form may influence the acquisition and retrieval of the other.\(^9^6\)

### 1.4.3 Memory consolidation

Memory consolidation was first proposed in 1900 as the process in which short-term memory is transformed into long-term memory.\(^9^7\) Consolidation involves the brain undergoing both synaptic and systemic changes including the formation of new synaptic connections, strengthening of existing connections and systemic reorganisation of memory storage and retrieval.\(^9^8\),\(^9^9\)

During systemic reorganisation, newly acquired information gradually becomes less reliant on the hippocampus and medial temporal lobe for storage and retrieval and is instead distributed throughout different cortical regions in the neocortex.\(^1^0^0\),\(^1^0^1\) Memory consolidation is therefore thought to explain why damage to the medial temporal lobe, particularly in the hippocampus, appears to affect memories made just before the onset of damage but does not affect distant memories.\(^1^0^2\)

Evidence of this comes from studies of amnesia. One of the best-known studies in humans is a patient named H.M who suffered from a severe form of epilepsy. In attempt to alleviate his condition, H.M underwent surgery in which parts of his medial temporal lobe were removed. Although this reduced the frequency of his seizures it left him with the inability to form new declarative memories (anterograde amnesia) as well as an inability to recall recent memories spanning the 11 years leading up to his surgery (retrograde amnesia). However, he was still able to recall memories from his childhood.\(^1^0^2\)-\(^1^0^5\)

Similar observations have been documented in other studies of retrograde amnesia using both animal and human models with varying amounts of recent memory loss depending on the extent and location of the damage sustained to the medial temporal lobe.\(^1^0^6\)-\(^1^0^8\)

Amnesia is typically the result of brain trauma and is not a consequence of normal ageing, although there is also a certain amount of memory decline that occurs as part of the ageing process with even more severe memory deficits observed in neurodegenerative diseases.\(^1^0^4\)
1.5 The brain in ageing

While ageing causes obvious changes to our outward appearance, there are more subtle structural and functional changes occurring in the brain. These changes can be reflected in slower reflexes, cognitive decline and deterioration of the senses e.g. hearing loss. 109

1.5.1 Structural changes

As our brains age, they undergo widespread structural alterations. Non-invasive neuroimaging techniques have been able to show that age-related changes in the brain vary widely in rate and extent in different brain regions and between individuals. 110 Ageing particularly affects the frontal and temporal lobes with less pronounced effects on the parietal and occipital lobes. 111 The volume of the brain peaks in the early 20s and gradually declines throughout the rest of adulthood, with a faster rate of decline after the age of 70. 112, 113 Changes in brain volume were initially believed to be due to substantial neuronal loss. Early research reported a 10-60% reduction in neuronal cell density in the brain, particularly in the hippocampus, from childhood to late adulthood. 114, 115 It has since been recognised that these studies were subject to various methodological issues and were not an accurate representation of neural plasticity in the ageing brain. 116 It is now widely accepted that the extent of neuronal loss in the neocortex and hippocampus in ageing is not as significant as once thought 117, 118 and that age-related changes in brain volume may be more a result of neuronal shrinkage, reduction in length and number of dendrites, loss of dendritic spines, myelin deterioration and synaptic injury which all contribute to slower cognitive processing and memory impairment. 119-122

1.5.2 Chemical changes

Mitochondria are the main source of reactive oxygen species (ROS) in the brain and are known to lose function and gain mutations with increasing age. 123 This can lead to an imbalance between ROS and antioxidant production and cause oxidative damage to lipids, proteins and DNA. 124 The brain is particularly susceptible to oxidative stress due to the high content of lipids in neuronal membranes and may experience loss in structural integrity and impaired cognitive ability as a result. 125 Age-related decline in cognitive and motor function may also be explained by the change in neurotransmitter systems in the brain with increasing age. Acetylcholine, dopamine and serotonin along with their respective receptors have been found to decline with age and can be a manifestation of age-related disease. 121, 126 The dopaminergic system appears to be
particularly susceptible to the aging process. Reduced concentrations of dopamine and dopaminergic receptors/neurons in old age have been associated with impaired higher order cognitive and motor function. Significantly reduced concentrations can lead to age-related disorders e.g. Alzheimer’s disease (AD), Parkinson’s disease (PD). Reduced concentrations of dopamine and dopaminergic receptors/neurons in old age have been associated with impaired higher order cognitive and motor function. Significantly reduced concentrations can lead to age-related disorders e.g. Alzheimer’s disease (AD), Parkinson’s disease (PD). 127-129 Hormonal changes with increasing age have also been linked to changes in cognition, particularly in older women.130 Many studies that have investigated the effects of oestrogen replacement therapy on cognition were subject to methodological limitations and have thus produced conflicting results.131, 132 While some clinical trials have reported that oestrogen use may protect against cognitive decline and reduce the risk of AD in women,133, 134 others found no cognitive benefit.135, 136 Due to inconsistent evidence and potential increased risk of cardiovascular disease137, cancers (e.g. ovarian, endometrial and breast cancer)138-140 and blood clots141, oestrogen replacement therapy is not currently recommended for the prevention or treatment of AD.

1.5.3 Age-related memory decline

Memory problems are some of the most common complaints amongst older adults but are not always indicative of neurodegenerative diseases. Multiple studies over the past 100 years have found that older adults have a markedly reduced performance in a variety of memory tasks compared to younger adults, however not all aspects of memory are equally affected by age.142 Semantic memory e.g. general and verbal knowledge, has been shown to slightly improve throughout adulthood and only begin to decline in older age.143 It has been suggested that semantic memory is more influenced by educational attainment than age.144 Contrastingly episodic memory appears to be one of the most age-sensitive memory systems, though the age of onset of episodic memory decline is unclear.145 Older adults typically show worse performance in tasks involving free/cued recall and recognition compared to younger adults.146 Several cross-sectional studies have reported a gradual decline that begins in the 20s or 30s and continues throughout life with a faster rate of decline in late adulthood,147-149 however adjusted longitudinal studies have shown episodic memory remains stable in early adulthood with decline beginning around age 60.149, 150 One source of inconsistency has been attributed to difference in educational level. Earlier cohorts will tend to have fewer years of education than those of a younger generation. Once the cross-sectional studies controlled for generational differences in educational attainment, they revealed a later age of onset similar to the longitudinal studies.149, 151 Age-related decline in episodic memory has been linked to an inability to form new connections and as such mostly affects recent memory rather than memories from childhood.152
Despite the significant evidence of age-related decline in explicit memory, implicit memory (i.e. unconscious recollection of a prior event) is thought to be preserved in older individuals.\textsuperscript{153, 154} Unlike episodic memory, implicit memory involves the strengthening of existing connections rather than formation of new ones which may explain why it appears to be preserved in ageing.\textsuperscript{151} This has been disputed by some studies that have found a marked decrease in implicit memory in older adults compared to younger individuals.\textsuperscript{155-157} A meta-analysis by La Voie and Light (1994) found a small yet significant effect of age on implicit memory.\textsuperscript{158} The inconsistency could be in part due to the small sample sizes used in former studies which were not able to detect any age-related change in implicit memory.\textsuperscript{159} It would therefore appear that implicit memory may also decline with age but to a much lesser extent than explicit memory.\textsuperscript{160}

The concept of a cognitive reserve is one which may explain the difference in the rate of cognitive decline observed in ageing and neurodegeneration between individuals. It proposes that the amount of intact brain influences cognitive function rather than amount of atrophy or disease pathology.\textsuperscript{161} This means that individuals with larger cognitive reserves will be able to compensate for age-related structural changes and disease pathology for longer than those with small reserves, thus delaying the onset of cognitive deficits.\textsuperscript{162} Cognitive stimulation and physical exercise are believed to increase cognitive reserves and may therefore protect against age-related cognitive decline and neurodegenerative diseases.\textsuperscript{163-165}

\textbf{1.6 Dementia and Alzheimer’s disease}

Dementia is a syndrome that encompasses a set of cognitive and psychiatric symptoms, including memory decline and changes in behaviour, that occur when the brain is damaged by disease.\textsuperscript{166} Dementia currently affects nearly 50 million people globally with an estimated 10 million new cases each year and is now one of the leading causes of death in the older population.\textsuperscript{2, 167} Projections have shown that by the year 2050 there may be as many as 152 million people living with dementia worldwide.\textsuperscript{168} AD is the most common cause of dementia, accounting for 60-70\% of all cases.\textsuperscript{2} Vascular dementia (VaD) is the second most common cause, accounting for up to 20\% of cases while dementia with Lewy bodies (DLB) accounts for around 15\%.\textsuperscript{168, 169} The global prevalence of frontotemporal dementia (FTD) is more uncertain but is believed to make up less than 5\% of all dementia cases.\textsuperscript{170}

It is possible for an individual to be diagnosed with more than one type of dementia (mixed dementia), this is typically seen with a mixed diagnosis of AD and VaD. Mixed dementias are thought to affect at least one in every ten people with dementia.\textsuperscript{167} All dementia subtypes are
progressive diseases and therefore symptoms will get worse over time.\textsuperscript{166}

1.6.1 AD progression

AD involves several stages ranging from pre-clinical AD to AD dementia (\textbf{Figure 1.3}).\textsuperscript{171} Early identification is key in finding a disease-modifying treatment that can prevent cognitive decline and AD progression. However, there is a certain amount of debate surrounding the diagnostic criteria for AD which makes it difficult to define the earliest stages.\textsuperscript{172-174} Amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) are hallmarks of AD pathology and are thought to begin accumulating around 20 years before the onset of symptoms; this is therefore referred to as the asymptomatic or pre-clinical stage.\textsuperscript{175} Advances in PET imaging and CSF analysis have enabled the detection and monitoring of AD pathology in vivo and may be clinically useful in identifying those in the pre-clinical stage who are at risk of developing AD later in life.\textsuperscript{171, 175}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{An illustration of the clinical progression of AD (adapted from Sperling et al. 2011).\textsuperscript{171}}
\end{figure}

AD is understood to become symptomatic when neuronal atrophy reaches a certain threshold.\textsuperscript{176} Typically, a significant amount of neuronal loss occurs in the medial temporal lobe (entorhinal cortex and hippocampus) and therefore the first symptom to appear is often a noticeable decline in memory, particularly an inability to remember recent events.\textsuperscript{177} This is the symptomatic predementia stage of AD and is referred to as mild cognitive impairment (MCI).\textsuperscript{178} MCI can have different clinical manifestations, e.g. amnestic MCI (aMCI) describes cognitive decline with an emphasis on memory loss that is not severe enough to interfere with daily life.\textsuperscript{179} It can be difficult to distinguish MCI from cognitive decline that is associated with normal ageing. Currently the diagnosis of MCI relies on evidence of cognitive decline in the absence of dementia or other neurodegenerative diseases and can be measured by
neuropsychological assessments such as the Mini Mental State Examination (MMSE) or the Montreal Cognitive Assessment (MoCA). Neuroimaging and biomarker analysis may also be used to aid the diagnosis of MCI and to predict those who are at risk of progressing to AD. Not all MCI patients will develop AD or any other dementia, however an estimated 10-15% will develop AD every year compared to 1-2% in non-cognitively impaired individuals. There are several external factors that may contribute to the development of MCI e.g. anxiety/depression, physical illness, side effects of medication. Once these factors are removed MCI may be reversed. Patients that develop AD experience progressive decline in cognition; their symptoms become more severe until the individual can no longer carry out day-to-day activities like eating, dressing and walking without help. During the terminal stages of the disease, also known as Alzheimer's dementia, the individual is usually bed-bound and requires round-the-clock care.

Unfortunately, there is no one test that can clinically diagnose AD. Instead, a combination of clinical and pathological approaches will be employed by primary care doctors and specialists. At the initial appointment, a detailed medical history will be recorded with the help of the patient’s primary carer/family member including any recent changes to the patient’s memory, behaviour or personality. A clinical diagnosis of dementia often relies on an impaired ability to perform activities of daily living (ADLs). ADLs are basic actions involved in daily self-care (e.g. bathing, dressing, cooking, housekeeping) and can be monitored using an ADL scale. ADL assessment is useful in determining both type and severity of the dementia.

A physical examination will also be performed in order to test motor and sensory neuron responses (e.g. reflexes, coordination, vision and hearing) followed by assessments of the different cognitive domains, usually using the Addenbrooke’s Cognitive Examination (ACE), MMSE or similar test. A key part of diagnosing AD is ruling out other conditions which may have similar clinical presentations – this can be achieved through laboratory testing of blood/urine samples or diagnostic imaging such as Magnetic Resonance Imaging (MRI), CT or PET scans that can help to identify signs of tumour or stroke which may be the underlying cause of AD-like symptoms.

Biomarkers are currently being studied to enable accurate preclinical diagnosis of AD for the earliest possible intervention. The ideal biomarker would detect the characteristic plaques and tangles associated with AD. It would be inexpensive and non-intrusive to obtain from the patient. It should have a high sensitivity to detect AD in the earliest stages of MCI and a high specificity to distinguish AD from other types of dementia. Many of the biomarkers already studied have come from the CSF however it would be ideal to identify a biomarker within the
blood plasma as this would be much less invasive to obtain from the patient. Aβ plaques and NFTs have been investigated as biomarkers as they are both present in the preclinical phase of AD. However, neither plaques nor tangles are specific to AD as both can be found in other neurodegenerative diseases as well as in the normal ageing population.

### 1.6.2 Risk factors

There are several risk factors associated with development of AD, some of which can be controlled and others which cannot. The most important risk factor is age. The Alzheimer's Society estimated that the risk for AD approximately doubles every 5 years of age. The majority of people who are diagnosed with AD are greater than 65 years old with more than 90% being over the age of 70. It is possible to develop AD under the age of 65 and while this is less common there are still an estimated 40,000 cases of early onset AD (EOAD) in the UK. As AD is a slowly progressive disease it is not uncommon for people to live for 10 or more years after being diagnosed.

In addition to age, other non-modifiable risk factors include biological sex and genetics. For reasons that are yet unclear, AD appears to affect more women than men. It has been suggested that the difference could be due to hormonal effects of oestrogen/testosterone or even the existence of predisposing genes on the X chromosome.

Genetic risk factors are mostly associated with the cases of AD which are inherited i.e. EOAD, also referred to as familial AD (FAD). In such cases, the disease generally develops under the age of 65 and is usually due to an inherited mutation in certain genes – the amyloid precursor protein (APP) gene and the Presenilin 1 (PS1) and Presenilin 2 (PS2) genes. These genes are involved in the processing of APP and so mutations can cause an increased burden of amyloid plaques which can further the progression of the disease. These early onset/familial cases are rare and only account for around 5% of all AD cases.

Down's syndrome is a genetic disorder caused by an extra copy of chromosome 21 (trisomy 21), the location of the APP gene. Thus individuals with Down’s syndrome are genetically predisposed to developing AD due to increased expression of APP, with most showing signs of AD pathology upon autopsy.

The apolipoprotein E (apoE) polymorphism is a well-established susceptibility gene for late onset or sporadic AD. ApoE is a protein that mediates the transportation and redistribution of cholesterol and phospholipids between cells of different types. The human apoE protein is polymorphic and thus has three alleles, APOε2, APOε3 and APOε4. APOε4 allele frequency is elevated in both familial and sporadic late-onset AD, reaching levels of 40-50% above that of the normal population. It has been established that the APOε4 allele increases the risk for AD 3-fold if inherited in the heterozygous pattern and 10 to 12-fold in the homozygous pattern. Contrastingly the APOε2 allele seems to reduce the risk of developing AD.
Environmental and lifestyle factors such as obesity, depression, smoking and educational attainment may account for up to 35% of the risk of AD. Targeting these modifiable risk factors may not only protect against AD but other age-related diseases.

Obesity is often thought to increase the risk of AD through systemic inflammation; white adipose tissues contain large numbers of activated macrophages which produce pro-inflammatory cytokines that contribute to neuronal injury. Certain clinical consequences of obesity have also been identified as risk factors for AD including high cholesterol and high blood pressure. A healthy lifestyle with a balanced diet and regular exercise can control weight, blood pressure and cholesterol levels while reducing the chance of developing diabetes or suffering from a stroke or ischaemic heart disease – all of which can be associated with an increased risk of AD.

Physical activity has been shown to decrease cortical amyloid burden and may increase neurogenesis in the hippocampus through the release of neurotrophic factors, thus improving long-term memory. The Mediterranean diet may disrupt amyloid aggregation while exerting antioxidant and anti-inflammatory effects, thereby reducing the risk of AD. However, both strategies can have issues with adherence in clinical trials.

Some reports have found an association between level of education and AD. It was suggested that those who are less educated are more at risk of developing AD; the reasons for this are mostly unknown. As aforementioned, a suggested explanation for this is that individuals with higher education will have greater cognitive reserves which can compensate for the initial neuronal loss for longer. Depression and head injuries have also been shown to increase the risk of AD.

### 1.6.3 Clinical symptoms

The presenting symptoms of AD are unique to each person and depend on the areas of the brain that are most affected by damage. Symptoms tend to be categorised as either cognitive or non-cognitive; those that are commonly seen in AD include difficulty finding the correct word (language), difficulty recalling recent events (memory), taking longer to complete daily tasks (complex attention), difficulty with planning and organising (executive function), getting lost in familiar places (perceptual motor/visuospatial function) and lack of interest/apathy (social cognition).

Early AD pathology originates in the temporal lobe and so cognitive symptoms, particularly episodic memory deficits, are usually the first to manifest in AD and will be noticeable to the patients themselves and those closest to them. Episodic memory deficits can lead to forgetting appointments, losing keys or potentially more dangerous incidents like forgetting to take medications or turn off the gas on a stove. While semantic memory is relatively well
preserved in normal ageing it appears to be disrupted in AD and can be reflected in reduced naming accuracy.\textsuperscript{226} It is important to note the effects of age on memory are mild compared to those seen in AD which more closely reflect retrograde amnesia.\textsuperscript{227} Non-cognitive symptoms, also known as behavioural and psychological symptoms of dementia (BPSD), can often include changes in personality e.g. becoming aggressive, apathetic, depressed or anxious.\textsuperscript{228} In later stages of AD some individuals may also experience hallucinations or delusions as well as disrupted sleep patterns and changes to appetite.\textsuperscript{229, 230} BPSD have been associated with altered glucose metabolism throughout the cerebral cortex, particularly in the frontal lobe.\textsuperscript{231-233} It is thought that individuals who show signs of mild behavioural impairment with no cognitive symptoms are at greater risk of developing dementia than those with MCI.\textsuperscript{234} Early behavioural symptoms are typically associated with FTD however almost all dementia cases will experience BPSD as they progress.\textsuperscript{235} BPSD can put considerable strain on the individual and their caregivers. and are a major reason behind early placement in nursing homes.\textsuperscript{236, 237}

1.6.4 Symptomatic treatment

The current treatments that exist for AD are symptomatic in nature and therefore only delay the course of the disease rather than preventing it. Biochemical examination of AD brain biopsies in the 1970s showed a significant reduction in the number of cholinergic neurons in the basal forebrain, cerebral cortex and hippocampus in the early stages of disease.\textsuperscript{238} A reduction in acetylcholine (ACh) synthesis was also identified due to a deficit in the ACh producing enzyme, choline acetyltransferase (ChAT).\textsuperscript{239, 240} ACh is thought to play an important role in memory storage and retrieval; it was therefore proposed that cholinergic dysfunction contributes to cognitive impairment in AD; this is known as the cholinergic hypothesis.\textsuperscript{241, 242} Cholinesterase inhibitors (ChEIs) were developed as potential treatments for memory symptoms in AD, they prevent the breakdown of ACh by acetylcholinesterase (AChE) and prolong neurotransmitter activity in cholinergic synapses with the aim of improving neuronal communication in the brain.\textsuperscript{243} There are currently three ChEIs that have been approved for the treatment of AD: donepezil, galantamine and rivastigmine.\textsuperscript{244} There is little evidence of a difference in efficacy between the three medications.\textsuperscript{245, 246} although donepezil is the only one of the three that is approved for the treatment of severe AD and has also been associated with reduced incidence of side effects with higher therapeutic doses compared to galantamine and rivastigmine.\textsuperscript{247, 248} Early administration of AChE inhibitors in patients with mild to moderate AD appears to improve memory performance and quality of life while delaying AD progression compared to
those on a placebo treatment. However, there is evidence to suggest that the effects of AChE inhibitors lasts a maximum of 2 years and therefore cannot be used for long-term prevention of cognitive decline in AD.

Another treatment for memory related symptoms in AD is the N-methyl-D-aspartate (NMDA) antagonist, memantine. NMDA receptors play a key role in excitatory transmission and are important in synaptic plasticity, memory and learning.

In AD, Aβ is believed to cause an excessive amount of glutamate to accumulate in synapses. Glutamate is an excitatory neurotransmitter and so excessive accumulation leads to over-activation of the NMDA receptors, causing a prolonged influx of Ca\(^{2+}\) into the postsynaptic neuron and resulting in cell death and progressive decline in cognitive function. While NMDA receptors have a role in glutamate excitotoxicity, they have also been implicated in tau induced neuronal damage. Memantine works by blocking the NMDA receptor, thereby preventing glutamate from binding and subsequent neuronal injury. There is evidence that memantine improves both cognitive and behavioural symptoms in patients with moderate to severe AD. Further improvement was observed when memantine was used in combination with donepezil or vitamin E, however these combination therapies did not have the same cognitive benefit in mild to moderate AD. Vitamin E is an antioxidant which has been shown to delay cognitive decline compared to a placebo in moderately severe AD, although the clinical significance of this appears to be minimal and the same effect was not replicated in individuals with MCI. Vitamin E supplementation is not currently used as a clinical intervention for AD due to limited and inconsistent evidence surrounding its efficacy and possible serious adverse side effects e.g. bleeding, haemorrhagic stroke, prostate cancer and increased mortality.

While ChEIs and memantine are established treatments for memory symptoms in AD, they have also been shown to improve behavioural symptoms e.g. apathy, depression, agitation. There are several treatment options for BPSD, both pharmacological and non-pharmacological. Pharmacological interventions such as antidepressants and antipsychotics are among those prescribed for BPSD however some studies have reported that the occurrence of adverse events outweigh the clinical benefits and so any use should be carefully considered and closely monitored.

Most guidelines recommend non-pharmacological interventions as the first choice for management of BPSD. These non-pharmacological approaches are patient-centred and may have to be tailored to the needs of each individual in order to get the most benefit – this can make interpreting results from clinical trials quite difficult. Behavioural therapy, reality orientation therapy and reminiscence therapy are all examples of standard non-pharmacological approaches that have demonstrated positive effects on both cognitive and behavioural symptoms, however reports on their efficacy are inconsistent due to the
variability in study designs from patient to patient. Alternative non-pharmacological interventions are less studied but have shown promising results in symptomatic treatment including bright light therapy, multisensory therapy, aromatherapy, music therapy and art therapy.

There are a number of interventions that have been studied for use in AD but have not been approved due to lack of cohesive evidence including statins, nonsteroidal anti-inflammatory drugs (NSAIDs), ginkgo biloba and omega-3 fatty acids.

1.6.5 Pathogenesis

As previously discussed, there is a certain amount of neuronal atrophy that occurs in healthy ageing, especially in the frontal cortex. It is therefore difficult to distinguish AD from normal ageing in older patients based solely on brain volume. However, in cases of EOAD, comparisons of brain weight with age-matched controls typically show an obvious difference. Gross examination of the AD brain may reveal significant atrophy in the hippocampus and entorhinal cortex which may not be present to the same extent in age-matched controls.

There are several different hypotheses that propose mechanisms behind AD pathogenesis including i) the amyloid cascade ii) tau hyperphosphorylation iii) cholinergic dysfunction iv) the mitochondrial cascade v) metabolic changes vi) vascular dysfunction. A definitive diagnosis of AD depends on the significant presence of extracellular Aβ plaques and intracellular NFTs in certain regions of the brain. These proteins are associated with inflammation and oxidative stress and therefore contribute to the destruction of neurons in the brain resulting in brain shrinkage and deterioration in cognitive function. Despite the belief that fibrillar Aβ triggers NFT formation, several studies have shown that the concentration of NFTs is more strongly correlated to the severity of cognitive decline in AD than Aβ plaque distribution.

Tau proteins are microtubule associated proteins that help to maintain microtubule stabilisation and have a role in their assembly – thus they are abundant in neuronal axons. There are six different isoforms of tau protein that are normally produced by neurons in the brain through alternative messenger RNA (mRNA) splicing. Tau undergoes post-translational modifications (e.g. phosphorylation, glycosylation) which can affect the affinity of tau for microtubules and under pathological conditions may promote misfolding and aggregation.

In AD, tau pathology begins in the medial temporal lobe structures (entorhinal cortex, hippocampus, amygdala) and spreads to the neocortex. Tau can become hyperphosphorylated (p-tau) and lose the ability to associate with microtubules, thus compromising microtubule stability and causing neurons to become more vulnerable to damage. P-tau can self-assemble to form paired helical filaments (PHFs) which can
aggregate to form neurotoxic NFTs in the cell bodies of neurons. However, NFTs are not specific to AD and are found in many different neurodegenerative diseases as well as in cognitively normal individuals, suggesting that tau aggregates may occur in response to a number of pathologic events such as oxidative stress, inflammation and, in some cases of FTD, mutations of the microtubule associated protein tau gene (MAPT).

The Aβ peptide is derived from the larger amyloid precursor protein (APP) and is produced by enzymatic cleavage. APP is initially cleaved by either β-secretase or α-secretase and subsequently cleaved by γ-secretase to form the Aβ and p3 peptides respectively. γ-secretase can cleave at multiple sites and can therefore produce Aβ peptides of different lengths, between 38 and 42 residues. Aβ40 is the most common, accounting for around 90%. Aβ42 accounts for less than 10% but has a major role in the pathogenesis of FAD. Mutations in APP, PS1 or PS2 genes increase the concentration of the more neurotoxic Aβ42 and promotes aggregation. Sporadic AD does not appear to overproduce Aβ42, but the elevated level could be explained by poor clearance mechanisms or increased aggregation.

The amyloid cascade hypothesis (Figure 1.4) proposes that an imbalance between the production and removal of Aβ leads to accumulation of Aβ plaques, induces the formation of NFTs and drives the progression of AD. However some studies have questioned the likelihood of this given that plaques and tangles originate in different areas of the brain and can be found in the non-cognitively impaired population as well. Unlike NFTs, Aβ plaques initially accumulate in areas of the brain that have a high metabolic demand e.g. association cortices, before becoming widespread throughout the cerebral cortex and cerebellum.

Figure 1.4 The amyloid cascade hypothesis (adapted from Hardy and Selkoe, 2002).
Many of the potential therapeutic treatments for AD have been aimed at reducing the burden of Aβ plaques in the brain with hopes of preventing further cognitive decline. While some studies may give positive initial results in animal models and pre-clinical studies, they were less successful in human trials with many phase III clinical trials failing to reach their endpoints. \(^{303-308}\) Schenk (2002) reported on the severe adverse reactions that some AD patients had in response to Aβ immunotherapy. \(^{309}\) Taking into consideration the extensive research on amyloid-based treatments and the lack of results, it may be time to investigate other potential therapeutic targets within the disease pathway. Oxidative stress \(^{310}\), blood-brain barrier dysfunction \(^{311}\), and neuroinflammation \(^{312}\) are also thought contribute to disease progression and may provide more useful therapeutic targets in the search for a preventative treatment for AD.

**1.7 Inflammation in AD**

Chronic neuroinflammation is a central mechanism in AD progression which drives AD pathology and neurodegeneration. \(^{312}\) In recent years, genome-wide association studies (GWAS) have identified polymorphisms in several genes that are associated with risk of AD. Many of these genes encode proteins that are involved in regulating microglial function and inflammatory pathways (Table 1.1); thus, it appears that inflammation is not only implicated in disease progression but also AD development. \(^{313-319}\)
Table 1.1. AD risk genes which encode immune/inflammatory proteins with their associated pathways and cell expression types (adapted from Verheijen and Sleegers, 2018). 320-322

<table>
<thead>
<tr>
<th>GENE</th>
<th>Chromosome</th>
<th>Primary Functions</th>
<th>Cellular Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1(^{313, 314})</td>
<td>1q32</td>
<td>Complement mediated immune response</td>
<td>Erythrocytes, B cells, T cells, monocytes, dendritic cells, neurons, microglia, choroid plexus.(^{323})</td>
</tr>
<tr>
<td>BIN1(^{313, 314})</td>
<td>2q14.3</td>
<td>Endocytosis, phagocytosis, synaptic transmission</td>
<td>Oligodendrocytes, neurons, astrocytes, microglia.(^{324, 325})</td>
</tr>
<tr>
<td>ABCA7(^{315})</td>
<td>19p13.3</td>
<td>Phagocytosis, lipid transport</td>
<td>Leukocytes, macrophages, microglia, neurons, oligodendrocytes.(^{326})</td>
</tr>
<tr>
<td>MS4A CLUSTER(^{315})</td>
<td>11q12</td>
<td>Regulates immune cell functions</td>
<td>B cells, monocytes, macrophages, microglia.(^{327})</td>
</tr>
<tr>
<td>CD33(^{315})</td>
<td>19q13.33</td>
<td>Regulates immune cell functions, phagocytosis</td>
<td>Leukocytes, macrophages dendritic cells, microglia.(^{328})</td>
</tr>
<tr>
<td>EPHA1(^{315})</td>
<td>7q34-q35</td>
<td>Immune response, endocytosis, cell adhesion</td>
<td>Epithelial cells.(^{329})</td>
</tr>
<tr>
<td>CD2AP(^{315})</td>
<td>6p12.3</td>
<td>Endocytosis, synaptic formation</td>
<td>Immune cells, epithelial cells, neurons.(^{330})</td>
</tr>
<tr>
<td>HLA-DRB5/DRBI(^{316})</td>
<td>6p21.32</td>
<td>Antigen presentation</td>
<td>B cells, dendritic cells, macrophages, microglia.(^{331})</td>
</tr>
<tr>
<td>INPP5D(^{316, 317})</td>
<td>2q37.1</td>
<td>Immune response, myeloid proliferation</td>
<td>Hematopoietic cells, monocytes, macrophages, mast cells, platelets, NK cells, microglia.(^{331})</td>
</tr>
<tr>
<td>MEF2C(^{316})</td>
<td>5q14.3</td>
<td>Immune cell proliferation, antigen presentation</td>
<td>Endothelia, B cells, microglia.(^{331})</td>
</tr>
<tr>
<td>TREM2(^{318, 319})</td>
<td>6p21.1</td>
<td>Immune response/phagocytosis</td>
<td>Macrophages, dendritic cells, microglia.(^{331})</td>
</tr>
</tbody>
</table>
1.7.1 Microglia

Microglia are the resident macrophages of the CNS which are involved in neuroinflammation and are the first line of defence against tissue injury in the brain. They are widespread throughout the CNS and exist in a range of morphological states with differing functions, from ramified microglial cells (RMCs) to amoeboid microglial cells (AMCs). RMCs are believed to reflect a quiescent/resting microglial state; they have numerous processes that extend into the microenvironment to monitor synapses and maintain brain tissue homeostasis. RMCs are thought to become activated in response to changes in the microenvironment, adopting graduated morphological changes such as the retraction of microglial processes. AMCs are phagocytic and migratory, often appearing during development or in response to injury or infection. They are morphologically similar to macrophages found outside of the CNS. They lack the processes found in RMCs, instead containing several phagocytic vacuoles. However it is unknown whether they originate from RMCs or from circulating monocytes which enter the brain in response to tissue damage. Microglia express pattern recognition receptors (PRRs) on the cell surface that can identify pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) and elicit an immune response to protect the brain against invading pathogens or aggregated/misfolded proteins. Under normal conditions the response stops once the stress factor is removed.

Active microglia can be categorised as classically activated (M1) or alternatively activated (M2). M1 microglia elicit an innate immune response through secretion of proinflammatory cytokines, nitric oxide (NO) and ROS which can result in chronic neuroinflammation and neuronal injury if not well regulated. M2 microglia produce anti-inflammatory cytokines and neurotrophic factors (brain-derived neurotrophic factor [BDNF] and nerve-derived growth factor [NGDF]) to promote wound healing while increasing their phagocytic activity for clearance of debris. Models of ageing have suggested that microglia become increasingly more dysfunctional with age and can enter a more inflammatory state with increased expression of PRRs and inflammatory markers such as major histocompatibility complex (MHC) antigens, resulting in increased activation. These dystrophic microglia have reduced phagocytic and migratory capacity and upregulate production of proinflammatory cytokines e.g. tumour necrosis factor-alpha (TNFα), interleukin-6 (IL-6) and interleukin-1-beta (IL-1β) which contribute to the heightened inflammatory status of the aged brain. In addition, microglia can multiply and become activated in a process called microglial priming, driven by changes to the CNS microenvironment (e.g. ageing, brain injury, neurodegenerative disease). Primed microglia are susceptible to secondary inflammatory stimuli, which can arise from the CNS or systemically, triggering an exaggerated inflammatory response. Microglial priming was first observed in
a mouse model of prion disease where a secondary inflammatory stimulus resulted in an upregulated population of resident microglia and increased synaptic degeneration compared to non-diseased controls.\textsuperscript{342}

In AD, both Aβ plaque deposition and NFT formation are associated with microglial activation and subsequent production of proinflammatory mediators. A transgenic mouse model of AD observed that microglia become progressively activated with age and that the level of microglial activation correlates with severity of Aβ plaque and NFT distribution\textsuperscript{343}, however it is not yet clear whether microglial activation and neuroinflammation induce AD pathology or are consequences of Aβ deposition.\textsuperscript{344} There is also debate surrounding the role of microglia in AD and whether they exert neuroprotective or neurotoxic effects. The neuroinflammatory hypothesis proposes that hyperactive microglia produce excess amounts of neurotoxins in response to Aβ plaque exposure whereas the microglial dysfunction hypothesis suggests that AD is caused by reduced phagocytic capacity of microglia, causing Aβ to accumulate as a result.\textsuperscript{345} Some researchers have suggested that microglia contribute more to amyloid production than phagocytic clearance of Aβ\textsuperscript{346}, while others propose that microglia are initially neuroprotective but become neurotoxic due to continuous exposure to Aβ plaques or secondary inflammatory stimulus e.g. brain injury, systemic inflammation.\textsuperscript{347} Despite the controversy surrounding the initial role of microglia, most agree that prolonged activation leads to the release of proinflammatory mediators including TNFα, ROS and NO which contribute to oxidative stress and neurodegeneration.\textsuperscript{348}

1.7.2 Non-steroidal anti-inflammatory drugs

NSAIDs are commonly used for symptomatic relief in a number of inflammatory conditions; they inhibit cyclooxygenase (COX) and subsequent prostaglandin synthesis and can therefore also be referred to as COX inhibitors.\textsuperscript{349}

NSAIDs have been increasingly studied as a potential preventative treatment for AD with most epidemiological studies agreeing that duration of NSAID use heavily influences the risk of developing AD. The Rotterdam study proposed that long term use of NSAIDs (24 months or longer) was associated with a reduced relative risk for AD compared to short-term users (one month or less) and non-users in age and sex matched controls.\textsuperscript{350}

A meta-analysis investigated the effects of NSAIDs on AD and agreed that long term use (24 months or longer) conferred protection against AD development.\textsuperscript{351} Contrastingly there are some epidemiological studies that found no association between NSAID use and a decreased risk of AD.\textsuperscript{352-355}

The Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT) was a longitudinal randomised controlled trial carried out to investigate two NSAIDs (naproxen and celecoxib) as potential AD treatments. It was initially concluded that neither were successful in slowing
the rate of neurodegeneration or the progression of AD.\textsuperscript{356} However the original conclusions were later revised to include that naproxen showed decreased incidence of AD after 2-3 years in those in the preclinical/asymptomatic stage but that it may have adverse effects on AD pathogenesis if administered in the more advanced stages.\textsuperscript{357}

Several mouse models of AD have investigated the effects on NSAIDs on AD pathology with varying results. Lim et al. (2000) and Yan et al (2003) found that long term administration of ibuprofen reduced the amount of Aβ, active microglia and proinflammatory mediators in the brain compared to non-treated mice.\textsuperscript{358, 359} Jantzen et al (2002) found similar effects of ibuprofen in a more severe model of AD and more dramatic reduction in Aβ burden with flurbiprofen treatment, however there was no reduction in amyloid pathology with celecoxib treatment.\textsuperscript{360} Interestingly, Kukar et al. (2005) found that celecoxib treatment in 3-month-old mice resulted in an almost 3-fold increase in Aβ42.\textsuperscript{361}

Despite the largely promising results from epidemiological and animal studies there is little clinical evidence for the use of NSAIDs as a preventative treatment for AD due to high withdrawal rates as a result of adverse side effects in pilot studies and a lack of longitudinal clinical trials.\textsuperscript{362-364}

\textbf{1.7.3 Systemic inflammation & TNF-alpha}

There is an increasing amount of interest in the theory that systemic inflammation can cause an exaggerated inflammatory response in the CNS which may drive the progression of neurodegenerative diseases.\textsuperscript{365-368} Activated macrophages are a major source of peripheral TNFα and other proinflammatory mediators which are typically produced in response to systemic infection or injury. While upregulation of these proinflammatory mediators is necessary for a rapid immune response, unregulated or sustained production can be detrimental resulting in serious pathological conditions such as sepsis or autoimmune disease.\textsuperscript{369} In the CNS, microglia are the main source of TNFα which is typically released in response to localised damage.\textsuperscript{370} Additionally, systemic inflammatory signals can be communicated to the brain by circulating cytokines produced during peripheral inflammation which can cross the BBB and exacerbate the central immune response.\textsuperscript{348, 371}

Mouse models of chronic neurodegeneration have shown that when microglial cells were primed, low levels of systemic inflammation resulted in an increased rate of brain atrophy and disease progression. The animals were injected with bacterial cell wall components (e.g. lipopolysaccharide (LPS)) to mimic a systemic infection – this appeared to amplify the production of proinflammatory IL-1β, TNFα and interferon-beta (IFNβ) in the CSF and plasma with accompanying deterioration in cognitive function compared to controls.\textsuperscript{372, 373}

Most inflammatory mediators appear to be elevated two- to four-fold in models of neurodegeneration however TNFα appears to be elevated to a greater extent and is thought to
be a signature of activated microglia.\textsuperscript{374}

TNFα is primarily synthesised as a transmembrane protein (tmTNFα) but can be processed by the metalloprotease, TNFα converting enzyme (TACE) to form soluble TNFα (sTNFα). tmTNF is involved in the innate immune response to infections while sTNFα is believed to facilitate inflammation.\textsuperscript{375}

Both forms of TNFα are active and exert their effects by interacting with two transmembrane receptors; TNFR1 and TNFR2.\textsuperscript{376} TNFR1, also known as p55, is present on most cell types and facilitates inflammation and apoptosis and can be activated by both tmTNFα and sTNFα. TNFR2, also known as p75, is involved in antiviral responses and tissue regeneration with expression restricted to specific cell types including microglia and astrocytes in the CNS. Unlike TNFR1, TNFR2 can only be activated by tmTNFα in response to neuronal injury or CNS infection.\textsuperscript{377, 378} In turn, TNFα initiates an immune reaction by upregulating the production of other proinflammatory mediators (cytokine cascade) and triggering inflammatory pathways.\textsuperscript{379} Under pathological conditions TNFα may have roles in cholinergic dysfunction, oxidative stress, hyperphosphorylation of tau and Aβ-associated neuronal death.\textsuperscript{380} TNFα also inhibits long term potentiation and therefore affects memory and learning.\textsuperscript{381}

In a relevant clinical study, 300 patients with mild to severe AD were observed over a 6-month period.\textsuperscript{382} The patients were thought to have activated microglia due the increased burden of Aβ plaques.\textsuperscript{374} Systemic inflammatory events (SIEs) were associated with a 2-fold increased rate of cognitive decline after 6 months and were believed to be closely associated with a raised level of TNFα. The group observed that those with raised serum TNFα at baseline who experienced SIEs had a 10-fold increase in rate of cognitive decline compared to those with low serum TNFα levels at baseline and who did not experience SIEs. They also noted that those who maintained low TNFα concentrations over the follow up period did not show signs of cognitive decline after 6 months.\textsuperscript{382} An elevated level of TNFα has also been associated with increased risk of conversion from MCI to AD\textsuperscript{383} and increased hippocampal atrophy.\textsuperscript{384} These studies led to the development of the hypothesis that reduction in TNFα levels may reduce the rate of neurodegeneration or even protect against development of AD.

\subsection{1.7.4 TNF inhibitors}

TNF inhibitors (TNFi) are a group of drugs that suppress inflammation by specifically targeting TNFα and its related inflammatory pathways. There are currently five TNFi that have been approved for use in several chronic inflammatory diseases including rheumatoid arthritis (RA), psoriatic arthritis (PsA) and Crohn’s disease.\textsuperscript{375} Infliximab, adalimumab and golimumab are immunoglobulin G (IgG) monoclonal antibodies. Both adalimumab and golimumab are
100% human while infliximab is chimeric (75% human, 25% mouse). Etanercept is a human dimeric fusion protein consisting of two TNF receptor (p75) subunits fused to the Fc portion of human IgG. Certolizumab pegol is an antigen binding fragment (Fab') of a humanised TNFα antibody that is chemically modified with polyethylene glycol.

Despite their different structures, all five TNFi have the same mechanism of action; they block the interaction of TNFα with both TNFR1 and TNFR2 receptors through high affinity binding with TNFα. Etanercept was the first TNFi to be approved for treatment of RA and is subcutaneously injected one to two times a week. Adalimumab and certolizumab pegol are also administered via subcutaneous injection every 2-4 weeks. Infliximab is administered via infusion over at least 2 hours with maintenance dosing every 4-8 weeks. Golimumab can be administered by subcutaneous injection or intravenous infusion over 30 minutes with a recommended dose of 50mg per month.

TNFα inhibition has been investigated in mouse models of AD and showed rapid reduction in both amyloid plaque burden and tau hyperphosphorylation upon administration of infliximab. A 6 month pilot study reported that peri-spinal administration of etanercept rapidly improved cognitive performance in subjects with mild to severe AD. There have been similar reports of rapid improvement after intrathecal administration of infliximab. TNFi cannot cross the blood-brain-barrier and so a peripheral mechanism was proposed in which TNFi are subcutaneously injected. A small 6 month randomised, placebo-controlled, phase 2 trial demonstrated the safety and tolerability of subcutaneous injection of etanercept in AD patients, however large randomized controlled trials (RCTs) are required to determine if there is any cognitive benefit before recommending TNFi as a viable treatment option for AD.

1.8 Rheumatoid arthritis

TNFα is also a major contributor to joint, cartilage and bone damage in rheumatoid arthritis (RA). RA is an autoimmune disease with a global prevalence rate between 0.3% and 1%. It involves chronic inflammation of the joint synovium which can cause swelling, pain, limited range of motion and increased mortality. If RA is not well managed the inflammation can cause joint erosion which over time may lead to deformity and disability. Joint damage is irreversible and thus there is no cure for RA though there are many treatment options that aim to slow the course of the disease, prevent joint damage and improve quality of life. RA tends to manifest between the ages of 20 and 40; in the early stages of disease the affected joints may not appear red or swollen but patients might
experience tenderness or pain which lasts for several weeks with pronounced stiffness in the morning. Joint symptoms in RA are typically symmetrical upon clinical presentation. While RA is primarily a joint disease it may also have adverse effects on other aspects of health due to inflammation e.g. fatigue, dry eyes or mouth, shortness of breath, chest pain, low red blood cell count, rheumatoid nodules under the skin. Joint pain makes exercise and strenuous physical activity more difficult and so some weight gain may occur in RA which may increase the risk for high cholesterol and blood pressure, diabetes and cardiovascular disease.

1.8.1 The immune system in RA

RA is an autoimmune disease and as such the main cells involved in its onset are immune cells, specifically macrophages and lymphocytes (B-cells, T-cells) residing in the joint synovium or circulating in peripheral blood. Under normal conditions, macrophages are involved in the innate immune response as a first line of defence against infection or injury while lymphocytes are involved the adaptive immune response where they are responsible for identifying and eliminating invading pathogens whilst retaining an immune memory. In RA, these cells identify host antigens as foreign and react destructively, leading to joint erosion. In an inflamed joint, macrophages of the M1 phenotype release proinflammatory cytokines (e.g. IL-1, IL-6, IL-8, IL-15, IL-18, IL-23 TNFα, IFN-γ) and chemokines (CCL5, CXCL-10) which contribute to recruitment of additional leukocytes, fibroblast proliferation and activation of osteoclasts to accelerate inflammation, joint destruction and bone resorption. In RA, macrophages also over-express MHC II and human leukocyte antigen (HLA) thus acting as antigen presenting cells (APCs) to support T-cell activation. The degree of macrophage infiltration and activation has been shown to correlate with the amount of pain, inflammation and damage in the affected joint.

Additionally, the central and peripheral B-cell tolerance checkpoints which remove autoreactive B-cells are typically defective in RA. This leads to accumulation of autoreactive mature naïve B-cells which release various chemokines and cytokines to promote inflammation of the joints (e.g. TNFα, IL-1, IL-6, IL-17) B-cells are the source of rheumatoid factor (RF) and anti-cyclic citrullinated protein (anti-CCP) antibodies which are well-established indicators of RA and disease severity. B-cells also act as APCs to support the activation and differentiation of T-cells.

T-cells can differentiate into T-helper (Th) or regulatory T (Treg) cells which contribute to pathogenesis of RA. Th1 cells are highly activated in RA and mediate cellular immunity through the release proinflammatory cytokines (e.g. IL-2, IFNγ, TNFα) and activation of
macrophages.\textsuperscript{410} Th2 cells mediate humoral immunity by secreting anti-inflammatory cytokines (e.g. IL-4, IL-5, IL-13) and trigger B-cell activation and release of effector antibodies.\textsuperscript{402} Th17 cells primarily secrete IL-17 which mediates inflammation and promotes bone loss.\textsuperscript{411, 412} Tregs act to suppress proliferation of autoreactive lymphocytes through release of IL-10 and transforming growth factor-beta (TGF-\(\beta\)), however Tregs cannot suppress the release of pro-inflammatory cytokines from lymphocytes or macrophages.\textsuperscript{413}

In RA, Treg function is compromised due to over-expression of co-stimulation molecules by APCs (e.g. class II HLA, CD80, CD86, CD40) and proinflammatory cytokines (e.g. TNF\(\alpha\), IL-7) and so autoreactive lymphocytes accumulate.\textsuperscript{414} Two additional subsets of T cell have been identified in RA: T follicular helper (Tfh) cells in the follicles of secondary lymphoid organs and T peripheral helper (Tph) cells within inflamed joint tissues. Both cell types have similar functions in supporting B-cell proliferation, differentiation and survival as well inducing antibody production.\textsuperscript{415} Other immune cells such as mast cells, dendritic cells and natural killer cells have been implicated in the pathophysiology of RA, with roles in proinflammatory cytokine production and T-cell activation.\textsuperscript{416-418} There are therefore many promising targets for anti-rheumatoid therapy.

1.8.2 Diagnosis
RA is one of the most common causes of disability in older adults. There are only an estimated 10\% of patients with RA that do not experience significant disability.\textsuperscript{419} The key to effectively managing RA is an early and accurate diagnosis by a specialist rheumatologist and early administration of treatment to prevent joint erosion and disability.\textsuperscript{420} The American College of Rheumatology (ACR) developed criteria for the diagnosis of RA in 1987, however it was criticised for lack of sensitivity in the early disease stages. In 2010, ACR in collaboration with the European League Against Rheumatism (EULAR) redefined RA diagnostic criteria with focus on increasing sensitivity in early disease stages.\textsuperscript{421, 422} The updated classification system for RA requires taking note of medical history, a physical examination and blood tests and particularly relies on the exclusion of other potential causes in order to achieve an accurate diagnosis.\textsuperscript{423} Medical history involves taking note of any family history of RA as well as the onset, duration and severity of joint symptoms. During the physical examination, the rheumatologist will assess the patient for joint pain, tenderness, swelling, range of motion and inflammation, taking note of how many small and large joints are affected.\textsuperscript{424} Blood tests analyse inflammatory markers and antibodies that are associated with RA including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF autoantibodies and anti-CCP antibodies. Abnormal ESR and CRP levels along with positive
RF and/or anti-CCP (seropositive) blood tests are often associated with RA, however serology results alone are not enough to confirm a diagnosis. Moreover, ESR and CRP are measures of inflammation and are not specific to RA. Although RF is positive in around 80% of RA cases, only around 40% of patients are RF positive upon clinical onset. RF is also not specific to RA and can be found in other autoimmune conditions or chronic infections. Anti-CCP is believed to be more specific to RA and appears earlier in the disease than RF however only an estimated 60-70% of RA patients are anti-CCP positive.

While ESR and CRP are useful in monitoring disease activity, RF and anti-CCP positive blood results tend to remain positive throughout the course of the disease despite high or low disease activity and are therefore not used to monitor patient response to treatment. Diagnostic imaging e.g. X-ray, ultrasound or MRI can be helpful in RA diagnosis and management in order to monitor joint erosion before and after commencing treatment.

### 1.8.3 Risk Factors

The exact cause of inflammation in RA is not known but there are certain genetic and environmental factors that may predispose disease development. Women are between two to three times more likely to develop RA than men and may be more likely to experience functional disability, indicating that hormonal factors may have a significant impact on RA risk and severity. This may be due in part to sex-related differences to treatment with some studies reporting that women are less likely to achieve remission, while others suggest that sex-related differences in severity of RA may be more a result of the measures of disease activity themselves.

Interestingly, pregnancy and breastfeeding have been linked with a reduced risk of developing RA. RA symptoms appear to improve during pregnancy, but several studies have reported over 90% of participants experience a flare in the year following delivery. Oral contraception and hormone replacement therapy may be associated with a reduced risk of RA and improved clinical outcome in post-menopausal women however the evidence for this is inconsistent.

Those with a family history of RA, particularly in first degree relatives, appear to be more likely to develop the disease with heritability estimated to be around 60%. Although certain twin studies have reported that a twin only has a 15% chance of developing RA if their sibling has the disease. GWAS have identified over 100 genetic markers that are associated with an increased risk of RA. In particular, HLA-DRB1 alleles (e.g. *0101, *102, *0401, *0404, *0405, *408, *1001) are believed to exhibit the strongest genetic influence in RA. The HLA-DRB1 alleles share a five amino acid sequence motif in the third hypervariable region of the DRβ1 chain; this shared epitope is believed to be present in over 80% of RA patients.
Previous studies have demonstrated an association between the shared epitope and susceptibility to severe RA.\textsuperscript{449-451} The susceptibility genes in Table 1.2 are believed to explain around 50% of the familial risk meaning that environmental factors must also contribute to heritability of RA.\textsuperscript{452}

<table>
<thead>
<tr>
<th>Susceptibility gene</th>
<th>Year</th>
<th>Encoded protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRB1\textsuperscript{453}</td>
<td>1978</td>
<td>Class II human leukocyte antigen/major histocompatibility complex</td>
</tr>
<tr>
<td>PADI4\textsuperscript{454}</td>
<td>2003</td>
<td>Peptidylarginine-deiminase 4</td>
</tr>
<tr>
<td>PTPN22\textsuperscript{455}</td>
<td>2004</td>
<td>Protein tyrosine phosphatase, non-receptor type 22</td>
</tr>
<tr>
<td>CTLA4\textsuperscript{456}</td>
<td>2005</td>
<td>Cytotoxic T-lymphocyte associated protein 4</td>
</tr>
<tr>
<td>TRAF1/C5\textsuperscript{457}</td>
<td>2007</td>
<td>Tumour necrosis factor receptor associated factor 1</td>
</tr>
<tr>
<td>STAT4\textsuperscript{458}</td>
<td>2007</td>
<td>Signal transducer and activator of transcription 4</td>
</tr>
<tr>
<td>TNFAIP3\textsuperscript{459}</td>
<td>2007</td>
<td>Tumour necrosis factor-alpha induced protein 3</td>
</tr>
<tr>
<td>IL2RA, IL2RB\textsuperscript{460}</td>
<td>2008</td>
<td>Interleukin 2A, Interleukin 2B</td>
</tr>
<tr>
<td>CD40\textsuperscript{461}</td>
<td>2008</td>
<td>CD40</td>
</tr>
<tr>
<td>CCL21\textsuperscript{461}</td>
<td>2010</td>
<td>CC chemokine ligand 21</td>
</tr>
<tr>
<td>FCGR\textsuperscript{462}</td>
<td>2010</td>
<td>Fc gamma receptor</td>
</tr>
<tr>
<td>CCR6\textsuperscript{463}</td>
<td>2010</td>
<td>CC chemokine receptor 6</td>
</tr>
<tr>
<td>IRF5\textsuperscript{463}</td>
<td>2010</td>
<td>Interferon-related factor 5</td>
</tr>
</tbody>
</table>

Smoking, particularly tobacco smoking, is perhaps the strongest lifestyle risk factor for RA and is often associated with seropositive cases.\textsuperscript{464} There appears to be a linear correlation between duration of cigarette use and risk for RA with ex-smokers remaining at an increased risk even 20 years after cessation.\textsuperscript{465} There is a certain amount of interaction between genetic and environmental factors e.g. smokers who carry the HLA-DRB1 shared epitope appear to be at a greater risk for developing seropositive RA compared to smokers who do not carry the shared epitope.\textsuperscript{466, 467} Contrastingly, alcohol consumption appears to decrease the risk for RA, an effect which has been observed to a greater degree in those that carry the shared epitope.\textsuperscript{468} Certain cardiovascular risk factors are often associated with RA such as diabetes, high blood pressure, high cholesterol and obesity. These factors may also contribute to increased mortality in RA.\textsuperscript{469} There also seems to be a certain impact of diet on RA with high amounts of red meat.
consumption and low intake of vitamin C and vitamin D being associated with an increased risk.\textsuperscript{450} Healthier diets have been associated with reduced risk for RA while certain dietary regimes e.g. Mediterranean and vegetarian diets have been investigated in the treatment of RA with varying results.\textsuperscript{471-473}

### 1.8.4 Treatment

The main goals of RA treatment are to achieve clinical remission status, alleviate pain, reduce inflammation, inhibit joint erosion and improve quality of life. There are four main groups of drugs that are used for managing RA: painkillers, NSAIDs, disease modifying anti-rheumatic drugs (DMARDs) and glucocorticoids (steroids).\textsuperscript{419} Painkillers and NSAIDs are symptomatic treatments often prescribed to relieve pain, inflammation and swelling caused by RA but have no effect in terms of reducing RA severity and so must be used in combination with other therapies.\textsuperscript{474} NSAIDs should be used sparingly and in low doses as they are often associated with adverse gastrointestinal and cardiovascular side effects.\textsuperscript{419}

Glucocorticoids such as prednisolone help to reduce inflammation and joint destruction in RA.\textsuperscript{475} ACR/EULAR criteria recommends short-term use of glucocorticoids at the beginning of treatment, during a flare-up or as a bridging therapy upon changing DMARD.\textsuperscript{476} Long-term steroid use is avoided due to adverse side effects e.g. osteoporosis, cardiovascular disease, thinning of skin, weight gain.\textsuperscript{477, 478}

DMARDs alleviate pain, inflammation and joint destruction in RA by suppressing the immune system. DMARDs can take up to 12 weeks take effect and so steroid injections may be offered for immediate pain relief in the meantime.\textsuperscript{479} There are 3 types of DMARDs; conventional DMARDs (csDMARDs), biologic DMARDS (bDMARDs) and targeted synthetic DMARDs (tsDMARDs). Joint damage occurs early in the disease process and so DMARDs have significant benefits when administered early.\textsuperscript{480}

Methotrexate (MTX) is the most common csDMARD and is typically the first line of treatment in patients with high disease activity while sulfasalazine (SSZ) tends to be used in patients with less severe RA.\textsuperscript{481, 482} Leflunomide (LEF) may be prescribed for patients who failed first line monotherapy and can often prescribed in combination with MTX.\textsuperscript{483} MTX, SSZ and LEF are all similar in terms of efficacy, tolerability and cost however hydroxychloroquine (HCQ) appears to be less toxic and associated with fewer side effects; it is also less effective and is therefore prescribed for very mild or palindromic RA - a type of inflammatory arthritis which causes recurring flare-ups of joint pain but exhibits no symptoms between these attacks.\textsuperscript{484, 485}

Common side effects of csDMARDs include nausea and vomiting which affects an estimated
20-65% of users and can result in poor adherence and discontinuation of treatment. One retrospective study of a large UK cohort found that one third of patients with RA or PsA stopped MTX therapy, most often due to poor tolerability. Other side effects can include hair loss, mouth sores, headaches, fatigue and hepatotoxicity. In rare cases more severe infections such as tuberculosis can occur. Due to their immunosuppressive nature and significant risk of adverse side effects, csDMARD use must be frequently monitored. Guidelines from the British Society of Rheumatology (BSR) and British Health Professionals in Rheumatology (BHPR) recommend blood tests for full blood count, kidney and liver function every two weeks until a stable dose is achieved with subsequent monitoring every 1-3 months thereafter. Those who are prescribed higher doses or change DMARD should again be monitored every 2 weeks until the dose stabilises.

It is possible for csDMARDs to be given in combination if response to monotherapy is inadequate. Combined use of MTX, SSZ and HCQ (triple therapy) has shown increased efficacy compared to monotherapy in RA. MTX and bDMARD combined therapy also appears to improve clinical outcome compared to MTX monotherapy. However combination therapies, particularly those involving bDMARDs, are associated with increased toxicity and risk of infection.

Biologics or bDMARDs are newer and typically more effective disease modifying treatments for RA that can take as little as two weeks to take effect. They are also much more expensive and therefore are typically prescribed after failure of one or two csDMARD therapies. Unlike csDMARDs, biologics target specific pathways in the inflammatory process and as such can be further subdivided into groups depending on their targets including: TNF inhibitors, Interleukin inhibitors, T-cell inhibitors and B-cell inhibitors. Each type of bDMARD is associated with their own side effects; some of the most common side effects of self-injectable bDMARDs are injection site reactions e.g., itching, swelling, redness or pain. Injection site reactions tend to be only a minor source of discomfort and should not be reason for discontinuing treatment.

Many studies have reported that bDMARDs are associated with an increased risk of serious infection compared to csDMARDs at both standard and high doses with some reporting that low doses do not confer an increased risk. Other studies have suggested that the risk of infection is more closely associated with high disease activity. The risk for serious infection must be weighed against the potential clinical benefits and discussed between rheumatologist and patient. With regular monitoring patients can be treated safely and effectively with bDMARDs however those with a history of recurrent infections should avoid biologic therapies where possible.

Another concern with bDMARDs, particularly TNFi, is an increased risk for malignant cancer e.g. non-Hodgkin’s Lymphoma, though it has been suggested that the risk of lymphoma...
may be more closely associated with RA than TNFi therapy. A systematic review which assessed the safety of biologics found that risk of cancer was not increased compared to csDMARDs, while data from the BSR Biologics Registry for Rheumatoid Arthritis (BSRBR-RA) suggests that mortality rates are similar between csDMARD and bDMARD patients.

An estimated 30-40% of patients show lack of response to bDMARDs. Combined biologic therapy does not appear to show additional clinical benefit compared to bDMARD monotherapy and may increase the incidence of serious adverse events. The guidelines on treating patients who discontinue initial TNFi therapy are still unclear. Upon failure of a TNFi, ACR recommends switching to a non-TNFi biologic, whereas EULAR recommends a second TNFi. Switching to a second TNFi has been shown to produce a similar or slightly improved clinical response in those that discontinued primary TNFi therapy, especially if the reason for discontinuation was due to adverse events. However, there is evidence to suggest that switching to a non-TNFi biologic significantly improves clinical outcomes compared to switching to a second TNFi, particularly in seropositive patients who failed to respond to initial TNFi therapy.

Lack of response to TNFi may be due to the formation of antidrug antibodies (ADAs) which can reduce efficacy and safety of the drug and are more common in anti-TNF monoclonal antibodies. Infliximab is a chimeric monoclonal antibody and so treatment over 1-2 years can lead to the formation of ADAs in approximately 10% of patients. Adalimumab and golimumab are 100% human monoclonal antibodies and so fewer patients form ADAs compared to those on infliximab. Those who develop ADAs to primary TNFi treatment are at greater risk for developing ADAs to subsequent TNFi treatments. It therefore may be more appropriate to switch to a non-TNFi biologic which has been shown to improve clinical outcome in those who show inadequate response to anti-TNF therapy.

More recently the European Medical Agency (EMA) and US Food and Drug Administration (FDA) approved the use of tsDMARDs and biosimilars in RA and other diseases. tsDMARDs are synthetic drugs that target specific pathways in the immune system including the Janus kinase (JAK) inhibitors, tofacitinib and baricitinib. tsDMARDs have been shown to be as effective as bDMARDs in managing RA and show improved clinical outcome in patients who failed to respond to TNFi treatment.

Biosimilars are similar to bDMARDs in terms of efficacy and safety but are much cheaper than their biological counterparts. The process of switching to a biosimilar appears to be relatively simple with little evidence of loss of efficacy or increase in adverse events.
1.8.5 Rheumatoid arthritis and Alzheimer’s disease

In recent years there has been increasing interest in using RA as a model of the accelerated effects of systemic inflammation in a number of disease areas, including cognitive decline associated with AD. This is of particular relevance in RA as these patients have higher levels of systemic inflammation than the general population, therefore if AD is driven by inflammatory processes these patients may be at an increased risk of cognitive impairment and dementia. Notably, both diseases have a strong genetic component explaining around 50-60% of disease risk. Figure 1.5 summarises the shared genetic, environmental and lifestyle risk factors associated with both RA and AD.

Figure 1.5. A summary of the shared genetic, environmental and lifestyle factors associated with RA and AD.

In addition, epidemiological studies suggest that patients with RA are less likely to develop AD compared to healthy controls either due to the protective role of RA itself or long-term use of NSAIDs or other anti-inflammatory agents. However these studies are often limited by small sample sizes and high heterogeneity in study design. Despite promising reports from epidemiological studies, results from longitudinal clinical trials (e.g. ADAPT) have found little evidence that long-term NSAID use protects against AD development. Other studies have reported that RA increases the risk of developing AD, with a number of studies reporting an increased prevalence of MCI in adults with RA compared to healthy
controls.\textsuperscript{536-538}

There is little published data surrounding the risk of AD in RA patients who are treated with TNFi. One clinical epidemiological study suggested that RA subjects were significantly more likely to develop AD compared to normal controls. The group also investigated different types of RA treatments i.e., steroids, csDMARDs and bDMARDs. TNFi, specifically etanercept, was the only therapy to reduce the risk of AD in RA subjects.\textsuperscript{535}

Data was analysed from the BBRBR-RA database which compared 13,500 RA patients being treated with TNFi to 4000 DMARD patients over an average of 9.5 years until specified date or death. This study found that RA patients treated with TNFi seemed to have a lower incidence of dementia although the model did not reach statistical significance. However, the BBRBR-RA database does not collect information on cognitive decline – the diagnosis of dementia was reliant on the cause of death on the death certificate. Dementia is commonly under-reported on death certificates, so the number of dementia related deaths was low (n=46).\textsuperscript{539}

Unpublished data from the pharmaceutical firm, Pfizer regarding analysis of patients with chronic inflammatory arthritis in two large medical insurance databases (Truven Health Analytics Marketscan and Optum Insight Optum) revealed that etanercept treatment was associated with a 60-70\% reduction in relative risk of AD compared to controls, an effect which was similar for adalimumab but not for NSAIDs Naproxen and Celecoxib. TNFi also appeared to exhibit neuroprotective effects in patients who were receiving treatment for just 0-2 years which suggests that the drug could be administered to those with MCI, 60\% of whom are likely to convert to AD over a 5-year period, to help slow or prevent the progression to AD dementia.\textsuperscript{540}

\section{Thesis Aims and Hypothesis}

This thesis is based on results from the rheumatoid arthritis medication and memory study (RESIST), an 18-month observational study which aims to observe cognitive decline in patients with both RA and MCI who on are TNFi and compare the rate of cognitive decline in this group to subjects with both RA and MCI who are on a csDMARD therapy. As RESIST is an ongoing multicentre study, the results presented in this thesis are preliminary, using all available data from Belfast, Antrim and Southampton at the time of analysis.
Overall Hypothesis: TNFi reduce the rate of cognitive decline in RA patients with MCI compared to RA patients with MCI who are being treated with csDMARDs.

Objectives:
1. Perform a cross-sectional analysis of RESIST screening data to explore the prevalence of MCI in a UK population of adults aged 55 years and older with RA and to identify potential demographic and clinical predictors of cognitive impairment.

2. a) Conduct a longitudinal analysis of cognitive performance at the 6-month timepoint to determine if TNFi reduce the rate of cognitive decline compared to csDMARDs, as measured by FCSRT and MoCA while adjusting for baseline cognitive scores.
   b) To determine if TNFi reduce the rate of cognitive decline at 6-months, independent of changes in mood, rheumatological disease stage and functional incapacity while adjusting for other confounding variables (age, sex, education, etc.).

3. a) Conduct a longitudinal analysis of cognitive performance at the 12-month timepoint to determine if TNFi reduce the rate of cognitive decline compared to csDMARDs, as measured by FCSRT and MoCA while adjusting for baseline cognitive scores.
   b) To determine if TNFi reduce the rate of cognitive decline at 12-months, independent of changes in mood, rheumatological disease stage, functional incapacity whilst adjusting for other confounding variables (age, sex, education, etc.).
Chapter 2: RESIST Protocol

2.1 Study Design

2.1.1 Summary
RESIST is an ongoing 18-month observational study of cognitive decline in patients who have both RA and aMCI. The study consists of two treatment groups (i) csDMARDs and (ii) TNFi. The primary objective is to determine if TNFi reduce the rate of cognitive decline in RA patients with aMCI compared to those on csDMARDs.
RESIST is a collaborative study between Queen’s University Belfast (QUB) and the University of Southampton (UoS), working alongside the Belfast Health and Social Care Trust (BHSCT), the Northern Health and Social Care Trust (NHSCT) and University Hospital Southampton NHS Foundation Trust (UHS). The study is funded by the Alzheimer’s Society and the Northern Ireland HSC Research and Development Department.
PhD candidate, BMcD contributed to data collection and as such was trained in administering assessments of cognition, global health and rheumatological disease severity. In addition, BMcD obtained a full UK driving licence and completed training and supervised practice in venepuncture in preparation for RESIST follow-up visits. BMcD screened 33% of participants within the RESIST Belfast cohort and completed 39% of baseline assessments, 30% of 6-month assessments and 25% of 12-month assessments within this cohort.

2.1.2 Study Endpoints
The primary outcome of this study is the mean change in Free and Cued Selective Reminding Test (FCSRT) score at 18 months between csDMARD and TNFi treatment groups, adjusting for baseline FCSRT score and other confounders (e.g. age, sex, mood, RA disease activity). Secondary outcomes will investigate the relationship between peripheral cytokine levels and cognitive decline and while observing the effects of the APOE ε4 allele on cognitive outcomes.

2.1.3 Ethical approval
This study and all related study documents were approved by the West Midlands – Black Country Research Ethics Committee (REC) within the NHS Health Research Authority (HRA) for participating NHS organisations in England (REC reference: 17/WM/0161). Research governance permission was also granted by local research and development departments in both Northern Ireland and Southampton (i.e. BHSCT, NHSCT, UHS).
2.2 Participants

2.2.1 Description of participants

Participants were recruited between May 2018 and March 2020 from rheumatology outpatient clinics within the BHSC (located in Royal Victoria Hospital, Belfast City Hospital and Musgrave Park Hospital), NHSCT (located in Antrim Area Hospital) and UHS (located in Southampton General Hospital). All patients ≥55 years of age who fulfilled ACR/EULAR criteria for RA and were taking any csDMARD or TNFi treatment were identified as suitable for the study by rheumatology consultants and specialist rheumatology nurses. Patients were not considered suitable if they were on any other biologic therapy (e.g. tocilizumab, rituximab) as these drugs work on different pathways in the inflammatory process that are unrelated to the TNFα cytokine.

2.2.2 Participant numbers

There are an estimated 1800 patients aged >55 years old that are on TNFi for RA in both Northern Ireland and Southampton. It was anticipated that around 1/3 of these patients would have short term recall problems and would achieve MoCA scores ≤27; therefore, we had access to approximately 600 TNFi treated patients who had aMCI. Power for the study was based on the Inflammation, Cognition, Stress (ICoS) study where aMCI subjects undergoing the same screening procedures declined by an average of 3.2 points in the FCSRT over an 18-month period. Assuming that TNFi decrease the mean reduction by 50% (i.e. 1.6-point decline over 18 months) then 108 subjects were needed in each arm of the study (assuming a 1:1 ratio between TNFi and csDMARDs) for over 80% power alpha = 0.05. A 10% drop out rate in each arm was assumed meaning that 120 participants were needed for each treatment group.

2.2.3 Inclusion criteria

- Participant was willing and able to give informed consent for participation in the study.
- Participant was willing and able to participate for the 18-month study or until the participant developed dementia.
- Participant was aged 55 years or older.
- Participant had a diagnosis of RA as per ACR/EULAR criteria.
- For the TNFi arm of study: participant was on a TNFi (Etanercept, Adalimumab, Certolizumab pegol, Golimumab, Infliximab). May also have been on a csDMARD
in conjunction.

- For the csDMARD arm of study: participant was on a conventional synthetic DMARD (Methotrexate, Sulfasalazine, Leflunomide, Hydroxychloroquine).
- Participant was cognitively impaired according to a MoCA cut-off score of ≤27.
- Fluent in the English language.

### 2.2.4 Exclusion Criteria

The participant was not considered eligible for the study if any of the following applied:

- Participant was unable to provide consent for the study.
- Participant lost capacity to provide consent during the study.
- Participant was not able to carry out cognitive tests due to visual/hearing impairment.
- Participant was not willing to take part in the follow-up.
- Participant was unlikely to cooperate in the study, not be able to be present at all scheduled visits or not able to follow study instructions.
- Participant was taking part in any other research study with administration of any investigational drug at time of enrolment.
- Participant had any previous or current medical condition that may impact on cognitive performance left to the Principal Investigator’s (BMcG/CH) judgement.
- Participant was taking cholinesterase inhibitor medication or memantine.

### 2.2.5 Participant Confidentiality

Personal information was recorded at screening (i.e. name, address, telephone number) for the purposes of participant recruitment and follow-up. This information was recorded and stored securely and separately to clinical information. All participants were assigned a unique identification number at screening which was maintained for the duration of the study. This identification number was used on all study documents and electronic databases to maintain anonymity - no other personal identifiers were used. All documents were stored securely in locked cabinets and were only accessible to members of the research team. All databases were
stored on encrypted, password protected computers and only available to authorised personnel. Any data transfer between sites was also encrypted and password protected. All study staff were trained in good clinical practice (GCP) prior to commencement of the study.

2.3 Study Procedures

2.3.1 Screening & Recruitment
Screening was carried out by trained research staff following identification of potential participants by rheumatologists/specialist nurses. Suitable patients were provided with a study information sheet (Appendix 2.1) during their regular scheduled appointment and asked if they would be willing to be screened for eligibility. Those who agreed were screened by BMcD or another member of the research team after obtaining written informed consent (Appendix 2.2). A data collection form (Appendix 2.3) was completed for each participant which captured demographics, concurrent medications and most recent RA serology results (i.e. ESR, Anti-CCP, RF) along with the specific ACR/EULAR criteria used for RA diagnosis and date of diagnosis where available. Any subjective memory concerns were recorded before the participant was cognitively assessed using the MoCA version 7.1 (Appendix 2.4).

MoCA scores of 27/30 and 19/30 were chosen a priori as upper and lower cut-offs respectively following a consensus meeting between the clinical leads of the RESIST study (BMcG and CH). Scores within this range were thought to best capture individuals that have MCI while excluding those that may have more severe forms of cognitive impairment including AD. Additionally, a cut-off score of 27/30 has previously demonstrated good sensitivity for the detection of MCI in the literature.\textsuperscript{542, 543} When amenable, subjects scoring \( \leq 19/30 \) were referred to a memory clinic (Appendix 2.5) for more extensive cognitive assessment and diagnosis if appropriate. Participants who scored within the eligible range and who were willing to take part were given a second information sheet (Appendix 2.6) with details about the longitudinal study and were followed up by phone after a minimum of one week to schedule a baseline visit if they wished to participate.

2.3.2 Follow-up
The follow-up period of the study consisted of four visits which took place in the participants’ homes (or an appropriate clinical setting if patient refused home visits). All willing participants were scheduled for a baseline visit which lasted no longer than 1 hour with subsequent visits at months 6, 12 and 18. Visits were carried out by trained research staff and where possible the same researcher carried out all visits for a given participant.
2.3.2.1 Baseline Visit (0 months)

The participant was asked to provide written informed consent (Appendix 2.7) for the study at the first visit - at subsequent visits verbal consent was satisfactory. A data collection form (Appendix 2.8) was completed which recorded a detailed medical history (e.g. smoking status, diabetes type 1/2, history of cardiovascular disease) and any changes to medication since screening. The participant then completed assessments of cognition, behavioural and psychological symptoms, functional ability, pain and RA disease activity using the following:

- MoCA (version 7.3) (Appendix 2.9)
- FCSRT (Appendix 2.10)
- Geriatric Depression Scale-Short Form (GDS) (Appendix 2.11)
- Health Assessment Questionnaire (HAQ) (Appendix 2.12)
- Visual Analogue Scale for pain (VAS)
- Disease Activity Calculator for Rheumatoid Arthritis (DAS28) (Appendix 2.13)

Once all assessments had been completed, a blood collection took place.

Different versions of the MoCA were used between screening (version 7.1) and follow-up visits (version 7.3) in order to reduce practice effects. Home visits were thought to provide a quieter and more relaxed environment for the participant and so MoCA score at baseline was considered to be a more accurate reflection of cognitive function compared to screening MoCA score. Participants who scored outside the eligible range in the MoCA (i.e. ≤19 or >27) at the baseline visit were withdrawn from the study.

2.3.2.2 Visit 2 (6 months +/- 2 weeks)

The participant was contacted after 6 months by phone to arrange the second visit. Verbal consent was obtained and documented along with any changes to medications and occurrence of any serious adverse events (SAEs) over the previous 6 months (Appendix 2.14). The participant then completed assessments of cognition, behavioural and psychological symptoms, functional ability, pain and RA disease activity using the following:

- MoCA (version 7.3)
- FCSRT
- GDS
• HAQ
• VAS
• DAS28

Once all assessments had been completed, a blood collection took place.

2.3.2.3 Visit 3 (12 months +/- 2 weeks)

Participant was contacted after 6 months by phone arrange the third visit. Verbal consent was obtained and documented along with any changes to medications and occurrence of any SAEs over the previous 6 months. The participant then completed assessments of cognition, behavioural and psychological symptoms, functional ability, pain and RA disease activity using the following:
  • MoCA (version 7.3)
  • FCSRT
  • GDS
  • HAQ
  • VAS
  • DAS28

Once all assessments had been completed, a blood collection took place.

2.3.2.4 Visit 4 (18 months +/- 2 weeks)

Participant was contacted after 6 months by phone or letter to arrange the fourth visit. Verbal consent was obtained and documented along with any changes to medications and occurrence of any SAEs over the previous 6 months. The participant then completed assessments of cognition, behavioural and psychological symptoms, functional ability, pain and RA disease activity using the following:
  • MoCA (version 7.3)
  • FCSRT
  • GDS
  • HAQ
  • VAS
Once all assessments had been completed, a blood collection took place.

This was the final study visit after which participants were thanked for taking part and are to be provided with adequate follow-up once the study ends.

2.4 Study Materials

2.4.1 Montreal Cognitive Assessment (MoCA) – Nasreddine, Z. (2005).

The MoCA was developed as a screening tool for the detection of MCI and is commonly used in both clinical and research settings. The MoCA is a 10-minute, 30-point pen and paper test which covers a wide range of cognitive domains including visuospatial/executive function, language, memory, attention and concentration, abstract reasoning, delayed recall and orientation. To account for differences in educational attainment an extra 1 point is awarded if the subject has spent 12 years or less in full-time education. A score of 26 and above indicates normal cognitive function and is a well validated cut off score, displaying higher sensitivity for MCI than the MMSE. The MoCA is also thought to be more sensitive to changes in cognitive function over time and may therefore be useful in identifying MCI patients who are at risk of developing dementia in longitudinal studies.

2.4.2 Free and Cued Selective Reminding Test with Immediate Recall (FCSRT-IR) - Grober and Buschke (1987).

FCSRT-IR is a 16-item controlled learning and recall task. It assesses episodic memory under conditions that control attention and cognitive processing thereby improving discriminative validity between AD and normal ageing. FCSRT is recommended by the International Working Group (IWG) for the diagnosis of early/prodromal AD (e.g. aMCI) and is also widely used in both research and clinical longitudinal studies to predict those at risk of developing AD and to distinguish AD from other types of dementia. There are two versions of the FCSRT available; one version presents items in picture format while the other presents the items as printed words. While the two versions are associated, they are not thought to be equivalent. On average, scores appear to be higher in the picture version, consistent with the pictorial superiority effect – a phenomenon which states that pictures are more easily remembered than words. FCSRT version should therefore be carefully considered and results should be interpreted with caution, especially when comparing
against other studies in the literature. The FCSRT-word version was used in RESIST. The test begins with a study phase, during which the participant is presented with a card containing four items presented as printed words. The participant is asked to identify the correct item (e.g. grapes) in response to a unique category cue (e.g. fruit). Once all four items have been correctly identified the card is removed and immediate cued recall of the four items is tested. If the participant fails to recall any of the items on their own, they are reminded (e.g. the sports equipment was a racquet). This process is repeated until all 16 items have been correctly identified and retrieved by immediate recall. The study phase is followed by three trials consisting of both free recall and cued recall. Each trial is preceded by a 20 second intervention in which the participant is asked to count backwards to prevent recall from short-term memory. During free recall, the participant is given a maximum of 2 minutes to recall as many of the 16 items as possible. For any items that are not retrieved by free recall, the participant is prompted with the category cue. If the participant fails to recall the item with the category cue, the item and category cue are presented together (e.g. the bird was an owl)

Free recall is defined as the cumulative sum of free recall across the three trials which is scored out of a maximum of 48. Total recall is the sum of both free and cued recall across the three trials and is also scored out of a maximum of 48. Both measures have been used to accurately identify prodromal AD in previous studies. Optimal cut-off scores for detecting AD will differ depending on the version used. Some studies have reported good sensitivity and specificity for detecting prodromal AD using cut-off scores of 17/48 for free recall and 40/48 in total recall in FCSRT-word.

2.4.3 Health Assessment Questionnaire (HAQ) –Fries et al. (2005).

The HAQ, introduced in 1980, is a self-reported questionnaire which measures patient-orientated outcomes in different day-to-day tasks and is used as a measure of functional status/disability in a variety of rheumatic diseases e.g. RA, PsA, lupus, ankylosing spondylitis. The HAQ is widely used throughout the world in both clinical and research settings with hundreds of reports regarding its validity and reliability. It is applicable in a range of languages and can be administered by mail, telephone or face-to-face with clinician/researcher.

The HAQ contains 8 sections: dressing, arising, eating, walking, hygiene, reach, grip and activities. Each section consists of 2-3 component questions for which the patient must score themselves on their ability to complete the specified task based on their previous week. Scoring for each question is as follows: 0 (without any difficulty), 1 (with some difficulty), 2 (with much difficulty), 3 (unable to do). The total score for a section is the highest score obtained within that section e.g. if three questions within a section are scored 1, 2 and 3 respectively then the total score for that section will be 3.
The patient is then asked to select any aids or devices they use from the list provided. E.g. cane, walker, crutches or wheelchair for walking. The patient is also asked to indicate whether they require help from another person for any of the 8 sections. If a patient has indicated that they require help from an aid/device or another person, the score for the corresponding section is changed to 2. If the score for that section is already 2 or more then it is left unchanged. The HAQ disability index (HAQ-DI) is a score between 0 and 3 which reflects the level of disability of the patient. It can be calculated by taking the sum of the section scores and dividing by the number of sections.\textsuperscript{561}

2.4.4 Geriatric Depression Scale (GDS) – Yesavage \textit{et al.} (1983).\textsuperscript{568}

The GDS was developed in 1983 as an instrument for diagnosing depression in older people. The original version is a 30-item questionnaire which has been adapted to a shorter 15-item version which is ideal for more time sensitive circumstances or patients who are easily fatigued/distracted. Both long and short versions show good validity and reliability.\textsuperscript{568-570} GDS has also been demonstrated to be reliable in assessing depression in those with MCI.\textsuperscript{571} Each question is answered in a yes/no format, the answer that indicates depression appears in bold font. Once the questionnaire is completed the number of bold answers selected are counted to obtain the score. In the GDS-short form, scores between 0 and 5 are considered normal, scores $\geq 5$ indicate probable depression with a sensitivity of 92% and specificity of 54% while scores $\geq 10$ almost always indicate depression.\textsuperscript{572, 573}

2.4.5 Visual Analogue Scale (VAS)

Various types of VAS are used in both clinical and research settings to measure severity of a range of symptoms. Pain VAS is a subjective measure of pain severity that is believed to have been first introduced by Hayes and Patterson in 1921 to help improve the quality of pain management.\textsuperscript{574} The pain scale is presented as a horizontal or vertical line of a fixed length (e.g. 10cm) with extreme limits at either end. The patient is asked to rate their pain on a continuous scale from 0 (no pain) to 10 (worst pain) where higher scores indicate greater pain intensity.\textsuperscript{575} Pain VAS is quick and easy to use and has demonstrated a high degree of sensitivity to treatment effects in both acute and chronic pain.\textsuperscript{576, 577}

2.4.6 Disease Activity Score Calculator for Rheumatoid Arthritis (DAS28) – Fransen, J. (2005).\textsuperscript{578}

DAS28 is a standardised measure of disease activity in RA and is routinely used for diagnosis and monitoring patient response to treatment. It requires a physical assessment of the 28 joints
associated with RA including those in the hands, wrists, elbows, shoulders and knees along with blood tests and personal assessment of health.\textsuperscript{578} The DAS28 uses four measures to calculate a composite score: (i) number of tender joints, (ii) number of swollen joints, (iii) ESR or CRP levels in blood as a measure of the degree of inflammation, (iv) Global assessment of health where the patient is asked to indicate how well they feel that day on scale from 0 (best health) to 10 (worst health). These measures are typically input to a computer or app which use a mathematical formula to calculate the DAS28 score.\textsuperscript{579}

The DAS28 scoring index for RA disease activity is as follows:

- \textless 2.6: disease remission
- 2.6-3.2: low disease activity
- 3.2-5.1: moderate disease activity
- \textgreater 5.1: high disease activity

RA treatments aim to achieve remission in a patient and so DAS28 assists in establishing a target score to aim for. This ‘treat to target’ method requires regular assessment of disease activity and appropriate therapeutic dose adjustments until target is reached. Low, moderate or high disease activity may warrant a change in treatment for some patients. A change in score of at least 0.6 indicates response to treatment while changes \textgreater 1.2 are considered clinically significant.\textsuperscript{580, 581}

\section*{2.4.7 Bloods}

Blood samples were obtained by venepuncture of a peripheral vein by a trained research member. Samples from the Northern Irish cohort were transported to the Centre for Public Health (QUB) or Antrim Area Hospital (NHSCT) within 4 hours of collection where they were processed and stored at -80°C (Appendix 2.15). Samples from the Southampton cohort were similarly transported, processed and stored at -80°C within 4 hours of collection. Blood samples were labelled using unique participant identification number only, no other personal information was used. Access to labs in QUB, NHSCT and UoS is strictly limited to authorised personnel only. All blood samples are to be sent to the University of Southampton at the end of the study where they will be analysed in the same batch, blind to clinical data to reduce the chances of measurement error and bias.

\section*{2.4.7.1 DNA analysis}

A venous blood sample was collected at baseline only using a 4ml ethylenediaminetetraacetic acid (EDTA) blood tube. The sample was inverted 8 times and stored at -80°C.
2.4.7.2 RNA analysis

Blood for RNA analysis was collected using specific PAXgene blood RNA tubes at each visit for a random sample of patients in each treatment group. Samples were left to rest for 2 hours (or overnight) at room temperature before being transferred to a -20°C freezer for 24 hours and finally stored at -80°C.

2.4.7.3 Analysis of immune response

Two serum samples and one venous blood sample were collected at each visit using 6ml serum and 6ml EDTA blood bottles respectively. Serum samples were left to clot for 30 minutes in the dark before processing. One 6ml serum was centrifuged at 3000rpm for 15 minutes and aliquoted into two 1.5ml serum preparations. The other 6ml serum sample was stored as whole blood at -80°C. The 6ml EDTA venous blood sample was stored at 4°C and centrifuged (3000rpm for 15 minutes) within 2 hours of collection. Plasma was then separated into two 1.5ml aliquots and stored at -80°C.

2.5 COVID-19

2.5.1 Summary of COVID-19 pandemic

The coronavirus disease (COVID-19) was a global pandemic that began its spread throughout the UK and Ireland in January/February 2020. Coronaviruses are known to cause respiratory infections with varying degrees of severity and are highly communicable, primarily spread through close contact with an infected person e.g. saliva/secretion droplets, touching contaminated objects or surfaces. While the majority of people experience mild symptoms and fully recover without need for hospital admission, an estimated 1 in 5 people become severely ill. There were over 45,000 COVID-19 related deaths reported in the UK and over 620,000 worldwide by July 2020.

2.5.2 Impact of COVID-19 in the UK and NI

In March 2020, both UK and Northern Ireland governments imposed a quarantine period where all non-essential workplaces were closed and the public were encouraged to work from home where possible. A 2-metre social distancing measure was introduced with emphasis on regular hand washing and face masks to help control the spread. Government guidelines
strongly advised high-risk groups to shield (i.e. stay at home and reduce social interactions with those outside their household) until the 31\textsuperscript{st} of July 2020.

2.5.3 Impact of COVID-19 on research

RESIST participants were considered a high-risk group due to their age and the immunosuppressant action of their RA medication. On the 16\textsuperscript{th} March 2020, the decision was made to temporarily suspend all study activity (including both screening and home visits) for the safety of the participants and research staff. As a result of the pandemic, the original recruitment target of 240 participants was not met and a smaller sample of participants had completed their 18-month follow-up than was anticipated for the purposes of this thesis. Consequently, data from the 6-month and 12-month timepoints was used for the longitudinal analysis which may limit the power of the study.

At least one time point was missed for most participants during the course of the pandemic. Prior to study recommencement, all research staff carrying out home visits received both doses of the COVID-19 vaccination and completed the necessary risk assessments/training. Upon study recommencement, priority was given to participants that were overdue their 18-month follow-up; participants that had missed their 6/12-month follow-up skipped ahead to the next scheduled timepoint.

Participants were contacted by phone prior to scheduling a home visit and were asked a series of questions to rule out COVID-19 symptoms (Appendix 2.16). Home visits were attended with full personal protective equipment (PPE) e.g. masks, gloves, visors, aprons and hand sanitiser while complying with social distancing measures as per government guidelines. At the study visit, the participant was given an updated participant information sheet (Appendix 2.17) and asked to sign a consent form agreeing to new COVID-specific measures (Appendix 2.18). A second COVID-19 questionnaire (Appendix 2.19) was completed on the day of the visit in which participants were asked about any symptoms experienced over the previous 6 months. Symptoms were rated on a severity scale from 0 (no symptoms) to 4 (acute respiratory distress requiring ventilation). In addition to blood samples normally collected, an antibody test for COVID-19 was offered at the final visit for each participant.
2.6 Statistical analysis

All analyses were conducted by BMcD using the Statistical Package for the Social Sciences (SPSS, version 26.0).

2.6.1 Missing Values

2.6.1.1 Screening cohort

Within the screening cohort there were a number of missing values in relation to RA clinical characteristics e.g. date of diagnosis, DAS28 score, ESR, RF and Anti-CCP status. This data was mostly obtained from hospital electronic care records (ECR) and therefore was dependent on how long the participant had been diagnosed and the level of detail written by the rheumatology consultant in their initial letter of diagnosis. There were also missing values with regards to the reporting of subjective memory complaints as this was added as an amendment to the study after screening had already started. Finally, educational attainment was not available for each participant at the time of analysis. As the analysis of screening data was exploratory, regression analysis excluded participants with any missing values.

2.6.1.2 Follow up cohorts

Within the 6-month cohort, FCSRT was incomplete for n=1 csDMARD participant at baseline. DAS28 score was missing for n=1 TNFi participant at baseline and n=4 participants (n=3 TNFi, n=1 csDMARD) at 6 months. This was typically due to a lack of access to the participant’s most recent ESR blood results. In the 12-month cohort there were no missing values. Due to the small number of missing values, longitudinal analysis of treatment effects on cognition proceeded using listwise deletion.

2.6.2 Analysis of screening data

Characteristics of all screened participants were summarized using descriptive statistics i.e. means and standard deviations (SD) for continuous variables and relative frequencies and percentages for categorical variables. An analysis was conducted on all participants who met the inclusion criteria to determine the proportion of participants with probable MCI (defined as a MoCA score of ≤27) in a population of older adults with RA. The proportion was determined along with a 95% confidence interval (CI). The association between subjective memory concerns and objective memory impairment was assessed through chi-squared test where all expected cell counts were greater than five. A linear regression model was created to identify potential predictors of cognitive impairment with MoCA score as the outcome which included age (years), sex, education (≤12 years or
>12 years), RA disease activity (DAS28 score), and RF status (positive or negative). Linearity, homoscedasticity and outliers were checked through visual inspection of scatterplots. Residuals were checked for normality using the Shapiro-Wilk test as well as visual inspection of normal probability plots/histograms. Independence of residuals was assessed by Durbin-Watson test.

2.6.3 Analysis of follow-up data

The primary outcome from longitudinal analysis was the mean change in FCSRT score between TNFi and csDMARD treatment groups, adjusting for baseline score. The data were checked for normality using the Shapiro-Wilk test and visual inspection of histograms/probability plots. Due to the limited variability and skewed distribution of FCSRT total recall scores, it was decided that tests of significance would not be appropriate. Therefore, the analysis of follow-up data proceeded using FCSRT free recall score only. This was deemed an appropriate response as there is evidence to suggest that impaired free recall is more indicative of prevalent and future dementia, although total recall should be taken into account in order to distinguish AD from non-AD dementias.

Demographic data for both 6-month and 12-month cohorts were summarized using mean and SD or relative frequencies and percentages. Independent samples t-tests were used for comparing continuous variables and Chi-square tests were used for comparing categorical variables. Diabetic status was initially split into three categories (N/A, type 1 or type 2), however due to the small number of responders with type 1 diabetes (n=1), the number of categories for diabetic status were collapsed into two (yes or no). ANCOVA was performed to calculate the difference in mean (and 95% CIs) FCSRT free recall score between treatment groups within the 6-month and 12-month cohorts, adjusting for baseline FCSRT score. An additional adjusted analysis accounted for age (years), sex, education (≤12 years or >12 years), history of cardiovascular disease (yes or no), presence of diabetes (yes or no), smoking status (non-smoker, ex-smoker, smoker), functional limitations (HAQ), depression (GDS) and disease activity (DAS28 score).

A secondary ANCOVA analysis calculated the difference in mean MoCA score between TNFi and csDMARD groups within the 6-month and 12-month cohorts, as previously described with adjustments for baseline MoCA score and other confounding variables. VAS pain score was highly correlated to DAS28 score and so was not included in ANCOVA analysis to avoid multicollinearity issues. Residual analysis was performed to test the various assumptions of ANCOVA. Normality was assessed by Shapiro-Wilks test and inspection of a histogram of the residual. Outliers (i.e. standardised residuals greater than +/- 3 SDs) were assessed by visual inspection of the data. Homogeneity of variances was assessed through
Levene’s test. Linearity and homoscedasticity were assessed through visual inspection of a scatterplot.

A final analysis was performed to evaluate the effects of long periods of social isolation and shielding on mental health in a group of older individuals with potential MCI. Depression scores were taken from the participants’ most recent pre- and post-lockdown visits and were compared using a paired samples T-test.
Chapter 3: Results

3.1 Prevalence of mild cognitive impairment in a population of older adults with rheumatoid arthritis: preliminary analysis using RESIST screening data.

3.1.1 Participant Eligibility and Recruitment

Participant recruitment from screening to baseline assessment is summarised in Figure 3.1. A total of 720 subjects were screened for the study between May 2018 and March 2020. N=4 participants were excluded after screening due to failure to meet inclusion criteria (n=1 was younger than 55 upon screening, n=2 did not have a diagnosis of RA and n=1 participant was unable to complete the MoCA due to severe RA in hands). Of those who met inclusion criteria N=512 participants (72%) screened positive for probable MCI according to a MoCA cut-off score of ≤27 while n=204 (28%) achieved scores >27 and were considered to be cognitively normal. N=34 participants (5%) scored ≤19 at screening and were believed to have more severe cognitive impairment that may be indicative of AD dementia; these participants were referred on to memory services for clinical assessment and diagnosis. Of the n=478 participants who were eligible after the initial screening, n=135 (28%) did not wish to proceed to the follow-up stage of the study. N=26 participants were withdrawn before baseline as they were on non-TNFi bDMARD or tsDMARD medication. A further n=3 participants were withdrawn due to medical reasons which meant they no longer met the inclusion criteria e.g. n=1 had been diagnosed with Parkinson’s disease and n=2 had a change in diagnosis from RA to PsA. This meant n=306 participants had completed their baseline assessment upon analysis of results.
Figure 3.1. Flow chart of patient screening and eligibility for the RESIST study from screening to baseline visit.
3.1.2 Demographic characteristics

Data for the 716 screened participants (67% female) who met initial inclusion criteria are summarised in Table 3.1. Participant age ranged from 55 to 89 with a mean age of 67.6 years (SD 8.1). The majority of screened participants had obtained secondary level education with 36% acquiring more than 12 years. Only 102 participants (25% of valid responses) reported any memory concerns before the MoCA was administered however 72% of participants screened positive for probable MCI according to a cut-off score of ≤27. To further explore this trend subjective memory concerns were compared against objective cognitive impairment in Table 3.2. The majority (75%) of participants asked were not concerned about their memory despite over two thirds (72%) screening positive for probable MCI according to a cut off score of ≤27 in the MoCA. Only 26% of those with probable MCI reported memory concerns prior to MoCA administration whereas 22% who did report a memory complaint were considered to be cognitively normal. While the odds of scoring of ≤27 in the MoCA were higher among those who reported memory concerns (OR 1.19, 95% CI 0.71, 1.97) the relationship between cognitive impairment and subjective memory concern was not statistically significant as assessed by Chi-squared test ($X^2 = 0.43, p = 0.512$).
Table 3.1. Summary of demographics and MoCA scores for screened participants (n=716).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>67.6 (8.1)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>235 (32.8)</td>
</tr>
<tr>
<td>Female</td>
<td>481 (67.2)</td>
</tr>
<tr>
<td>Subjective memory concerns, n (%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>104 (14.5)</td>
</tr>
<tr>
<td>No</td>
<td>320 (44.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>292 (40.8)</td>
</tr>
<tr>
<td>Educational attainment, n(%)</td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>420 (58.7)</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>260 (36.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>36 (5.0)</td>
</tr>
<tr>
<td>Education-adjusted MoCA score, mean (SD)</td>
<td>25.4 (3.2)</td>
</tr>
<tr>
<td>Cognitive status(^b)</td>
<td></td>
</tr>
<tr>
<td>≤27 / Probable MCI, n (%)</td>
<td>511 (71.5)</td>
</tr>
<tr>
<td>&gt;27 / Cognitively normal, n (%)</td>
<td>204 (28.5)</td>
</tr>
</tbody>
</table>

Demographics are summarised as mean and SD for continuous variables or frequency and percentages for categorical variables.

\(^a\) Education-adjusted MoCA score was missing for n=34 participants (4.7%) who were excluded when calculating means.

\(^b\) N=1 participant with missing educational attainment had a raw MoCA score of 27 and was not grouped into either category.
Table 3.2. Subjective memory concern versus objective cognitive impairment according to MoCA cut-off (≤27/30).

<table>
<thead>
<tr>
<th>Subjective memory concerns</th>
<th>Probable MCI (MoCA ≤27)</th>
<th>Cognitively Normal (MoCA &gt;27)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Yes, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o % within memory concerns</td>
<td>76</td>
<td>26</td>
<td>102</td>
</tr>
<tr>
<td>o % within memory concerns</td>
<td>74.4% 71.2%</td>
<td>25.5% 28.8%</td>
<td>100% 100%</td>
</tr>
<tr>
<td>• No, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o % within memory concerns</td>
<td>222</td>
<td>90</td>
<td>312</td>
</tr>
<tr>
<td>o % within memory concerns</td>
<td>71.2%</td>
<td>28.8%</td>
<td>100% 100%</td>
</tr>
<tr>
<td>Total, n</td>
<td>297</td>
<td>117</td>
<td>414</td>
</tr>
<tr>
<td>o % of total</td>
<td>71.7%</td>
<td>28.3%</td>
<td>100% 100%</td>
</tr>
</tbody>
</table>

*Subjective concerns were added to the screening data collection form as part of an amendment after study commencement therefore responses were not collected for all participants at time of screening.
3.1.3 Clinical characteristics

RA specific clinical characteristics are summarised in Table 3.3. Sixty-one percent of screened participants were on a csDMARD treatment while 32% were on a TNFi treatment. The remaining 7% were on alternative therapies such as biologic DMARDs (e.g. rituximab), JAK inhibitors (e.g. baracitinib) or steroids (e.g. prednisolone). These participants did not meet revised inclusion criteria and were excluded from further participation in the study. Mean age of participants upon diagnosis of RA ranged from 11 to 85 with a mean age of 54.4 years (SD 14.2). RA duration was rounded to the closest year and ranged from 0 to 73 years with a mean duration of 13 years (SD 12.9). DAS28 score upon commencement of treatment ranged from 0.63 (remission status) to 7.85 (high disease activity) with a mean of 3.51 (SD 1.5) (moderate disease activity). ESR ranged from 1mm/hr to 137mm/hr with a mean of 22.5mm/hr (SD 21.2) which lies between the normal ranges for men and women over the age of 50.\textsuperscript{18} Forty-one percent of participants were anti-CCP positive while 45% tested positive for RF antibodies, 28% of participants were positive for both anti-CCP and RF antibodies.
Table 3.3. Summary of RA specific clinical characteristics for screened participants

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA Medication, n (%)</td>
<td></td>
</tr>
<tr>
<td>• csDMARD</td>
<td>434 (60.6)</td>
</tr>
<tr>
<td>• TNFi</td>
<td>230 (32.1)</td>
</tr>
<tr>
<td>• Other</td>
<td>52 (7.3)</td>
</tr>
<tr>
<td>Duration of RA in years, mean (+/-SD) (n=596)*</td>
<td>13.0 (12.9)</td>
</tr>
<tr>
<td>Age at diagnosis in years, Mean (+/-SD) (n=596)*</td>
<td>54.4 (14.2)</td>
</tr>
<tr>
<td>DAS28 score, mean (+/-SD) (n=373)*</td>
<td>3.51 (1.5)</td>
</tr>
<tr>
<td>ESR in mm/hr, mean (+/-SD) (n=582)*</td>
<td>22.5 (21.2)</td>
</tr>
<tr>
<td>Anti-CCP, n (%)</td>
<td></td>
</tr>
<tr>
<td>• Positive</td>
<td>293 (40.9)</td>
</tr>
<tr>
<td>• Negative</td>
<td>170 (23.7)</td>
</tr>
<tr>
<td>• Unknown</td>
<td>253 (35.3)</td>
</tr>
<tr>
<td>RF, n (%)</td>
<td></td>
</tr>
<tr>
<td>• Positive</td>
<td>324 (45.3)</td>
</tr>
<tr>
<td>• Negative</td>
<td>141 (19.7)</td>
</tr>
<tr>
<td>• Unknown</td>
<td>251 (35.1)</td>
</tr>
</tbody>
</table>

*Values were obtained from hospital electronic care records and were not available for all participants.
### 3.1.4 MoCA performance

Raw MoCA scores were available for all participants and ranged from 12 to 30 with a mean of 24.8 (SD 3.35). Education adjusted MoCA scores were available for n=683 participants (mean 25.4, SD 3.16). There was a negative correlation between age and screening MoCA score ($r = -0.292, p < 0.001$). Age-stratified mean MoCA scores are presented in Table 3.4; five age groups were chosen for comparison as each was comprised of a similar number of participants. As expected, the overall mean MoCA score decreased with age.

As shown in Figure 3.2, females on average achieved higher scores compared to males (mean 25.6 vs 25.1) though this was not significant ($p = 0.089$). Education on the other hand had a significant impact on test results. Before correction, those with >12 years full-time education scored significantly higher than those with ≤12 years (mean 26.31, SD 2.79 vs mean 23.85, SD 3.28; $p < 0.001$). After awarding an extra 1 point to those with ≤12 years education, MoCA score was still significantly higher among those with higher education ($p < 0.001$).

Overall, participants performed best in the orientation task with 94% of participants achieving the maximum number of points. The lowest scoring averages were observed in delayed recall (78% scored ≤4/5), cube (46% scored 0/1) and verbal fluency tasks (41% scored 0/1).

**Figure 3.2.** Relative frequency histogram of screening MoCA score for males and females with line of fit indicating the upper cut off score ($≤27/30$).
Table 3.4. Age stratified mean MoCA scores for screened participants adjusted for education (n=683).

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>n</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-59</td>
<td>131</td>
<td>18</td>
<td>30</td>
<td>26.28</td>
<td>2.67</td>
</tr>
<tr>
<td>60-64</td>
<td>137</td>
<td>17</td>
<td>30</td>
<td>26.18</td>
<td>2.99</td>
</tr>
<tr>
<td>65-69</td>
<td>131</td>
<td>17</td>
<td>30</td>
<td>26.17</td>
<td>2.85</td>
</tr>
<tr>
<td>70-74</td>
<td>140</td>
<td>12</td>
<td>30</td>
<td>24.69</td>
<td>3.34</td>
</tr>
<tr>
<td>75+</td>
<td>144</td>
<td>13</td>
<td>30</td>
<td>23.94</td>
<td>3.19</td>
</tr>
<tr>
<td>Total</td>
<td>683</td>
<td>12</td>
<td>30</td>
<td>25.42</td>
<td>3.17</td>
</tr>
</tbody>
</table>

Education-adjusted MoCA scores were unavailable for n=34 participants. Means are calculated based on remaining n=683 participants.
3.1.5 Regression analyses

The associations between screening MoCA score and various characteristics are presented in Table 3.5. A multivariable regression model with age, sex, education, DAS28 score and RF status explained 12% of the variance in screening MoCA score (adjusted $R^2 = 0.12$, $F = 9.28$, $p < 0.001$).

For each 10-year increase in age there was on average a 1.11-point decrease in MoCA score (95% CI -1.42, 0.09). After adjusting for covariates, MoCA score was predicted to decrease by an average of -0.70 points per 10-year increase in age. (95% CI -1.06, -0.34; $p < 0.001$). Similarly, for every 1-point increase in DAS28 score the MoCA score decreased by 0.29 points (95% CI -0.48, -0.09; $p = 0.004$). After adjustment for covariates this was little altered ($\beta = -0.30$, 95% CI 0.07, 1.30; $p = 0.029$).

Individuals with more than 12 years of education had higher MoCA scores than those with 12 years or less by an average of 1.49 (95% CI 1.01, 1.96; $p<0.001$). When adjusting for covariates a similar association was observed ($\beta = 0.68$, 95% CI 0.07, 1.30; $p = 0.029$).

Individuals who were RF positive had lower MoCA scores than those who were RF negative by an average of 0.64 (95% CI -1.22, -0.07; $p = 0.029$) before adjustment and 0.84 (95% CI -1.46, -0.21; $p = 0.009$) after adjustment.
Table 3.5. Summary of simple and multiple linear regression analyses with screening MoCA score as the outcome.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>N</th>
<th>Mean (SD)</th>
<th>Difference in mean(^c) (95% CI)</th>
<th>P</th>
<th>Adjusted difference in mean(^d) (95% CI)</th>
<th>Adjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (per 10-year increase)(^a)</strong></td>
<td>683</td>
<td>25.42 (3.16)</td>
<td>-1.11 (-1.42, -0.09)</td>
<td>&lt;0.001</td>
<td>-0.70 (-1.06, -0.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sex(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Male</td>
<td>223</td>
<td>25.12 (3.12)</td>
<td>0.00 (reference)</td>
<td>0.089</td>
<td>0.00 (reference)</td>
<td>0.084</td>
</tr>
<tr>
<td>* Female</td>
<td>460</td>
<td>25.56 (3.18)</td>
<td>0.44 (-0.07, 0.95)</td>
<td></td>
<td>0.54 (-0.74, 1.15)</td>
<td></td>
</tr>
<tr>
<td><strong>Education(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* ≤12 years</td>
<td>420</td>
<td>24.83 (3.25)</td>
<td>0.00 (reference)</td>
<td>&lt;0.001</td>
<td>0.00 (reference)</td>
<td>0.029</td>
</tr>
<tr>
<td>* &gt;12 years</td>
<td>260</td>
<td>26.32 (2.78)</td>
<td>1.49 (1.01, 1.96)</td>
<td></td>
<td>0.68 (0.07, 1.30)</td>
<td></td>
</tr>
<tr>
<td><strong>DAS28(^a)</strong></td>
<td>368</td>
<td>25.79 (2.89)</td>
<td>-0.29 (-0.48, -0.09)</td>
<td><strong>0.004</strong></td>
<td>-0.30 (-0.49, -0.10)</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td><strong>RF(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Negative</td>
<td>137</td>
<td>26.00 (2.89)</td>
<td>0.00 (reference)</td>
<td><strong>0.029</strong></td>
<td>0.00 (reference)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>* Positive</td>
<td>315</td>
<td>25.36 (2.87)</td>
<td>-0.64 (-1.22, -0.07)</td>
<td></td>
<td>-0.84 (-1.46, -0.21)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Variable was entered into regression model as continuous variable to give increase in MoCA score per unit increase.

\(^b\) Variable was entered into regression model as dummy variable to give increase in MoCA score compared to reference category. Dummy variables were coded as follows: Sex (Male = 0, Female = 1), Education (≤12 years = 0, >12 years = 1), RF status (Negative = 0, Positive = 1)

\(^c\) Unstandardized regression coefficient and 95% CI from simple regression models of each independent variable with screening MoCA score as the outcome (\(p<0.05\))

\(^d\) Unstandardized regression coefficient and 95% CI from multiple regression model which includes age, sex, education, DAS28 score and RF status with screening MoCA score as the outcome (n = 299), (\(p<0.05\)).
3.2 TNFi for the prevention of Alzheimer’s disease: analysis of RESIST data at both 6 and 12 months.

3.2.1 Follow-up recruitment

RESIST participant recruitment between June 2018 and March 2020 is detailed in Figure 3.3. As mentioned earlier in this chapter, n=306 participants had completed a baseline MoCA assessment before the temporary suspension of visits due to COVID-19. N=57 participants (19%) scored outside of the eligible range in the MoCA at baseline and were excluded from further participation in the study. N=12 participants (4%) were withdrawn as they were on a non-TNFi bDMARD and did not meet inclusion criteria; n=1 participant was withdrawn as their diagnosis of RA had been changed to PsA. N=19 participants (6%) who completed the baseline assessment declined further participation in the study and did not proceed to the 6-month follow-up visit.

Of the n=216 participants that were eligible for follow-up after the baseline visit, n=130 (60%) have completed their 6-month assessment with the remaining n=86 (40%) still to be scheduled once visits are allowed to resume. N=7 participants withdrew from the study before their 12-month follow-up, leaving 209 currently eligible. N=69 participants have gone on to complete the 12-month follow up and n=7 have completed their final 18-month assessment.
Figure 3.3. Diagram summarising participant recruitment over an 18-month follow-up between June 2018 and March 2020 for the RESIST study.

Baseline visit completed  
$n = 306$

Excluded ($n = 90$)
- Scored $\leq 19$ ($n = 4$)
- Scored $> 27$ ($n = 53$)
- Declined participation ($n = 19$)
- Withdrawn due to medication ($n = 13$)
- Withdrawn due to change in diagnosis ($n = 1$)

Eligible ($n = 216$)

Visit 2 completed  
$n = 130$

Still to complete Visit 3  
$n = 140$

Still to complete Visit 4  
$n = 202$

Visit 3 completed  
$n = 69$

Excluded ($n = 7$)
- Declined participation ($n = 7$)

Eligible ($n = 209$)

Visit 4 completed  
$n = 7$

Eligible ($n = 209$)
3.2.2 Baseline characteristics

The characteristics of all RESIST participants who completed the baseline assessment (n=306) are summarised in Table 3.6. The age of participants at baseline ranged from 55-86 years with a mean of 68.7(SD 7.9) years. Two thirds (66%) of study participants were female. Sixty-two percent were on csDMARD monotherapy or combination therapy at baseline and were allocated to the csDMARD arm of the study. Thirty-four percent were on a TNFi (either alone or in conjunction with a csDMARD) and were assigned to the TNFi group. As aforementioned, the remaining 4% of participants were on non-TNFi bDMARD therapies at baseline and were withdrawn from the study. Forty-two percent of participants at baseline reported history of cardiovascular disease, only 10% had diabetes and 10% were smokers. Just over one third (35%) of participants had spent more than twelve years in full-time education. MoCA score at baseline ranged from 18 to 30 with a mean of 25.2 (SD 2.5) points, this was significantly higher than the mean MoCA score at screening for the same sample (mean difference 0.5, 95% CI 0.22, 0.78, p < 0.001). A large majority (83%) of participants scored ≤27/30 in the MoCA and were classed as having probable MCI at baseline.

As only n=7 participants had completed their final 18-month follow-up visit upon analysis of results, the analysis going forward for the purposes of this thesis will focus on data from participants who had completed their 6-month (n=130) and 12-month (n=69) assessments before March 2020.

3.2.3 Comparison of cognitive performance between screening and baseline

Baseline assessments were completed on average 11 weeks after screening. Cognitive performance was compared for the 306 subjects who had completed the MoCA at both visits. The biggest discrepancies were found in the 3D shape task (screening mean 0.50, SD 0.50 vs baseline mean 0.84, SD 0.37), verbal fluency task (screening mean 0.52, SD 0.50 vs baseline mean 0.68, SD 0.46) and abstraction task (screening mean 1.44, SD 0.67 vs baseline mean 1.76, SD 0.45). Paired analysis revealed that participants on average achieved higher scores in the baseline MoCA (version 7.3) compared to the screening MoCA (version 7.1) with a mean increase of 0.51 points (screening mean 24.64, SD 2.12 vs baseline mean 25.15, SD 2.52; p < 0.001).
Table 3.6. Summary of demographics and MoCA scores for participants at baseline (n=306).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years, mean (+/-SD)</strong></td>
<td>68.7 (7.9)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>• Male, n (%)</td>
<td>104 (34.0)</td>
</tr>
<tr>
<td>• Female, n (%)</td>
<td>202 (66.0)</td>
</tr>
<tr>
<td><strong>RA treatment group</strong></td>
<td></td>
</tr>
<tr>
<td>• csDMARDs, n (%)</td>
<td>189 (62.1)</td>
</tr>
<tr>
<td>• TNFi, n (%)</td>
<td>105 (34.0)</td>
</tr>
<tr>
<td>• Biologics, n (%)</td>
<td>12 (3.9)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
</tr>
<tr>
<td>• Non-smoker, n (%)</td>
<td>147 (48.0)</td>
</tr>
<tr>
<td>• Ex-smoker, n (%)</td>
<td>102 (33.3)</td>
</tr>
<tr>
<td>• Smoker, n (%)</td>
<td>29 (9.5)</td>
</tr>
<tr>
<td>• Unknown, n (%)</td>
<td>28 (9.2)</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
</tr>
<tr>
<td>• N/A, n (%)</td>
<td>246 (80.4)</td>
</tr>
<tr>
<td>• Type 1, n (%)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>• Type 2, n (%)</td>
<td>29 (9.5)</td>
</tr>
<tr>
<td>• Unknown, n (%)</td>
<td>30 (9.8)</td>
</tr>
<tr>
<td><strong>History of cardiovascular disease</strong></td>
<td></td>
</tr>
<tr>
<td>• Yes, n (%)</td>
<td>128 (41.8)</td>
</tr>
<tr>
<td>• No, n (%)</td>
<td>152 (49.7)</td>
</tr>
<tr>
<td>• Unknown, n (%)</td>
<td>26 (8.5)</td>
</tr>
<tr>
<td><strong>Subjective memory concerns</strong></td>
<td></td>
</tr>
<tr>
<td>• Yes, n (%)</td>
<td>50 (16.3)</td>
</tr>
<tr>
<td>• No, n (%)</td>
<td>145 (47.4)</td>
</tr>
<tr>
<td>• Unknown, n (%)</td>
<td>111 (36.3)</td>
</tr>
<tr>
<td><strong>Educational attainment</strong></td>
<td></td>
</tr>
<tr>
<td>• ≤12 years, n (%)</td>
<td>196 (64.1)</td>
</tr>
<tr>
<td>• &gt;12 years, n (%)</td>
<td>107 (35.0)</td>
</tr>
<tr>
<td>• Unknown, n (%)</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td><em><em>MoCA score</em>, mean (+/-SD)</em>*</td>
<td></td>
</tr>
<tr>
<td>• Screening</td>
<td>25.2 (2.5)</td>
</tr>
<tr>
<td>• Baseline</td>
<td>24.7 (2.1)</td>
</tr>
<tr>
<td><strong>Cognitive status</strong></td>
<td></td>
</tr>
<tr>
<td>• &gt;27 / Cognitively normal, n (%)</td>
<td>53 (17.3)</td>
</tr>
<tr>
<td>• ≤27 / Probable MCI, n (%)</td>
<td>253 (82.7)</td>
</tr>
</tbody>
</table>

*Education-adjusted MoCA score missing for n=3 participants. Mean MoCA score was calculated based on n=303 valid participants.
3.2.4 Six-month cohort

The baseline characteristics of the RESIST 6-month cohort (n=130) are summarised for both treatment groups in Table 3.7. Ages ranged from 55-85 years with an overall mean of 68.5 (SD 7.9) years. The TNFi group was slightly younger than the csDMARD group (67.1 years vs 69.2 years) but this was not found to be statistically significant ($p = 0.122$).

Although the male to female ratio was approximately equal across treatment groups in the 6-month cohort, there was a slightly larger proportion of males in this sample (40%) compared to both screening (33%) and baseline (34%).

Over half (52%) of the 6-month cohort reported a history of cardiovascular disease at baseline; only 11% had diabetes and 12% were smokers. Just over one third (34%) of participants had spent more than twelve years in full-time education; there was no association between treatment group and educational attainment ($p = 0.932$). Furthermore, cognitive performance, as assessed by MoCA, was not significantly different between treatment groups at screening ($p = 0.333$).

At baseline, the TNFi group scored significantly higher in the MoCA compared to the csDMARD group (mean difference 0.84, 95% CI 0.12, 1.55, $p = 0.023$). Compared to screening, the TNFi group showed slight improvement in cognitive performance at baseline although this was not statistically significant (mean difference 0.33, 95% CI -0.41, 1.08, $p = 0.372$). On the other hand, the csDMARD group showed a decline in MoCA score from screening to baseline indicating worse cognitive performance but again this result was not significant (mean difference, -0.11, 95% CI -0.62, 0.40, $p = 0.659$).
Table 3.7. Summary of baseline demographics and MoCA scores at screening and baseline for RESIST6-month cohort (n=130).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>All Participants</th>
<th>csDMARD</th>
<th>TNFi</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>130</td>
<td>88</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>68.55 (7.88)</td>
<td>69.22 (8.47)</td>
<td>67.14 (6.31)</td>
<td>0.122</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50 (38.5%)</td>
<td>35 (26.9%)</td>
<td>15 (11.5%)</td>
<td>0.656</td>
</tr>
<tr>
<td>Female</td>
<td>80 (61.5%)</td>
<td>53 (40.8%)</td>
<td>27 (20.8%)</td>
<td></td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>68 (52.3%)</td>
<td>45 (34.6%)</td>
<td>23 (17.7%)</td>
<td>0.864</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>47 (36.2%)</td>
<td>32 (24.6%)</td>
<td>15 (11.5%)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>15 (11.5%)</td>
<td>11 (8.5%)</td>
<td>4 (3.1%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>116 (89.2%)</td>
<td>78 (60.0%)</td>
<td>38 (29.2%)</td>
<td>0.752</td>
</tr>
<tr>
<td>Yes</td>
<td>14 (10.8%)</td>
<td>10 (7.7%)</td>
<td>4 (3.1%)</td>
<td></td>
</tr>
<tr>
<td>History of cardiovascular disease, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>62 (47.7%)</td>
<td>43 (33.1%)</td>
<td>19 (14.6%)</td>
<td>0.699</td>
</tr>
<tr>
<td>Yes</td>
<td>68 (52.3%)</td>
<td>45 (34.6%)</td>
<td>23 (17.7%)</td>
<td></td>
</tr>
<tr>
<td>Educational attainment, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>86 (66.2%)</td>
<td>58 (44.6%)</td>
<td>28 (21.5%)</td>
<td>0.932</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>44 (33.8%)</td>
<td>30 (23.1%)</td>
<td>14 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>MoCA score, mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>24.55 (2.14)</td>
<td>24.42 (2.19)</td>
<td>24.81 (2.02)</td>
<td>0.333</td>
</tr>
<tr>
<td>Baseline</td>
<td>24.58 (1.96)</td>
<td>24.31 (1.98)</td>
<td>25.14 (1.83)</td>
<td><strong>0.023</strong></td>
</tr>
</tbody>
</table>

* Between group comparisons were made using independent samples t-test for continuous variables or chi-squared test for categorical variables (p<0.05).
3.2.4.1 Assessments

Mean assessment scores across both baseline and 6-month timepoints for the MoCA, FCSRT, HAQ, GDS, DAS28 and VAS are summarised by treatment group in Table 3.8.

Cognitive assessments (FCSRT & MoCA)

Both the FCSRT and MoCA were used to measure cognitive function with higher scores in both assessments indicating greater cognitive ability. At baseline, FCSRT free recall score ranged from 9-39 out of a maximum of 48 with a mean of 25.3 (SD 6.3). Total recall score ranged from 40-48 out of 48 with a mean of 47.2 (SD 1.60). At 6 months there was a significant mean increase in free recall score and MoCA score. Compared to the csDMARD group, the TNFi group obtained higher free and total recall scores at both timepoints but only free recall score was statistically significantly different between treatment groups at baseline (mean difference 3.92, 95% CI 1.83, 6.00, \( p < 0.001 \)) and 6 months (mean difference 3.45, 95% CI 0.96, 5.93, \( p = 0.007 \)). After adjusting for baseline scores, between group differences were no longer significant (mean difference 0.578, 95% CI -1.40, 2.56, \( p = 0.565 \)). Similarly, the TNFi group significantly outperformed the csDMARD group in the MoCA at both baseline (mean difference 0.84, 95% CI 0.12, 1.55, \( p = 0.023 \)) and 6 months (mean difference 1.08, 95% CI 0.25, 1.91, \( p = 0.011 \)). However, this mean difference between groups at 6 months did not remain significant after adjusting for baseline MoCA scores (mean difference 0.569, 95% CI -0.15, 1.29, \( p = 0.120 \)).

Functional ability (HAQ)

The HAQ provided a measure of functional ability where higher scores indicate greater physical disability. Scores at baseline ranged from 0 (no incapacity) to 3 (full incapacity) with an overall mean of 1.23 (SD 0.90). There was no significant difference in HAQ score between baseline and 6-month assessments (\( p = 0.156 \)). Compared to the csDMARD group, TNFi participants had statistically higher HAQ scores at both baseline (\( p = 0.024 \)) and 6 months (\( p = 0.009 \)) which may indicate greater degrees of physical disability in TNFi participants. When adjusting for baseline assessment scores, between group differences at 6 months did not remain significant (mean difference 0.103, 95% CI -0.06, 0.26, \( p = 0.204 \)).
Table 3.8. Means (SD) and mean differences (95% CI) in the FCSRT, MoCA, HAQ, GDS, DAS28 and VAS scores by treatment group across a 6-month follow-up (n=130).

<table>
<thead>
<tr>
<th>Assessment /maximum score</th>
<th>Baseline</th>
<th>6 months</th>
<th>6 months</th>
<th>6 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>csDMARD</td>
<td>TNFi</td>
<td>P Value</td>
<td>csDMARD</td>
<td>TNFi</td>
</tr>
<tr>
<td>N</td>
<td>88</td>
<td>42</td>
<td>-</td>
<td>88</td>
<td>42</td>
</tr>
<tr>
<td>aFCSRT free recall/48</td>
<td>24.01 (6.41)</td>
<td>27.93 (5.16)</td>
<td>&lt;0.001</td>
<td>26.10 (6.90)</td>
<td>29.55 (6.25)</td>
</tr>
<tr>
<td>MoCA/30</td>
<td>24.31 (1.98)</td>
<td>25.14 (1.83)</td>
<td>0.023</td>
<td>24.66 (2.28)</td>
<td>25.74 (2.11)</td>
</tr>
<tr>
<td>HAQ/3</td>
<td>1.11 (0.90)</td>
<td>1.49 (0.85)</td>
<td>0.024</td>
<td>1.15 (0.89)</td>
<td>1.59 (0.89)</td>
</tr>
<tr>
<td>GDS/15</td>
<td>3.66 (3.75)</td>
<td>2.74 (2.72)</td>
<td>0.115</td>
<td>3.42 (3.30)</td>
<td>3.43 (3.35)</td>
</tr>
<tr>
<td>bDAS28/9.4</td>
<td>3.44 (1.52)</td>
<td>3.35 (1.57)</td>
<td>0.752</td>
<td>3.71 (1.81)</td>
<td>3.39 (1.75)</td>
</tr>
<tr>
<td>VAS/100</td>
<td>34.30 (28.08)</td>
<td>36.90 (26.51)</td>
<td>0.615</td>
<td>38.27 (30.33)</td>
<td>45.36 (29.48)</td>
</tr>
</tbody>
</table>

<sup>a</sup>FCSRT was incomplete for n=1 participant who was excluded when calculating mean FCSRT free recall scores and between group comparisons.  
<sup>b</sup>DAS28 score was missing for n=1 participant in the TNFi group at baseline and n=4 participants (n=3 TNFi, n=1 csDMARD) at 6 months. These participants were excluded when calculating means and between group comparisons.  
<sup>c</sup>Between group comparisons were made using an independent samples t-test for continuous variables (p<0.05).  
<sup>d</sup>Between group comparisons were made using ANCOVA models which adjusted for baseline assessment scores (p<0.05).
Depression (GDS)

Depression scores ranged from 0-14 out of 15, where higher scores indicate more severe depression. The overall mean of 3.36 (SD 3.47) at baseline and 3.42 (SD 3.3) at 6 months lie within the normal range (<5). There was no significant difference in overall mean depression score at 6 months ($p = 0.796$).

As shown in Table 3.9, participants were grouped into three categories (normal, mild/moderate and moderate/severe depression) based on different cut-off scores in the GDS. The vast majority of participants in both treatment groups scored within the normal range at baseline although a larger proportion of csDMARD participants displayed mild to severe depressive symptoms compared to the TNFi group (26% vs 12%). There was a noticeable increase in the number of participants in the TNFi group who exhibited depressive symptoms at 6 months with this group obtaining a higher mean compared to baseline, though this was not significant ($p = 0.068$). In contrast, the csDMARD group had less participants in the severe category and therefore exhibited a small decrease in mean depression score, though again this was insignificant ($p = 0.428$). Furthermore, there was little evidence that GDS score at 6 months was significantly different between treatment groups in unadjusted ($p = 0.990$) and adjusted analyses ($p = 0.300$).

Disease activity (DAS28)

Disease activity at baseline ranged from 0.49 (remission) to 7.58 (high activity) with an overall mean of 3.41 (SD 1.53) (moderate activity). At 6 months, mean DAS28 score for the cohort as a whole had increased slightly though this was not found to be significant as assessed by paired samples t-test ($p = 0.113$).

In Table 3.9, RA disease activity is grouped into four categories based on different DAS28 cut-offs. The proportion of participants in remission was approximately equal within each group at both baseline and 6-month assessments. Compared to the TNFi group, there was a larger proportion of participants in the csDMARD group that were in the high disease activity category at baseline (18% vs 13%) with even more csDMARD participants at 6 months with high disease activity (23% vs 13%). Additionally, the csDMARD group achieved marginally higher DAS28 scores at both timepoints indicating higher disease activity compared to the TNFi group, though these mean differences were not significant at baseline ($p = 0.752$) or 6 months ($p = 0.356$). Between group differences at 6 months remained insignificant after adjusting for baseline scores ($p = 0.352$).
Table 3.9. Classification of depressive symptoms and disease activity by treatment group and tests of association at baseline and 6-months.

<table>
<thead>
<tr>
<th></th>
<th>Baseline csDMARD</th>
<th>Baseline TNFi</th>
<th>6 months csDMARD</th>
<th>6 months TNFi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>88</td>
<td>42</td>
<td>88</td>
<td>42</td>
</tr>
<tr>
<td><strong>Depression (GDS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (0–5)</td>
<td>65 (73.9%)</td>
<td>37 (88.1%)</td>
<td>66 (75.0%)</td>
<td>31 (73.8%)</td>
</tr>
<tr>
<td>Mild/Moderate (&gt;5)</td>
<td>16 (18.2%)</td>
<td>4 (9.5%)</td>
<td>18 (20.5%)</td>
<td>10 (23.8%)</td>
</tr>
<tr>
<td>Moderate/Severe (&gt;10)</td>
<td>7 (8.0%)</td>
<td>1 (2.4%)</td>
<td>4 (4.5%)</td>
<td>1 (2.4%)</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>87</td>
<td>38</td>
<td>87</td>
<td>38</td>
</tr>
<tr>
<td><strong>Disease activity (DAS28)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission (DAS≤2.6)</td>
<td>28 (32.2%)</td>
<td>14 (36.8%)</td>
<td>32 (36.8%)</td>
<td>15 (39.5%)</td>
</tr>
<tr>
<td>Low (2.6&lt;DAS≤3.2)</td>
<td>16 (18.4%)</td>
<td>2 (5.3%)</td>
<td>10 (11.5%)</td>
<td>4 (10.5%)</td>
</tr>
<tr>
<td>Moderate (3.2&lt;DAS≤5.1)</td>
<td>27 (31.0%)</td>
<td>17 (44.7%)</td>
<td>25 (28.7%)</td>
<td>14 (36.8%)</td>
</tr>
<tr>
<td>High (DAS&gt;5.1)</td>
<td>16 (18.4%)</td>
<td>5 (13.2%)</td>
<td>20 (23.0%)</td>
<td>5 (13.2%)</td>
</tr>
</tbody>
</table>

Depression and disease activity categories are summarised by frequencies and percentages within each treatment group.
Pain (VAS)
Pain scores for the 6-month cohort ranged from 0 (no pain) to 100 (worst pain imaginable) with a mean of 35.14 (SD 27.51) at baseline. At 6 months, VAS pain score for the cohort as a whole had significantly increased (mean difference 5.42, 95% CI 0.81, 10.34, \( p = 0.022 \)) Within the TNFi group there was a significant mean increase from baseline to 6 months as assessed by a paired samples t-test (mean difference 8.45, 95% CI 2.85, 14.05, \( p = 0.004 \)). While the csDMARD group also exhibited higher pain scores at 6 months the mean difference was not significant (\( p = 0.214 \)). Though the TNFi group obtained higher mean pain scores across both timepoints compared to the csDMARD group, these mean differences were not significant (\( p > 0.05 \)). Between group differences in VAS pain score at 6 months remained insignificant after adjusting for baseline scores (mean difference 5.45, 95% CI -3.71, 14.60, \( p = 0.242 \)).

3.2.4.2 ANCOVA analysis of treatment effects on cognitive performance at 6 months with additional adjustments

FCSRT
As shown in Table 3.10, after adjustment for covariates (baseline FCSRT, HAQ, GDS, DAS28, age, sex, education, history of cardiovascular disease, diabetes and smoking status) the TNFi group actually appeared to show the greatest improvement in free recall at 6 months, but this was not significant (mean difference 0.78, 95% CI -1.90, 3.47, \( p = 0.562 \)).

MoCA
Similarly the TNFi group appeared to show greater improvement in the MoCA compared to the csDMARD group after adjustment for covariates in Table 3.10, although there was little evidence that this was significant (mean difference 0.28, 95% CI -0.67, 1.24, \( p = 0.553 \)).
Table 3.10. Mean difference (95% CI) between treatment groups in FCSRT and MoCA score changes from baseline to 6 months.

<table>
<thead>
<tr>
<th></th>
<th>FCSRT</th>
<th>MoCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adjusted model</td>
</tr>
<tr>
<td>csDMARD</td>
<td>0.00 (reference)</td>
<td>0.00 (reference)</td>
</tr>
<tr>
<td>TNFi</td>
<td>0.78 (-1.90, 3.47)</td>
<td>0.28 (-0.67, 1.24)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.562</td>
<td>0.553</td>
</tr>
</tbody>
</table>

Model is adjusted for baseline FCSRT/MoCA score, baseline HAQ, GDS and DAS28 scores plus demographic confounders (age, sex, education, history of cardiovascular disease, diabetes and smoking status).
3.2.5 Twelve-month cohort

The baseline characteristics of the RESIST 12-month cohort (n=69) are summarised for both treatment groups in Table 3.11. Ages ranged from 55-85 years with an overall mean of 67.8 years. The TNFi group was slightly younger than the csDMARD group (67.2 years vs 68.3 years) but this was not found to be statistically significant (p = 0.532). Following the same trend as the RESIST screening cohort, almost two thirds (62%) of study participants in the 12-month sample were female with approximately equal distribution of males and females across treatment groups. Over half (51%) of the sample reported a history of cardiovascular disease at baseline, 14% had diabetes and 12% were smokers. Just over one third (36%) of participants had spent more than twelve years in full-time education with no significant differences in educational attainment between the treatment groups. Furthermore, there was no significant difference in MoCA performance between treatment groups at screening or baseline (p > 0.05). Compared to screening, the TNFi group showed slight improvement in cognitive performance at baseline although this was not statistically significant as assessed by paired samples t-test (mean difference 0.27, 95% CI -0.61, 1.14, p = 0.539). On the contrary, the csDMARD group showed a statistically significant decline in MoCA score from screening to baseline (mean difference, -0.85, 95% CI -1.45, -0.24, p = 0.008).
Table 3.11. Summary of baseline demographics and MoCA scores at screening and baseline for RESIST 12-month cohort (n=69).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>All participants</th>
<th>csDMARD</th>
<th>TNFi</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>69</td>
<td>39</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>67.78 (7.40)</td>
<td>68.26 (8.30)</td>
<td>67.17 (6.12)</td>
<td>0.532</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26 (37.7%)</td>
<td>14 (20.3%)</td>
<td>12 (17.4%)</td>
<td>0.727</td>
</tr>
<tr>
<td>Female</td>
<td>43 (62.3%)</td>
<td>25 (36.2%)</td>
<td>18 (26.1%)</td>
<td></td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>38 (55.1%)</td>
<td>22 (31.9%)</td>
<td>16 (23.2%)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>23 (33.3%)</td>
<td>12 (17.4%)</td>
<td>11 (15.9%)</td>
<td>0.851</td>
</tr>
<tr>
<td>Smoker</td>
<td>8 (11.6%)</td>
<td>5 (7.2%)</td>
<td>3 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>59 (85.5%)</td>
<td>33 (47.8%)</td>
<td>26 (37.7%)</td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>1 (1.4%)</td>
<td>0 (0.0%)</td>
<td>1 (1.0%)</td>
<td>0.431</td>
</tr>
<tr>
<td>Type 2</td>
<td>9 (13.0%)</td>
<td>6 (8.7%)</td>
<td>3 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>History of cardiovascular disease, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (50.7%)</td>
<td>18 (26.1%)</td>
<td>16 (23.2%)</td>
<td>0.554</td>
</tr>
<tr>
<td>No</td>
<td>34 (49.3%)</td>
<td>21 (30.4%)</td>
<td>14 (20.3%)</td>
<td></td>
</tr>
<tr>
<td>Educational attainment, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>44 (63.8%)</td>
<td>24 (34.8%)</td>
<td>20 (29.0%)</td>
<td>0.660</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>25 (36.2%)</td>
<td>15 (21.7%)</td>
<td>10 (14.5%)</td>
<td></td>
</tr>
<tr>
<td>MoCA score, mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>25.00 (1.67)</td>
<td>25.13 (1.56)</td>
<td>24.83 (1.82)</td>
<td>0.472</td>
</tr>
<tr>
<td>Baseline</td>
<td>24.64 (1.90)</td>
<td>24.28 (1.88)</td>
<td>25.10 (1.86)</td>
<td>0.076</td>
</tr>
</tbody>
</table>

* Between group comparisons were made using independent samples T-test for continuous variables or Chi-squared test for categorical variables (p<0.05).
3.2.5.1 Assessments

Mean assessment scores across each timepoint for the MoCA, FCSRT, HAQ, GDS, DAS28 and VAS are summarised by treatment group in Table 3.12.

Cognitive assessments (FCSRT & MoCA)

At baseline, free recall score for the cohort as a whole ranged from 14-38 out of a maximum of 48 with a mean of 26.4 (SD 5.4). Total recall score ranged from 42 to 48 out of 48 with a mean of 47.4 (SD 1.3). At 12 months there was a significant mean increase in free recall score (mean difference 1.81, 95% CI 0.54, 3.08, \( p = 0.006 \)). There was also a significant increase in MoCA score at 12 months (mean difference 1.26, 95% CI 0.71-1.82, \( p < 0.001 \)).

Similar to the 6-month cohort, the TNFi group in the 12-month cohort obtained higher free recall, total recall and MoCA scores across each timepoint compared to the csDMARD group. Baseline free recall score was significantly different between treatment groups (mean difference 2.94, 95% CI 0.52-5.36, \( p = 0.018 \)). However, there was little evidence a difference between treatment groups in unadjusted (\( p = 0.582 \)) and adjusted (\( p = 0.381 \)) analyses at 12 months.

The TNFi group also performed better than the csDMARD group in the MoCA at each timepoint although between group differences were not statistically significant at baseline (\( p = 0.076 \)), 12 months unadjusted (\( p = 0.575 \)) or 12 adjusted analyses (\( p = 0.940 \)).

Functional ability (HAQ)

HAQ scores at baseline ranged from 0-3 with an overall mean of 1.20 (SD 0.93). There was no significant mean difference in HAQ score between baseline and 12-month assessments (\( p = 0.205 \)).

TNFi participants in the 12-month sample had statistically higher HAQ scores at baseline (\( p = 0.016 \)), 6 months (\( p = 0.001 \)), and 12 months (\( p = 0.014 \)) again suggesting that the TNFi group experiences greater difficulty carrying out daily activities. When adjusting for baseline HAQ scores, between group differences remained significant at 6 months (mean difference 0.25, 95% CI 0.03, 0.47, \( p = 0.024 \)), but not at 12 months (mean difference 0.05, 95% CI -0.14, 0.23, \( p = 0.611 \)).
Table 3.12. Means and standard deviations in the FCSRT, MoCA, HAQ, GDS, DAS28 and VAS by treatment group across a 12-month follow-up (n=69).

<table>
<thead>
<tr>
<th>Assessment /maximum score</th>
<th>Baseline</th>
<th></th>
<th></th>
<th>12 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>csDMARD</td>
<td>TNFi</td>
<td>P Valuea</td>
<td>csDMARD</td>
<td>TNFi</td>
</tr>
<tr>
<td>N</td>
<td>39</td>
<td>30</td>
<td>-</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td>FCSRT/48</td>
<td>25.13 (5.92)</td>
<td>28.07 (4.15)</td>
<td>0.018</td>
<td>27.87 (6.41)</td>
<td>28.67 (5.20)</td>
</tr>
<tr>
<td>MoCA/30</td>
<td>24.28 (1.88)</td>
<td>25.10 (1.86)</td>
<td>0.076</td>
<td>25.77(2.27)</td>
<td>26.07 (2.05)</td>
</tr>
<tr>
<td>HAQ/3</td>
<td>0.97 (0.91)</td>
<td>1.51 (0.87)</td>
<td>0.016</td>
<td>1.03 (0.92)</td>
<td>1.56 (0.84)</td>
</tr>
<tr>
<td>GDS/15</td>
<td>3.56 (4.36)</td>
<td>2.63 (2.66)</td>
<td>0.278</td>
<td>3.62 (4.05)</td>
<td>2.43 (3.14)</td>
</tr>
<tr>
<td>DAS28/9.4</td>
<td>3.42 (1.50)</td>
<td>3.57 (1.56)</td>
<td>0.689</td>
<td>3.69 (1.85)</td>
<td>3.73 (1.51)</td>
</tr>
<tr>
<td>VAS/100</td>
<td>33.97 (29.69)</td>
<td>39.33 (26.90)</td>
<td>0.442</td>
<td>44.74 (31.64)</td>
<td>43.50 (28.62)</td>
</tr>
</tbody>
</table>

a Between group comparisons were calculated using independent samples T-test (p<0.05).
b Between group comparisons were calculated using ANCOVA models which adjusted for baseline assessment scores (p<0.05).
Depression (GDS)

In the 12-month cohort, baseline depression scores ranged from 0-14 out of 15 with a mean of 3.16 (SD 3.72). There was no significant difference in overall mean depression score at 12 months ($p = 0.897$).

Participants were grouped into categories based on different cut-off scores in the GDS (Table 3.13). Comparable to the 6-month cohort, a large majority (80%) of the 12-month sample scored within the normal range in the GDS at baseline. In comparison to the TNFi group, a larger proportion of csDMARD participants exhibited mild to severe depressive symptoms at both baseline (26% vs 13%) and 12 months (33% vs 13%). Furthermore, the csDMARD group showed a small increase in depression score over at 12 months while the TNFi group declined which is the opposite to what was observed in the 6-month cohort. There was little evidence that mean differences between treatment groups at 12 months were statistically significant in unadjusted ($p = 0.191$) and adjusted models ($p = 0.360$).

Disease activity (DAS28)

For the 12-month cohort, disease activity at baseline ranged from 0.56 (remission) to 7.58 (high activity) with an overall mean of 3.48 (SD 1.52) (moderate activity). The mean DAS28 score was lower in the csDMARD group at baseline (3.42 vs 3.57) with a greater percentage of participants in this group achieving remission compared to the TNFi group (33% vs 17%), although there was also a greater percentage of csDMARD participants in the high disease category at both timepoints (Table 3.14).

Though not statistically significant, DAS28 score had increased at 12 months ($p = 0.157$). Over half (58%) of participants achieved higher DAS28 scores at 12 months with one third of participants being grouped in a higher disease activity category compared to baseline. Unlike at baseline, the TNFi group achieved lower DAS28 scores and had a larger proportion of participants achieving remission compared to the csDMARD group (30% vs 26%). Between group differences in DAS28 score were not significant at baseline ($p = 0.689$) or 12 months (unadjusted $p = 0.927$, adjusted $p = 0.803$).
Table 3.13. Classification of depressive symptoms and disease activity by treatment group and tests of association at baseline and 12-months.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>12 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>csDMARD</td>
<td>TNFi</td>
<td>csDMARD</td>
<td>TNFi</td>
</tr>
<tr>
<td>N</td>
<td>39</td>
<td>30</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td><strong>Depression (GDS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (0-5)</td>
<td>29 (74.4%)</td>
<td>27 (90.0%)</td>
<td>28 (71.8%)</td>
<td>26 (86.7%)</td>
</tr>
<tr>
<td>Mild/Moderate (&gt;5)</td>
<td>6 (15.4%)</td>
<td>2 (6.7%)</td>
<td>9 (23.1%)</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Moderate/Severe (&gt;10)</td>
<td>4 (10.3%)</td>
<td>1 (3.3%)</td>
<td>2 (5.1%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>30</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td><strong>Disease activity (DAS28)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission (DAS≤2.6)</td>
<td>10 (25.6%)</td>
<td>9 (30.0%)</td>
<td>13 (33.3%)</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td>Low (2.6&lt;DAS≤3.2)</td>
<td>10 (25.6%)</td>
<td>2 (6.67%)</td>
<td>4 (10.3%)</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>Moderate (3.2&lt;DAS≤5.1)</td>
<td>13 (33.3%)</td>
<td>15 (50.0%)</td>
<td>13 (33.3%)</td>
<td>9 (30.0%)</td>
</tr>
<tr>
<td>High (DAS&gt;5.1)</td>
<td>6 (15.4%)</td>
<td>4 (13.3%)</td>
<td>9 (23.1%)</td>
<td>5 (16.7%)</td>
</tr>
</tbody>
</table>

Depression and disease activity categories are summarised by frequencies and percentages within each treatment group.

* Association between treatment group and classification of GDS/DAS28 score was assessed through Chi-square test ($p<0.05$).
Pain (VAS)
Pain scores for the 12-month cohort ranged from 0 (no pain) to 100 (worst pain imaginable) with a mean of 36.30 (SD 28.43) at baseline. At 12 months pain score had significantly increased (mean difference 7.90, 95% CI 1.40, 14.40, \( p = 0.018 \)) with both groups exhibiting higher degrees of pain compared to baseline, however the mean increase was only significant within the csDMARD group (mean difference 10.77, 95% CI 1.42, 20.12, \( p = 0.025 \)). Though not statistically significant, the TNFi group obtained a higher mean pain score at baseline (\( p = 0.442 \)) but a lower mean score at 12 months (\( p = 0.867 \)) compared to the csDMARD group. Between group differences in VAS pain score at 12 months remained insignificant after adjusting for baseline scores (\( p = 0.457 \)).

3.2.5.2 ANCOVA analysis of treatment effects on cognitive performance at 12 months with additional adjustments

FCSRT
Between group differences remained insignificant after adjusting for covariates in Table 3.14 (baseline FCSRT, HAQ, GDS, DAS28, age, sex, education, history of cardiovascular disease, diabetes, smoking) with the csDMARD group maintaining the greatest improvement (mean difference -0.70, 95% CI -4.43, 2.93, \( p = 0.694 \)).

MoCA
When adjusting for covariates in Table 3.14 the between group differences remained statistically insignificant however the difference was reversed with TNFi group appearing to demonstrate more of an improvement (mean difference 0.54, 95% CI -1.14, 2.22, \( p = 0.514 \)).
Table 3.14. Mean difference (95% CIs) in cognitive test score change from baseline to 12 months between treatment groups for the FCSRT and MoCA

<table>
<thead>
<tr>
<th></th>
<th>FCSRT</th>
<th>MoCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted model</td>
<td></td>
</tr>
<tr>
<td>csDMARD</td>
<td>0.00 (reference)</td>
<td>0.00 (reference)</td>
</tr>
<tr>
<td>TNFi</td>
<td>-0.70 (-4.34, 2.93)</td>
<td>0.54 (-1.14, 2.22)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.694</td>
<td>0.514</td>
</tr>
</tbody>
</table>

Model 1 adjusts for baseline HAQ, GDS and DAS28 scores plus demographic confounders (age, sex, education, history of cardiovascular disease, diabetes and smoking status).
3.2.6 Association between RA treatment group and probable MCI classification.

Cognitive status is summarised by treatment group for both 6-month and 12-month cohorts in Table 3.15, where probable MCI was defined as MoCA scores ≤ 27.

In the 6-month cohort, the vast majority (86%) of participants scored ≤ 27 in the MoCA at the 6-month assessment and were considered to have probable MCI. Within the csDMARD group the risk of being cognitively impaired was 7.8 times greater than being classed as cognitively normal (MoCA >27), whereas within the TNFi group the risk of being cognitively impaired was 4.25 times higher than being cognitively normal. Compared to the csDMARD group, the odds of having probable MCI was lower in the TNFi group with an odds ratio of 0.545 (95% CI 0.198, 1.501) although the association between treatment group and cognitive status was not statistically significant, as assessed by Chi-squared test ($\chi^2(1) = 1.41, p = 0.236$).

Of the 69 participants (39 csDMARD, 30 TNFi) that had completed a 12-month visit, n=52 participants (75%) were classed as having probable MCI. Of the csDMARD group, n=31 participants (79%) scored 27 or less in the MoCA compared to n=21 (70%) in the TNFi group. The risk of being cognitively impaired was higher than being cognitively normal in both groups. Compared to the csDMARD group, the odds of scoring ≤ 27 in the MoCA was lower in the TNFi group with an odds ratio of 0.602 (95% CI 0.20, 1.81). However, the association between RA treatment group and cognitive status was not found to be statistically significant, as assessed by Chi-squared test ($\chi^2(1) = 0.82, p = 0.365$).
**Table 3.15.** Contingency table showing the classification of cognitive status at both 6 and 12 months according to a MoCA cut-off of ≤ 27.

<table>
<thead>
<tr>
<th>Cognitive status</th>
<th>csDMARDs</th>
<th>TNFi</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>csDMARDs</th>
<th>TNFi</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>88</td>
<td>42</td>
<td>-</td>
<td>39</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td><strong>Probable MCI (MoCA ≤ 27)</strong></td>
<td>78 (88.6%)</td>
<td>34 (81.0%)</td>
<td>0.236</td>
<td>31 (79.5%)</td>
<td>21 (70.0%)</td>
<td>0.365</td>
</tr>
<tr>
<td><strong>Cognitively normal (MoCA &gt; 27)</strong></td>
<td>10 (11.4%)</td>
<td>8 (19.0%)</td>
<td></td>
<td>8 (20.5%)</td>
<td>9 (30.0%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Association between treatment group and cognitive status was assessed by Chi-square test (p<0.05)
3.2.7 Impact of COVID-19 lockdown on depression scores

Forty-seven participants to date have completed the GDS since the restart of study visits on 03/09/20. Post-lockdown depression scores increased in 19 participants (40%), decreased in 14 (30%) and did not change in 14 (30%). Table 3.16 summarises the classification of depression according to different cut-offs for both pre- and post-lockdown visits. There was an overall increase in number of participants who were categorised as having mild-severe depression post-lockdown (30% vs 21%) however there was no statistically significant mean increase in depression score between pre- and post-lockdown visits (mean difference 0.40, 95% CI -0.31, 1.12, \( p = 0.260 \)).

Table 3.16. Classification of depression scores both pre- and post-lockdown (n=47)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Pre-lockdown, n (%)</th>
<th>Post-lockdown, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 (normal)</td>
<td>37 (78.7%)</td>
<td>33 (70.2%)</td>
</tr>
<tr>
<td>5-8 (mild depression)</td>
<td>6 (12.8%)</td>
<td>12 (25.2%)</td>
</tr>
<tr>
<td>9-11 (moderate depression)</td>
<td>2 (4.3%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>12-15 (severe depression)</td>
<td>2 (4.3%)</td>
<td>1 (2.1%)</td>
</tr>
</tbody>
</table>
Chapter 4: Discussion and Conclusion

4.1 Summary of screening analyses

The analysis of RESIST screening data aimed to produce descriptive data for a large sample of older adults with RA that were screened for the study using the MoCA and to investigate the impact of demographic and clinical characteristics on cognitive status in this population. An unexpectedly high proportion (71.5%) of screened participants had probable MCI according to a cut-off score of 27/30 in the MoCA with two thirds (66.8%) of those screened meeting the inclusion criteria for the RESIST study (i.e. MoCA scores between 20 and 27). While the mean MoCA score for the RESIST screening sample was 2 points lower than original normative data published by Nasreddine et al. (2005), age stratified mean MoCA scores for this cohort were similar to normative data published in recent population-based studies.\textsuperscript{543, 584-586}

A MoCA cut-off of ≤26 is widely used in the literature to indicate MCI as this was the score originally proposed by Nasreddine et al (2005).\textsuperscript{543} When applied to the RESIST screening cohort, almost half of the participants (44.9%) fell below this cut-off with the biggest deficits in delayed recall, visuospatial/executive function and verbal fluency tasks. Our findings are analogous to previous studies which assessed cognitive function in patients with RA. A study by Bartolini et al.\textsuperscript{587} reported cognitive impairment in 38-71% of their RA cohort with worst cognitive outcomes observed in the visuospatial/executive function tasks. A study by Shin et al.\textsuperscript{537} observed that cognitive impairment in RA ranged from 8% on semantic fluency tasks to 29% on visuospatial/memory tasks with 31% of their cohort being classified as cognitively impaired overall. Similarly, Appenzeller et al.\textsuperscript{588} found 30% of their RA cohort to be cognitively impaired in a battery of neuropsychological assessments. We found lower prevalence rates compared to a recent study by Vitturi et al.\textsuperscript{538} who observed cognitive impairment in 98% of their RA population using a cut-off score of 26/30 points in the MoCA. This discrepancy may be due to low educational attainment in that study as 46% of RA participants had less than 4 schooling years and 3% were illiterate. The main limitation of these studies is that they consisted of small RA sample sizes.\textsuperscript{538, 587, 588} One systematic review explored the levels of cognitive impairment in patients with RA across fifteen studies. Although the authors were unable to determine the prevalence of MCI as only three studies reported this information, they found evidence that individuals with RA significantly underperform in cognitive assessments compared to healthy controls - particularly in memory, verbal fluency and attention tasks. In addition, age, education, disease activity and depression were identified as factors associated with cognitive impairment, although this was inconsistent across individual studies and based on small sample sizes ranging from 13 to 157 participants.\textsuperscript{536}

While methodology varies between these observational studies making direct comparisons
difficult, they imply that the burden of cognitive impairment in RA is significant and highlights the need for standardised longitudinal assessment of cognition in these patients.

RESIST participants on average performed better in their baseline cognitive assessment compared to initial screening however this should be interpreted with caution as different versions of the MoCA were used at screening (version 7.1) and baseline (version 7.3). Nevertheless, higher scores might be expected at baseline due to the difference in environment. Screening was conducted in a busy clinical setting while baseline and all subsequent assessment took place in participants’ homes, providing a quieter and more relaxed environment which has been shown to impact cognitive performance.

A relationship between subjective memory concern and cognitive impairment was proposed by Petersen and has since been adapted into the criteria for diagnosing MCI. There is evidence that subjective memory complaints may be a predictor of cognitive impairment and dementia up to two decades later. However, the results presented in this thesis found that the agreement between subjective memory concern and objective cognitive impairment (according to a MoCA score of ≤27) was poor and contributes to the growing body of research which suggests that subjective memory complaints may not be a reliable indicator of cognitive impairment and should perhaps be reconsidered as part of the diagnostic criteria for MCI. However, it should be noted that self-reported memory complaints were completed as part of a screening data collection form during face-to-face interviews and as such could be subject to a certain amount of socially desirable response bias (i.e. subjects choose the more favourable response which may not reflect their true thoughts). This may explain why 75% of participants with objective cognitive impairment did not report any memory complaints prior to screening.

As previously discussed in Chapter 1: Literature review, age is the biggest risk factor for AD with risk approximately doubling every 5 years of age. This preliminary analysis of RESIST screening data revealed that the mean MoCA score decreased with increasing age which is consistent with other studies that used the MoCA to measure cognitive performance. Educational attainment also had a significant impact on MoCA performance with those who spent more than 12 years in full-time education outperforming those with 12 years or less. This concurs with the majority of the literature which suggests that those who are less educated are at a greater risk for developing AD and supports the theory that those with higher education possess a larger cognitive reserve which is able to compensate for neuronal loss for longer. Participants who were positive for RF antibodies (seropositive RA) on average achieved lower scores in the MoCA compared to those who were seronegative. A linear relationship between DAS28 score and MoCA score was discovered which is analogous to previous studies. Moreover, high RA disease activity was associated with worse cognitive outcomes. Together,
these findings imply that higher levels of chronic systemic inflammation may significantly affect cognitive function which has implications beyond the scope of RA. Thus strategies to prevent cognitive impairment and reduce risk of AD should include measures to modulate inflammation.

4.2 Summary of longitudinal analyses

4.2.1 Primary objective
The primary objective of this research was to test the hypothesis that TNFi treatment slows the rate of cognitive decline in a group of older adults with both RA and MCI, compared to a group of individuals with RA and MCI on csDMARD treatment.

Both treatment groups in the 6-month cohort demonstrated a significant improvement in free recall performance over time, however there was no evidence of a difference between TNFi and csDMARD use in the FCSRT (mean difference 0.58, 95% CI -1.40, 2.56, p = 0.565) or MoCA (mean difference 0.57, 95% CI -0.15, 1.29, p = 0.120) after adjustment for baseline.

In analysis of the RESIST 12-month cohort both groups demonstrated better cognitive function overall, though only the csDMARD group significantly improved. After adjustment for baseline there was no difference in the FCSRT (mean difference -1.09, 95% CI -3.57, 1.38, p = 0.381) or MoCA (mean difference -0.04, 95% CI -1.06, 0.98, p = 0.940) between treatment groups at 12 months.

Furthermore, with regards to the classification of probable MCI according to a MoCA cut-off score of 27, there no association between TNFi use and reduced risk of cognitive impairment within the 6- or 12-month cohorts.

4.2.2 Secondary Objective
The secondary objective of this thesis was to determine whether TNFi slow the rate of decline in a population of adults with RA and MCI, independent of mood and disease severity.

In analyses of both 6-month and 12-month follow-up cohorts, there was no evidence of an association between TNFi and better cognitive performance in the FCSRT or MoCA after adjustment for mood (GDS), disease severity (DAS28), functional limitations (HAQ) and other demographic confounders.


4.3 Discussion

Overall, these results suggest that there may be no meaningful cognitive benefit to using TNFi over traditional anti-rheumatic treatment in patients with both RA and MCI. There is conflicting evidence surrounding the efficacy of TNFi on cognition due to the lack of longitudinal studies. As discussed in Chapter 1: Literature review, early evidence for TNFi as an AD treatment came from a prospective pilot study of fifteen patients with mild to severe AD. Patients were given 25-50mg of etanercept by perispinal injection once a week for six months. After six months patients exhibited significant improvement in the MMSE, the Alzheimer’s Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and the Severe Impairment Battery (SIB). However, this route of administration is invasive and therefore not ideal for most patients, particularly those who may be more vulnerable.

Another pilot study investigated the effects of subcutaneous adalimumab on cognition. Fifteen patients with RA received 40mg of adalimumab by subcutaneous injection every 2 weeks as part of their routine clinical care. After 12 weeks, patients showed significant improvement in full scale, verbal and performance IQ while also showing signs of reduced RA disease activity. Discrepancies between these studies and RESIST may be due in part to differences in methodology e.g. the studies mentioned above were proof of concept studies and thus consisted of small sample sizes and used different tools to assess cognitive function. Moreover the patient populations differed in terms of cognitive status at baseline and overall demographics e.g. the mean age of participants reported by Raferty et al. was very young at 48.9 years of age.

In addition, a number of epidemiological studies have found that TNFi reduce the risk of cognitive impairment in RA. A recent retrospective case-control study examined electronic health records of 56 million adults with an inflammatory disease in order to test the hypothesis that systemic inflammation involving TNFα is associated with an increased risk of AD and that this could be mitigated using TNFi. RA was associated with a higher risk of AD compared to a non-inflammatory disease group. Furthermore, RA treatment with etanercept, adalimumab or infliximab was associated with a significantly reduced risk of AD. The benefit of etanercept was also replicated in RA patients with a diagnosis of dementia. However, there is also evidence in the literature of a non-existent or negative association between TNFi and cognitive function and risk of AD. A randomised double-blind phase 2 clinical trial of patients with mild to moderate AD randomly allocated participants to receive a subcutaneous injection containing 50mg of etanercept or placebo once a week for 24 weeks. While etanercept was well tolerated, the researchers found no evidence that it improved cognitive function, global function or behaviour compared to the placebo group. Another study suggested that TNFi administration led to a case of reversible rapidly progressive dementia in a patient with ankylosing spondylitis which appeared to improve upon TNFi
withdrawal – suggesting that TNFi may be involved in the manifestation of dementia.\textsuperscript{607} A recently published study by Oláh \textit{et al.} (2020) investigated the effects of different anti-rheumatic treatments on cognitive function in 60 female patients with RA. Patients were over 18 years of age and were on a stable dose of methotrexate or biologic therapy for at least six months prior to the study. There were 20 participants who were receiving methotrexate and 40 who were receiving biologics (20 on infliximab, 20 on tocilizumab) in combination with methotrexate. Additionally, a control group consisting of 39 healthy female participants was included. Participants were cognitively assessed using a battery of neuropsychological tests including the MoCA where cognitive impairment was defined as scores of <26/30. MoCA scores were found to be significantly lower amongst the RA population compared to the control group. Furthermore, researchers found a negative association between TNFi use and cognition with those on biologic treatments appearing to be at greater risk of cognitive impairment compared to those on csDMARDs.\textsuperscript{608} However, this study was cross-sectional in design and did not investigate treatment effects over time. The sample sizes were relatively small, particularly within the csDMARD group (n=20). Moreover, the bDMARD group included both TNFi (infliximab) and non-TNFi biologics (tocilizumab), therefore treatment effects within this group cannot be attributed to TNFi alone.\textsuperscript{608}

There is also a lack of research surrounding the effects of csDMARDs on cognition with both protective and detrimental effects being previously reported. A retrospective case-control study using data from the Taiwan National Health Insurance Research Database investigated the effect of treatment on dementia incidence in 1,914 patients with RA. The control group, consisting of subjects with RA, was compared against patients with both RA and dementia. csDMARD use was associated with a 1.63-fold higher risk for dementia, particularly vascular dementia. Methotrexate, sulfasalazine, hydroxychloroquine and leflunomide all conferred significant risk for dementia, and even greater risk when used in combination therapies. There was no significant risk of dementia amongst bDMARD or NSAID users.\textsuperscript{609} Another study reported that higher doses of methotrexate were associated with poorer cognitive performance but still remained within the normal range.\textsuperscript{610} A double-blind placebo control trial investigated the effects of hydroxychloroquine on cognitive function in patients with early AD found no difference in the rate of cognitive decline between placebo and control group after 18 months. However, subjects recruited to this study did not have rheumatoid arthritis and as such were only assigned the treatment groups at the beginning of the study. Thus, participants would not exhibit any long-term effects this medication may have.\textsuperscript{611} On the contrary, one retrospective population-based study using data from the UK Clinical Practice Research Datalink identified 11,772 patients with RA, 8312 of which were on a
csDMARD. After a median follow-up of 6.5 years, csDMARDs were associated with a reduced risk of dementia compared to non-users, particularly amongst methotrexate users.612

As discussed in Chapter 1: Literature review, TNFi are typically prescribed to patients after the failure of multiple csDMARD therapies and as such are often associated with more severe disease states.613, 614 While measures of pain, disease activity, depression and functional limitations were found to be correlated,615-618 they did not differ between csDMARD and TNFi treatment groups in this study.619-621

The observed increase in cognitive performance was especially surprising given that both treatment groups experienced slight increases in functional incapacity, disease severity and pain over time, all of which have been previously linked to poorer cognitive outcomes.605, 622-625

Depression is a well-known comorbidity of RA; many studies have found depression to be more common in RA patients compared to healthy controls.626, 627 Other research has found that depression may also be associated with cognitive impairment and AD.628, 629 A meta-analysis carried out on seven case-control studies and six prospective studies put forward three potential hypothesis to explain the association between depression and dementia risk. The first hypothesis is that depression is a prodromal feature of dementia, the second is that depression accelerates the clinical manifestation of dementia and the third is that depression damages the hippocampus via the glucocorticoid cascade, thereby causing dementia. While the evidence was found to support the association between depression and dementia, it did not favour any one hypothesis.530

One study used both case-control and co-twin control study designs to investigate whether a history of depression was associated with an increased likelihood of dementia and if this association was dependent on the timing of the first depressive episode. Participants in the study had taken part in the Study of Dementia in Swedish Twins (HARMONY).631 The co-twin sample consisted of 146 twin pairs aged 65 and older; analysis revealed that twins with a history of depression were three times more likely to have dementia compared to their co-twin after controlling for shared genetic and environmental risk factors. Furthermore, the case-control analysis, which consisted of 12,680 individuals aged 65 and older, revealed that the occurrence of the first depressive episode within 10 years of dementia onset was associated with a two and a half times greater likelihood of AD and nearly a four times greater likelihood of dementia. Therefore, this study supported the hypothesis that depression is a prodromal feature of dementia.628 There are other studies which have found that early onset depression is associated with increased risk of dementia, suggesting that depression is not a prodrome, however this was only true for those with low educational levels.632, 633

Despite finding no significant difference in the occurrence of depressive symptoms between treatment groups in the present study, it was interesting to find that in both the 6-month and
12-month cohorts the group that experienced a decrease in depressive symptoms over time was the group that showed the greatest improvement in the MoCA. This could be indicative of the association between depression and cognitive impairment, although there were no significant correlations between HAQ, GDS, VAS OR DAS28 and cognitive outcomes. This may be due to the relatively low levels registered in each measurement as there is evidence to suggest that only very severe symptoms are associated with cognitive impairment.634, 635

4.4 Strengths

There is little evidence in the literature which evaluates cognitive performance in individuals with RA with most of the existing studies tending to be cross-sectional in design.536, 608 To the researcher’s knowledge, this is one of the first longitudinal approaches to evaluating the effects of different anti-rheumatic medications, particularly TNFi, on cognition in older RA subjects with MCI. The preliminary analysis of screening data, while mainly explorative, provided useful information on the prevalence of probable MCI in a UK population of adults with RA whilst identifying possible predictors of cognitive impairment. The addition of the FCSRT in the longitudinal study design adds strength and reliability to the measurement of cognitive change. Furthermore, the longitudinal phase of study was rather comprehensive in controlling for possible confounding variables by administering various assessments for the measurement of disease activity, pain, mood and functional incapacity.

4.5 Limitations

While the results presented in this research yielded some interesting findings, there are certain limitations that should be considered in order to better interpret the results.

4.5.1 Screening analyses

The intention of screening was to rapidly identify subjects with possible cognitive impairment rather than to formally diagnose MCI and so cognitive function was assessed using the MoCA alone which may limit sensitivity. Moreover, the sensitivity and specificity of the cut-off score used was not able to be evaluated due to the lack of known MCI diagnoses, thus there may be a larger number of false positives (i.e. the misclassification of cognitively normal individuals as cognitively impaired) than would be obtained when using a battery of neuropsychological assessments or different cut-off scores.636 At the end of the 18-month follow-up period planned for RESIST a diagnosis of MCI (following Petersen criteria590) will be reached by consensus
clinical opinion (BMcG, CH) for each participant and will provide insight as to which is the most appropriate MoCA cut-off score for detecting MCI in this RA population. RESIST is a multicentre study and therefore involved several different interviewers. While each interviewer was trained in administering the MoCA (and subsequent follow-up assessments) and scores were calculated following the standardised marking scheme and checked by BMcD prior to analysis, the potential for interviewer error must still be considered.637

There was a large amount of missing data in relation to RA disease characteristics recorded at screening, this is due to the fact that this information was largely obtained from hospital electronic care records and as such depended on the duration of disease and the level of detail in the initial diagnosis letter written by rheumatology consultants. Consequently, the regression analysis was conducted on a reduced sample and may not be representative of the entire screening cohort. Important information on comorbidities (e.g. cardiovascular disease, smoking status, diabetes) and psychological conditions (e.g. depression) which are known risk factors for AD were not recorded at screening and therefore could not be assessed in regression analyses for identifying predictors of cognitive impairment.

4.5.2 Longitudinal analyses

The main limitations of the longitudinal analysis were the small sample sizes and short follow up periods. Power for the study was initially calculated based on a sample size of 240. While there were no real issues encountered in screening, there was a larger than expected withdrawal of participants before the baseline assessment - 30% as opposed to the 10% in each arm that was accounted for in sample size calculations. The sample sizes were further limited by the COVID-19 pandemic which forced a temporary halt to all study visits in March 2020. Consequently, the number of participants who had completed 6-month and 12-month assessments was less than initially expected with very few participants who had completed the 18-month assessment upon analysis of results. In order to analyse as many participants as possible the research presented in this thesis is based on shorter follow up periods, over which cognition is less likely to significantly change.

Longitudinal analysis did not account for adherence to medication or changes in medication throughout the follow up, though this applied to very few participants in these analyses. Participants who had changed to a different type of RA medication during the course of their follow up (n=5 in the 6-month cohort; n=5 in the 12-month cohort) followed the intention to treat principle and so remained in the same treatment group that they were assigned at baseline. Although the MoCA is a well validated cognitive assessment and is widely used as a screening tool, it may be subject to practice effects when repeatedly administered during longitudinal
The FCSRT adds reliability to the measurement of cognitive change, especially given that a different list of words was used at each visit, however the potential for practice effects must still be considered. Despite the comprehensive adjustment for confounders in the longitudinal analysis, residual confounding may still persist e.g. this study design did not record data in relation to sleep quality or fatigue which have been found to affect physical and cognitive health.\textsuperscript{642, 643} Ideally an analysis of treatment duration and effect on cognitive performance would have been conducted however due to time constraints and limited availability of treatment start dates, this was not possible to include in this thesis. Furthermore, due to COVID and time restraints it was not possible to analyse blood samples and evaluate the effect of peripheral inflammatory biomarkers and genetic risk factors on cognitive outcomes.

4.6 Implications and future research

The exact mechanisms behind neurodegeneration in RA are unknown however one explanation for the seemingly high prevalence of MCI this population is the potential association between chronic systemic inflammation and AD development and progression.\textsuperscript{644, 645} As discussed in Chapter 1: Literature review, neuroinflammation in AD is believed to be a result of Aβ plaque deposition and subsequent microglial activation accompanied by the upregulation of inflammatory markers.\textsuperscript{603} Chronic systemic inflammation is a characteristic of RA but is also believed to contribute to neuroinflammation in AD despite the BBB.\textsuperscript{646-648} Furthermore, there have been several inflammatory biomarkers which have been identified in both diseases,\textsuperscript{649, 650} including the TNFα cytokine which contributes to joint destruction in RA and has also been found to be associated with more rapid cognitive decline in patients with AD.\textsuperscript{382, 394, 395, 651} Early observational studies suggested that anti-inflammatory treatments such as glucocorticoids or NSAIDs may lower the risk of AD.\textsuperscript{532, 652, 653} While results of several epidemiological studies were initially promising, there has been little success in subsequent clinical trials.\textsuperscript{362, 363, 654-657}

Since TNFi have been successfully repurposed for other chronic inflammatory conditions it was hypothesised that patients with AD may also benefit from these medications, especially given the raised level of serum TNFα previously observed in these patients.\textsuperscript{382} Perispinal administration of TNFi has shown promising results however is not ideal due to the invasiveness of this route of administration.\textsuperscript{604, 658, 659} As TNFi cannot cross the BBB, it was thought that subcutaneous administration could suppress the level of peripheral TNFα in the early stages of AD could help to prevent neurodegeneration. Though results from epidemiological studies look promising,\textsuperscript{391, 539} there have been few longitudinal clinical trials that have investigated this approach in patients with AD or MCI.
The RESIST study presents one of the first longitudinal assessments of TNFi on cognition in patients with RA and MCI. The preliminary analysis of RESIST screening data is the largest cross-sectional study to date to investigate cognition in RA, demonstrating a seemingly high prevalence of MCI in a population of adults with RA in Northern Ireland and Southampton while also identifying age, education, disease activity and rheumatoid factor status as possible predictors of cognitive impairment. This research contributes to the existing evidence base surrounding the risk of AD amongst individuals with RA and suggests that it may be worthwhile implementing a routine cognitive screening tool, such as the MoCA, in rheumatology clinics for the early identification of cognitive impairment.

While the analysis of RESIST longitudinal data found little evidence that TNFi slow the rate of cognitively decline or reduce the risk of cognitive impairment compared to csDMARDs, this analysis is to be repeated at the close of the 18-month follow-up period planned for RESIST with further investigation into the duration of treatment, combination therapies, immune response analysis and possession of genetic risk factors and their subsequent effects on cognitive performance. Ideally, this type of research would monitor cognition over several years however this is dependent on funding and may be made difficult due to the age and health status of the participants involved. In the meantime, the RESIST study will help to inform whether longer studies would be worthwhile.

The inclusion of the blood analyses will strengthen the results considerably. As mentioned in **Chapter 2: Methods**, blood samples are to be analysed in one batch at the end of the study, blind to clinical data in order to minimise measurement error and bias. Venous blood samples will be analysed for the possession of the APOE-ε4 allele, a well-established risk factor for AD which has also been implicated as a modulator in systemic inflammation. Serum and plasma will be analysed for common pro and anti-inflammatory molecules (IFNγ; TNFα; IL-6; IL-10; IL-12p70; TGF-β; IL-4; IL-13) and acute phase reactants (high sensitivity CRP) to determine if the rate of cognitive decline is related to the peripheral inflammatory profile. Analysis of RNA samples will focus on the role of inflammatory markers and will be used to help to identify new treatment targets for both RA and AD.

Overall further research is needed to determine if there is any statistically significant cognitive benefit of TNFi treatment in subjects at risk of cognitive impairment.
4.7 Conclusion

As dementia is now one of the biggest global public health concerns, finding a preventative intervention is of vital importance especially as the ageing population continues to grow. In recent years there has been increasing interest in the potential role of systemic inflammation in the pathogenesis of Alzheimer’s disease (AD). This is of particular relevance in RA as these patients have higher levels of systemic inflammation than the general population, therefore if AD is driven by inflammatory processes, then these patients may be at an increased risk of cognitive impairment and dementia. Additionally, cognitive impairment in RA can have significant impact on ability to plan and successfully complete daily activities and is also important for adhering to treatment regimens. The analysis of RESIST screening data suggests that routine cognitive screening in rheumatology clinics may be a useful tool in RA disease management. Additionally, higher levels of RA disease activity were associated with poorer cognitive performance, suggesting that the association between reduced cognitive function is, at least in part, driven by the effects of systemic inflammation rather than through patient related outcomes such as pain, depression, or anxiety. This raises the intriguing possibility that controlling the systemic inflammatory response in RA using DMARDs might have benefits to cognitive function. This could also be transferable to other situations in which inflammation appears to drive cognitive decline, such as AD.

The longitudinal analysis in this thesis aimed to compare the effects of TNFi and csDMARD treatment on cognitive change in older adults with RA and MCI, independent of changes in mood, disease activity, function, and other demographic confounders. This preliminary research, despite some promising trends, found little evidence of a relationship between TNFi and better cognitive performance. This relationship will be better described at the conclusion of the RESIST study. Nevertheless, this study is a valuable contribution in a topic that is largely under researched and will help to inform any future research on the repurposing of TNFi for the prevention of AD.
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Appendix 2.1 – RESIST: Initial screening participant information sheet (BHSCT version)

Short Study Title: RESIST
RhEumatoid arthritis, medication and memory Study

MREC Reference: 17/WM/0161 Trust Study Number: 17004BMcG-SP
Local Investigator: Dr Bernadette McGuinness
Local Co Investigator: Dr Chris Cardwell

PATIENT INFORMATION SHEET AND INFORMED CONSENT

You are being invited to take part in a research study. Before you decide to participate or not, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Thank you for reading this.

Study of TNF inhibitors in mild cognitive impairment.

The Belfast Health and Social Care Trust and Queen’s University Belfast are conducting a programme of research assessing participants with rheumatoid arthritis. To do this, we wish to make comparisons between people with mild memory problems on TNF inhibitors and those on traditional drugs (DMARDs) such as methotrexate.

What is the aim of this study?

This initial screening test will determine if you are eligible to take part in the full study. We wish to screen all patients with rheumatoid arthritis (RA) over the age of 55 years. We aim to determine if you have a mild problem with your memory.

Why am I being asked to take part in this study?

You are being asked to take part because you have been identified as someone who has RA and is aged 55 years or older.
Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you can withdraw at any time and without giving a reason.

Refusal to take part in this study will not affect your care or treatment in any way.

What does my participation involve?

Firstly, we will ask if you would be willing to take part. If you agree we will speak to you about the study, give you study related documentation and ask you to sign a consent form.

While you wait in the outpatient clinic/day-centre a rheumatology specialist nurse will carry out a short test of cognition, the Montreal Cognitive Assessment (MoCA).

How long will my participation last?

If you wish to take part in this initial part of the study your involvement will last approximately 10 minutes. There are no restrictions or requirements as part of this study.

Is there any treatment in this study?

This study does not involve any treatment or tests that are harmful.

What are the possible benefits of taking part?

This study will provide you with some baseline knowledge regarding your memory and other aspects of cognition. If you are suitable you will be asked to take part in a longer study which will monitor your cognition over time.

What do I do if I feel I have been harmed?

It is very unlikely you will have any problems during this project but if you have any concerns please contact the supervisors whose contact details are listed above.
Will my taking part in this study be kept confidential?

All personal information collected about you during the course of the research will be kept strictly confidential. We will assign you a unique study identity number. The unique study ID will be used in the database that will be created with the information obtained from the study. This database will be kept on encrypted computers along with copies of your signed consent form and will be securely stored at Queen’s University Belfast. With your consent, we will inform your GP if you require a medical follow-up of any of the tests undertaken as part of this study.

What will happen to the results of the research study?

In any publications/reports that arise from this study, the role that volunteers played will be acknowledged. No individual will be identified in any of these releases.

Who is organising and funding the research?

Funding has been provided by the Alzheimer’s Society.

Who has approved this study?

This study has been reviewed by the office of research ethics West Midlands- Black County Research Ethics Committee.

The researchers would like to thank you for taking part in this study.

All volunteers will be provided with their own copy of this Patient Information Sheet along with a copy of the consent form which you sign.

Complaints: If you have any concerns about this study, you can raise these directly with the chief investigator Dr B McGuinness. If you are not satisfied, then you should contact staff at the Belfast Health and Social Care Trust Research Office (Tel: 028 90636366). You may also make a formal complaint through the Belfast Heath and Social Care Trust’s Complaints Department. You can contact them at Complaints Department, Musgrave Park Hospital, McKinney House, 7th Floor, Stockman’s Lane, Belfast BT9 7J. Tel: (028) 9504 8000. Email: complaints@belfasttrust.hscni.net.
Harm: If you feel you have been harmed during the research study, the normal National Health Service complaints mechanisms are available to you. Also, if you are harmed as a consequence of someone’s negligence, then you may have grounds for legal action for compensation against Queen’s University Belfast or the Belfast Health and Social Care Trust but you may have to pay your legal costs.

Dr Bernadette McGuinness, Dr Chris Cardwell
Centre for Public Health, Queen’s University Belfast, Grosvenor Road, Belfast, BT12 6BA
Prof Clive Holmes, Prof Chris Edwards
MARC, Moorgreen Hospital, Southampton, SO30 3JB

The researchers would like to thank you for taking part in this study.
Appendix 2.2 – RESIST screening participant consent form

RESIST
Rheumatoid arthritis, medication and memory Study: Screening Questionnaire
Participant Identification Number:

Name of Chief Investigator: Dr Bernadette McGuinness

1. I confirm that I have read and understand the information sheet dated 6 August 2018, Version 3.

2. I understand that my participation in this screening test is voluntary.

3. I agree to the memory and thinking skills test and the use of results for the research study if applicable.

4. I agree to take part in the above study.

5. I agree to being asked regarding follow-up over the full study period.

6. I give my permission for my GP to be informed of any abnormal cognitive function results.

________________________   _______________   _______________
Name of Patient                  Date                     Signature

________________________   _______________   _______________
Name of Person taking consent (If different from researcher)     Date                     Signature

________________________   _______________   _______________
Researcher                                Date                     Signature

When completed, 1 for patient, 1 for researcher site file, 1(original) to be kept in medical notes
**Appendix 2.3 – RESIST: Screening data collection form**

### RESIST Study: DATA COLLECTION FORM – Screening

<table>
<thead>
<tr>
<th>Screening Site Location</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant screening ID</td>
<td></td>
</tr>
</tbody>
</table>

#### Consents

<table>
<thead>
<tr>
<th>Consent Item</th>
<th>Completed Y/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant information sheet provided</td>
<td></td>
</tr>
<tr>
<td>Participant consent form</td>
<td></td>
</tr>
<tr>
<td>Name of researcher obtaining consent</td>
<td></td>
</tr>
<tr>
<td>Participant meets inclusion criteria</td>
<td></td>
</tr>
<tr>
<td>Reasons for exclusion</td>
<td></td>
</tr>
</tbody>
</table>

#### Participant Information

<table>
<thead>
<tr>
<th>Information Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Date of diagnosis of RA</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP antibody titre level</td>
<td>+ / -</td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>Date:</td>
</tr>
<tr>
<td>DAS28 score (at beginning of treatment)</td>
<td>Date:</td>
</tr>
<tr>
<td>RF</td>
<td>+ / -</td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Subjective memory loss?</td>
<td></td>
</tr>
</tbody>
</table>

#### Joint ACR / EULAR Criteria

<table>
<thead>
<tr>
<th>Involvement Description</th>
<th>Tick</th>
<th>Criteria Description</th>
<th>Tick</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 Large Joint</td>
<td></td>
<td>Acute Phase Reactants</td>
<td></td>
</tr>
<tr>
<td>1 - 2-10 Large Joints</td>
<td></td>
<td>0 - Normal ESR and CRP</td>
<td></td>
</tr>
<tr>
<td>2 - 1-3 Small Joints (with or without large joint involvement)</td>
<td></td>
<td>1 - Abnormal ESR or CRP</td>
<td></td>
</tr>
<tr>
<td>3 - 4-10 Small Joints (with or without large joint involvement)</td>
<td></td>
<td>Symptom Duration</td>
<td></td>
</tr>
<tr>
<td>5 - &gt; 10 joints (at least one small joint)</td>
<td></td>
<td>0 - &lt;6 weeks</td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td></td>
<td>1 - &gt;6 weeks</td>
<td></td>
</tr>
<tr>
<td>0 - Negative RF / ACPA</td>
<td></td>
<td>Diagnose as RA if ≥ 25</td>
<td></td>
</tr>
<tr>
<td>2 - Low Positive RF / ACPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - High Positive RF / ACPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>Dose</td>
<td>Amount</td>
<td>Frequency</td>
</tr>
<tr>
<td>------------</td>
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</tr>
</tbody>
</table>

MoCA (Version ___)
MoCA score at screening

<table>
<thead>
<tr>
<th>Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Recruited</td>
</tr>
</tbody>
</table>

Recorded by:  Signature:  Date: ___/___/____

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Appendix 2.4 – Montreal Cognitive Assessment (MoCA) version 7.1

Montreal Cognitive Assessment (MoCA)
Version 7.1 Original Version

VISUOSPATIAL / EXECUTIVE
- Copy cube
- Draw CLOCK (Ten past eleven) (3 points)

NAMING
- Contour
- Numbers
- Hands

MEMORY
- Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.
  - 1st trial
  - 2nd trial

ATTENTION
- Read list of digits (1 digit/sec.). Subject has to repeat them in the forward order
  - 7 4 2
- Subject has to repeat them in the backward order
  - 5 8 1

Serial 7 subtraction starting at 100
- 9 3
- 8 6
- 7 9
- 6 5

LANGUAGE
- Repeat: I only know that John is the one to help today.
- The cat always hide under the couch when dogs were in the room.

ABSTRACTION
- Similarity between e.g. banana - orange - fruit
- train - bicycle
- watch - ruler

DELAYED RECALL
- His to recall words WITH NO DUE

Optional
- Multiple choice cue

ORIENTATION
- Date
- Month
- Year
- Day
- Place
- City

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30

Administered by: ______________________

TOTAL /30

Add 1 point if ≤ 12 yr. edu
Appendix 2.5 – RESIST: GP memory referral letter

PRIVATE AND CONFIDENTIAL

DATE

TITLE NAME
ADDRESS 1
ADDRESS 2
ADDRESS 3
POSTCODE

Dear Dr

RE: [INSERT PATIENT NAME] [DATE OF BIRTH] [ADDRESS]

This gentleman/lady has been an active participant in the TiIF Inhibitors in the Prevention of Alzheimer's Disease (RESIST) study at Queen's University Belfast.

Part of the recruitment process involves an assessment of cognitive function using the Montreal Cognitive Assessment (MoCA), performed by a specialist Dementia Research Nurse.

I am writing to inform you that this gentleman's/lady's MoCA score ([insert score]) was found to be below the normal reference value and indicative of impaired cognitive function. He/she would therefore benefit from a medical review of his/her cognitive status and onward referral to a memory clinic if you deem appropriate.

Yours Sincerely,

[Signature]

Dr. Bernadette McGuinness
MD PhD FRCP
Appendix 2.6 – RESIST: Second screening participant information sheet

Short Study Title: RESIST
RhEumatoid arthritis, medication and memory Study

MREC Reference: 17/WM/0161 Trust Study Number: 17004BMcG-SP
Local Investigator: Dr Bernadette McGuinness
Co Investigators: Dr Chris Cardwell, Prof. Clive Holmes, Prof. Chris Edwards

PATIENT INFORMATION SHEET AND INFORMED CONSENT

You are being invited to take part in a research study. Before you decide to participate or not, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Thank you for reading this.

Study of TNF inhibitors in the prevention of Alzheimer’s disease.

The Belfast and Northern Trusts, Queen’s University Belfast, University Hospital Southampton, Southern Health Foundation Trust and the University of Southampton are conducting a programme of research assessing participants with rheumatoid arthritis (RA). To do this, we wish to make comparisons between people with mild memory problems on TNF inhibitors and those on traditional drugs (DMARDs) such as methotrexate.

What is the aim of this study?

This main study will determine if patients with RA and mild memory problems are protected against memory decline and Alzheimer’s disease if they are on different types of RA treatments. We wish to follow patients with RA over the age of 55 years who are on TNF inhibitors and DMARDs.
This research will combine patient information collected separately by clinics in Northern Ireland and Southampton. This may allow opportunities for future randomised controlled trials of RA drugs in patients with mild cognitive impairment.

Why am I being asked to take part in this study?

You are being asked to take part because you have been identified as someone who has RA, mild memory problems, is aged 55 years or older and is on a TNF inhibitor or a DMARD.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you can withdraw at any time and without giving a reason.

Refusal to take part in this study will not affect your care or treatment in any way.

What does my participation involve?

Firstly, we will ask if you would be willing to take part. If you agree we will speak to you about the study and give you study related documentation.

A research nurse/PhD student will visit your home at a time convenient to you and ask you and your carer to sign a consent form. We will administer tests of thinking and memory, mood, and functional ability and will ask you about your pain levels and disease activity. You will also be asked to provide a blood sample. With your permission, we would like to store some of the samples you have given us at the end of the study for future use. These samples will be stored in Queen’s University Belfast and the University of Southampton. Storage of these samples will enable us to undertake more research if new knowledge and technology becomes available. We do not know for sure what these future studies will be but they may involve genetic analysis, sharing samples with collaborators abroad or research in collaboration with partners such as commercial companies.
Any samples shared with collaborators will be anonymised and you will not be identifiable. You can indicate on the consent form if you agree to let us retain samples for future use in other ethically approved studies.

How long will my participation last?

If you wish to take part in this second part of the study your involvement will last approximately 60 minutes. We will repeat the study visit procedure each six-months for eighteen months so there will be 4 study visits in total. These will all take part in your home at a time convenient to you and your partner.

You may choose not to attend this research appointment by advising the researcher in advance. Withdrawal will not have any adverse impact on their clinical treatment. There are no restrictions or requirements as part of this study.

Is there any treatment in this study?

This study does not involve any treatment or tests that are harmful. You will continue on your prescribed RA treatment as planned. There is a small risk of bruising and fainting associated with providing a blood sample. A fully trained individual will take the blood samples to ensure that any discomfort is kept to a minimum.

What are the possible benefits of taking part?

This study will provide you with some baseline knowledge regarding your memory and other aspects of thinking (cognition). As we are monitoring your cognition over time we will be able to alert you to any significant changes quickly and we will prompt a referral to your GP. You will also potentially be helping future patients with mild cognitive problems if either treatment is found to be beneficial.

What do I do if I feel I have been harmed?

It is very unlikely you will have any problems during this project but if you have any concerns please contact the supervisors whose contact details are listed below.
Will my taking part in this study be kept confidential?

All personal information collected about you during the course of the research will be kept strictly confidential. We will assign you a unique study identity number. The unique study ID will be used in the database that will be created with the information obtained from the study. This database will be kept on encrypted computers along with copies of your signed consent form and will be securely stored at Queen’s University Belfast and the University of Southampton. With your consent, we will inform your GP if you require a medical follow-up of any of the tests undertaken as part of this study.

What will happen to the results of the research study?

In any publications/reports that arise from this study, the role that volunteers played will be acknowledged. No individual will be identified in any of these releases.

Who is organising and funding the research?

Funding has been provided by the Alzheimer’s Society.

Who has approved this study?

This study has been reviewed by the office of research ethics West Midlands- Black County Research Ethics Committee.

The researchers would like to thank you for taking part in this study.

All volunteers will be provided with their own copy of this Patient Information Sheet along with a copy of the consent form which you sign.

Complaints: If you have any concerns about this study, you can raise these directly with any of the researchers. If you are not satisfied, then you should contact staff at the Belfast Health and Social Care Trust Research Office (Tel: 028 90636366). You may also make a formal complaint through the Belfast Heath and Social Care Trust’s Complaints Department. You can contact them at Complaints Department, Musgrave Park Hospital, McKinney House, 7th Floor, Stockman’s Lane, Belfast BT9 7JB. Tel: (028) 9504 8000, Email: complaints@belfasttrust.hscni.net.
**Harm:** If you feel you have been harmed during the research study, the normal National Health Service complaints mechanisms are available to you. Also, if you are harmed as a consequence of someone’s negligence, then you may have grounds for legal action for compensation against Queen’s University Belfast or the Belfast Health and Social Care Trust but you may have to pay your legal costs.

Dr Bernadette McGuinness, Dr Chris Cardwell, Centre for Public Health, Queen’s University Belfast, Grosvenor Road, Belfast, BT12 6BA

b.mcguinness@qub.ac.uk

Prof Clive Holmes, Clinical and Experimental Sciences, University of Southampton, Southampton, SO17 1BJ

c.holmes@soton.ac.uk

Prof Chris Edwards, University of Southampton, Southampton, SO17 1BJ

c.edwards@soton.ac.uk

The researchers would like to thank you for taking part in this study.
Appendix 2.7 – RESIST: Study consent form

Patient Consent Form

<table>
<thead>
<tr>
<th>Short study title:</th>
<th>RESIST RhEumatoid arthritis, medication and memory Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study doctor:</td>
<td>Dr Bernadette McGuinness</td>
</tr>
<tr>
<td>Protocol number:</td>
<td>17/WM/0161</td>
</tr>
<tr>
<td>Patient identification number:</td>
<td></td>
</tr>
</tbody>
</table>

Study Patient Consent Form

1. I confirm I have read and understand the information sheet dated 6 August 2018. Version 3 for the above study. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. I will get a signed copy of this form for my records.

2. The study researcher has answered my questions in a way that makes sense to me. I have had time to consider taking part in this study.

3. I understand that relevant sections of my medical records and data collected during the study may be looked at by individuals from the sponsor for this study, from UK regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records, providing strict confidentiality is maintained.

4. I voluntarily agree to allow study staff to collect, use and share my health data. I understand that I am not giving up any of my legal rights by signing this form. I understand the study is being conducted by researchers from Queen's University Belfast and University of Southampton that my personal information will be held securely on University (or Trust) premises and handled in accordance with the provisions of the Data Protection Act 1998.

5. I understand that the storage of blood samples for future research is entirely optional.

6. I agree that the blood samples I have given and the information gathered about me can be stored by Queen's University Belfast and the University of Southampton for possible use in future research projects.

7. I agree for genetic testing to be performed on my blood samples.

8. I agree to tests which assess my memory and thinking ability, other related tests and blood samples and the use of results for the research study.

9. I agree for my study partner to provide information about my memory, functional ability and medical history.
<table>
<thead>
<tr>
<th></th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>I understand that I am free to stop any assessment, test or questionnaire at any time. I understand that I do not have to answer study questions or provide a reason to study staff for refusing to answer a question.</td>
</tr>
<tr>
<td>11</td>
<td>I understand that if my condition deteriorates and I am no longer able to fully consent to my involvement in this study that mine and my study partner’s participation will be stopped immediately with no further study procedures carried out.</td>
</tr>
<tr>
<td>12</td>
<td>I agree to take part in the above study.</td>
</tr>
<tr>
<td>13</td>
<td>I give my permission for my GP to be informed of any abnormal cognitive function results.</td>
</tr>
</tbody>
</table>

**PATIENT**

Print name: ____________________________________________

Signature: ____________________________________________

Date: ________________________________________________

**INVESTIGATOR**

- I have carefully explained to both the patient and the study partner the nature and purpose of the above study.
- There has been an opportunity for both the patient and the study partner to ask questions about this research study.
- I have answered all questions that the patient and study partner have about this study.

Print name: ____________________________________________

Signature: ____________________________________________

Date: ________________________________________________
## RESIST Study:
### DATA COLLECTION FORM – Visit Number: 1

<table>
<thead>
<tr>
<th>Participant study ID</th>
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<tbody>
<tr>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>Consents</th>
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<tbody>
<tr>
<td>Participant information sheet provided</td>
<td>Y / N</td>
</tr>
<tr>
<td>Participant consent form</td>
<td>Y / N</td>
</tr>
<tr>
<td>Name of researcher obtaining consent</td>
<td></td>
</tr>
<tr>
<td>Participant meets inclusion criteria</td>
<td>Y / N</td>
</tr>
<tr>
<td>Reasons for exclusion</td>
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</table>

<table>
<thead>
<tr>
<th>Medical History</th>
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<tbody>
<tr>
<td>Smoking Status</td>
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<tr>
<td>Diabetic</td>
</tr>
<tr>
<td>Family history of Cardiovascular disease</td>
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<tr>
<td>Other relevant history</td>
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<table>
<thead>
<tr>
<th>Changes to Medication</th>
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<tbody>
<tr>
<td>Medication</td>
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<tr>
<td>------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Questionnaires</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>FCSRT (Form A, B or C)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MoCA (Version ___ )</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
</tr>
<tr>
<td>Health Assessment Questionnaire</td>
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<tr>
<td>DAS28</td>
</tr>
<tr>
<td>Visual Analogue Scale Pain Score</td>
</tr>
</tbody>
</table>

**Blood Sample**

- Date of blood sampling
- Time of blood sampling (hour 0-23, minute 0-59)

**Serious Adverse Event**

- Recorded by: [ ]
- Signature: [ ]
- Date: __/___/____

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Appendix 2.9 – Montreal Cognitive Assessment (MoCA) version 7.3
Appendix 2.10 – Free and Cued Selective Reminding Test (FCSRT)

Form A – Words (1)

OWL  RACQUET

DESK  GRAPES
CHIMNEY  CANOE

SHARK    PAPER CLIP
Form A – Words (3)

CACTUS

RULER

TELESCOPE

RATTLE
RAZOR

STEERING WHEEL

PITCHER

GUITAR
CLOUDS

ANCHOR

WATCH

ROLLING PIN
Form B – Words (3)

NINE   BALLOONS

VEST   BENCH
Form B – Words (4)

WREATH

AX

BASKET

PIPE
Form C – Words (1)

SKATE

ONION

BROOM

BEAR
CAKE   VOLCANO

DOMINOES   TRAIN
<table>
<thead>
<tr>
<th>CROWN</th>
<th>TULIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIANGLE</td>
<td>CABIN</td>
</tr>
</tbody>
</table>
Form A – Score sheet

Site ID _____  Patient ID _____  Assessor Initials _____  Date _____ / _____ / _____  Form: A  [LST]

<table>
<thead>
<tr>
<th>CATEGORY CUE</th>
<th>ITEM</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
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</thead>
<tbody>
<tr>
<td>1 Fruit</td>
<td>Grapes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Sports Equipment</td>
<td>Racquet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Bird</td>
<td>Owl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Furniture</td>
<td>Desk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Part of a Building</td>
<td>Chimney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Type of Boat</td>
<td>Canoe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 A Fastener</td>
<td>Paper Clip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 A Fish</td>
<td>Shark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Used for Measuring</td>
<td>Ruler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 For Seeing</td>
<td>Telescope (spyglass)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Kind of Plant</td>
<td>Cactus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Used by Babies</td>
<td>Rattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Part of a Car</td>
<td>Steering Wheel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 For Holding Liquids</td>
<td>Pitcher (jug)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 A Toiletry</td>
<td>Razor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Musical Instrument</td>
<td>Guitar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTALS**

\[
\text{[IDEN]} \ [\text{NME}] \ [\text{ICR}] \ [\text{FR1}] \ [\text{CR1}] \ [\text{FR2}] \ [\text{CR2}] \ [\text{FR3}] \ [\text{CR3}]
\]

\[
\text{[SUMFREE3]} = \_\_\_\_\_\_\_\_\_ \ [\text{SUMTOT3}] = \_\_\_\_\_\_\_\_\_
\]
Form B – Score sheet

<table>
<thead>
<tr>
<th>CATEGORY CUE</th>
<th>ITEM</th>
<th>I</th>
<th>N</th>
<th>ICR</th>
<th>FR1</th>
<th>CR1</th>
<th>FR2</th>
<th>CR2</th>
<th>FR3</th>
<th>CR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Insect</td>
<td>Spider</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Part of the Body</td>
<td>Foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Snack Food</td>
<td>Pretzel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Found in Hospitals</td>
<td>Wheelchair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Weather Phenomenon</td>
<td>Clouds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6 Part of a Ship</td>
<td>Anchor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7 Jewelry</td>
<td>Watch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 For Baking</td>
<td>Rolling Pin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Clothing</td>
<td>Vest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Toy</td>
<td>Balloons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 A Number</td>
<td>Nine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 For Sitting</td>
<td>Bench</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Tool</td>
<td>Ax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 For Smoking</td>
<td>Pipe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Xmas Decoration</td>
<td>Wreath</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 For Carrying Things</td>
<td>Basket</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTALS**

[SUMFREE3] = ___________ [SUMTOT3] = ___________
# Form C – Score sheet

<table>
<thead>
<tr>
<th>CATEGORY CUE</th>
<th>ITEM</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-Footed Animal</td>
<td>Bear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Worn on Feet</td>
<td>Skate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Vegetable</td>
<td>Onion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>For Cleaning Up</td>
<td>Broom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Earth Formation</td>
<td>Volcano</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Transportation</td>
<td>Train</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>A Game</td>
<td>Dominoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Dessert</td>
<td>Cake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Worn on the Head</td>
<td>Crown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>A Shape</td>
<td>Triangle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Kind of Building</td>
<td>Cabin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>A Flower</td>
<td>Tulip</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Type of Light</td>
<td>Candle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Weapon</td>
<td>Sword</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>For Sewing</td>
<td>Thread</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Kitchen Appliance</td>
<td>Toaster</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTALS**

[IDEN] [NME] [ICR] [FR1] [CR1] [FR2] [CR2] [FR3] [CR3]

**SUMFREE3** = ____________  **SUMTOT3** = ____________
Appendix 2.11 – Geriatric Depression Scale (GDS)

GERIATRIC DEPRESSION SCALE (GDS)

NAME: __________________________ DATE: __________________________

<table>
<thead>
<tr>
<th>Question</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Are you basically satisfied with your life?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Have you dropped many of your activities or interests?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3. Do you feel that your life is empty?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4. Do you often feel bored?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5. Are you in good spirits most of the time?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6. Are you afraid that something bad is going to happen to you?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7. Do you feel happy most of the time?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Do you often feel helpless?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>9. Do you prefer to stay at home, rather than going out and doing new things?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>10. Do you feel you have more problems with your memory than most?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>11. Do you think it is wonderful to be alive?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>12. Do you feel pretty worthless the way you are now</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>13. Do you feel full of energy?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>14. Do you feel that your situation is hopeless?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>15. Do you think that most people are better off than you are?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

> 5 problems (answers in BOLD) indicates probable depression

TOTAL: __________________________

THE GERIATRIC DEPRESSION SCALE (GDS)

1. The GDS short form (15 questions) has been derived from the 30 question version. It has been designed for the assessment of depressive symptomatology in elderly people and excludes any questions relating to the physical symptoms of depression common in old age.

2. The GDS is a screening device and should not be used as a diagnostic tool. It can be used to monitor the client’s emotional state in relation to treatment or change in physical health. The questionnaire can guide further clinical interviews and when used this way has been found very acceptable to clients.

3. The questions are read out and the patient is asked how they have felt over the past week using a Yes/No response format. No further explanation or interpretation should be given to the questions.

4. Each answer indicating depression (bold ‘yes’ or ‘no’) counts one point. Scores greater than 5 are indicative of probable depression.
### HEALTH ASSESSMENT QUESTIONNAIRE (HAQ)

**Date:** [empty]  
**Patient Name:** [empty]

Please tick the one response which best describes your usual abilities over the past week

<table>
<thead>
<tr>
<th>1. DRESSING and GROOMING</th>
<th>Without ANY difficulty</th>
<th>With SOME difficulty</th>
<th>With MUCH difficulty</th>
<th>UNABLE to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Dress yourself, including tying shoelaces and doing buttons?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Shampoo your hair?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. RISING</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Stand up from an armless straight chair?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Get in and out of bed?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. EATING</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Cut your meat?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Lift a full cup or glass to your mouth?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>c. Open a new carton of milk (or soap powder)?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. WALKING</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Walk outdoors on flat ground?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Climb up five steps?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

**PLEASE TICK ANY AIDS OR DEVICES THAT YOU USUALLY USE FOR ANY OF THESE ACTIVITIES:**

<table>
<thead>
<tr>
<th>Cane (W)</th>
<th>Walking frame (W)</th>
<th>Built-up or special utensils (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crutches (W)</th>
<th>Wheelchair (W)</th>
<th>Special or built-up chair (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

Devices used for dressing (button hooks, zipper pull, shoe horn) [ ]

**PLEASE TICK ANY CATEGORIES FOR WHICH YOU USUALLY NEED HELP FROM ANOTHER PERSON:**

<table>
<thead>
<tr>
<th>Dressing and Grooming</th>
<th>Eating</th>
<th>Rising</th>
<th>Walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>
Please tick the one response which best describes your usual abilities over the past week

### 5. HYGIENE
Are you able to:

a. Wash and dry your entire body? | Without ANY difficulty | With SOME difficulty | With MUCH difficulty | UNABLE to do
b. Take a bath? | | | |

c. Get on and off the toilet? | | |

### 6. REACH
Are you able to:

a. Reach and get down a 5 lb object (e.g. a bag of potatoes) from just above your head? | |

b. Bend down to pick up clothing off the floor? | |

### 7. GRIP
Are you able to:

a. Open car doors? | |

b. Open jars which have been previously opened? | |

c. Turn taps on and off? | |

### 8. ACTIVITIES
Are you able to:

a. Run errands and shop? | |

b. Get in and out of a car? | |

c. Do chores such as vacuuming, housework or light gardening? | |

**PLEASE TICK ANY AIDS OR DEVICES THAT YOU USUALLY USE FOR ANY OF THESE ACTIVITIES:**

- Raised toilet seat (H)
- Bath seat (H)
- Bath rail (H)
- Long handled appliances for reach (R)
- Jar opener (for jars previously opened) (G)

Other (specify)  

**PLEASE TICK ANY CATEGORIES FOR WHICH YOU USUALLY NEED HELP FROM ANOTHER PERSON:**

- Hygiene
- Gripping and opening things
- Errands and housework

ID  

For office use only
Appendix 2.13 – Disease Activity Score Calculator for Rheumatoid Arthritis (DAS28)

28-JOINT SWOLLEN AND TENDER JOINT COUNT

Which joints are *tender*? (please tick)

Which joints are *swollen*? (please tick)

Global VAS: Overall well-being: Please indicate on the scale below

<p>| 0 | 100 |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Best Imaginable</td>
<td>Worst Imaginable</td>
</tr>
<tr>
<td>Health State</td>
<td>Health State</td>
</tr>
</tbody>
</table>

ESR

CRP
Appendix 2.14 – RESIST: Data Collection Form (Visits 2/3/4)

**RESIST Study:**
DATA COLLECTION FORM – Visit Number:

| Participant study ID |

<table>
<thead>
<tr>
<th>Consents</th>
<th>Completed</th>
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<tbody>
<tr>
<td></td>
<td>V 2</td>
</tr>
<tr>
<td>Participant’s verbal consent obtained</td>
<td>Y/N</td>
</tr>
<tr>
<td>Name of researcher obtaining consent</td>
<td></td>
</tr>
<tr>
<td>Participant meets inclusion criteria</td>
<td>Y/N</td>
</tr>
<tr>
<td>Reasons for exclusion</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Changes to Medication</th>
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</thead>
<tbody>
<tr>
<td>Medication</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Questionnaires</th>
<th>Completed</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y/N</td>
<td></td>
</tr>
<tr>
<td>FCSRT (Form A, B or C)</td>
<td>SUMFREES</td>
<td>SUMTOT3</td>
</tr>
<tr>
<td>MoCA (Version ___)</td>
<td>Referral to Memory Service?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health Assessment Questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual Analogue Scale Pain Score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of blood sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of blood sampling (hour 0-23, minute 0-59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serious Adverse Event</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Recorded by:</th>
<th>Signature:</th>
<th>Date: <em><strong>/</strong></em>/____</th>
</tr>
</thead>
</table>
Appendix 2.15 – RESIST Blood Scheme

**RESIST STUDY BLOOD SCHEME**

**a)**
2 x 0 ml clotted (red top)  
Store at room temperature  
In dark for at least 10 mins  
COLLECT AT VISITS  
0, 6, 12 & 18 months

Spin 1 x 6ml red top 3000 rpm  
For 15 min  
Serum into 1.5ml aliquots

**b)**
1 x 4 ml EDTA (purple top)  
COLLECT AT VISIT 0 ONLY

Invert 8 times  
1 purple top Stored for genotyping (DNA)

**c)**
1 x Paxgene blood DNA tube (2.5mls whole blood)  
COLLECT AT VISITS  
0, 6, 12 & 18 months

Leave tube in rack for at least 2 hours at room temperature (if necessary these tubes can be left in the rack on the bench overnight).  
Freeze upright at -20°C for at least 24 hours and then transfer to -80°C.
d) 1 x 6 ml EDTA (purple top)
Store at 4°C and centrifuge within 2 hours
COLLECT AT VISITS
0, 6, 12 & 18 months

Spin 3000 rpm
For 15 min

Purple cap

Plasma into 1.5ml aliquots
## COVID-19 Telephone Checklist Prior to Home Visit

**Participant ID:**

<table>
<thead>
<tr>
<th>Question</th>
<th>✓</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Would you be willing to complete a home visit appointment?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Are you currently displaying any COVID-19 symptoms?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• A new, continuous cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Loss of smell or taste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Have you previously had COVID-19?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• If yes, ask question 5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Have you been in contact with anyone that has had COVID-19?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• If yes, ask question 5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. If applicable, have you completed a two-week isolation period?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Are there additional people in your household?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• How many?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• What age?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Are any of the additional people in your household at a higher risk of contracting COVID-19?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Would you (and your carer if applicable) be willing to wear a PPE face mask provided by a nurse on arrival?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Signature:**

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**Date:**

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COVID-19 TELEPHONE CHECKLIST  
VERSION 1, 30 JUNE 2020
Appendix 2.17 – RESIST: Participant information sheet for COVID-19

Study Title: RESIST
RhEumatoid arthritiS, medication and memory Study

MREC Reference: 17/WM/0161. IRAS Project ID: 223104
Local Investigator: Dr Bernadette McGuinness
Co Investigators: Dr Chris Cardwell, Prof. Clive Holmes, Prof. Chris Edwards, Dr Gary Meenagh

What is the aim of this study?
You are currently taking part in a study (RhEumatoid arthritiS, medication and memory Study, or RESIST study) which aims to determine if patients with RA and mild memory problems are protected against memory decline and Alzheimer’s disease if they are on different types of RA treatments. In that study we are following patients with RA over the age of 55 years who are on TNF inhibitors and DMARDs.

Due to the recent worldwide infection with the COVID-19 virus questions have been raised as to whether patients taking TNF inhibitors or DMARDs are at increased or decreased risk of developing a COVID infection or symptoms and also whether COVID infection has any effect on memory performance.

Why am I being asked to take part in this study?
You are being asked to take part because you are taking part in the RESIST study which is monitoring patients with RA over the age of 55 years who are on TNF inhibitors and DMARDs and who may or may not have been infected with COVID virus.

Do I have to take part?
It is up to you to decide whether or not to take part in this additional part of the study. If you do decide to take part, then you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you can withdraw at any time and without giving a reason.

Refusal to take part in this study will not affect your care or treatment in any way.
What does my participation involve?
Firstly, we will ask if you would be willing to take part. If you agree we will speak to you about the study and give you study related documentation. At your final planned study visit for the RESIST study we would ask you some additional questions, taking approximately 3 minutes of your time, about your experience of any COVID infection or any symptoms you may have had. With your permission, we would like to store this data on the study database on encrypted computers which will enable us to undertake more research in future if new knowledge and technology becomes available. In addition, at your final planned study visit, whilst we are taking a blood sample for the RESIST study we will take an additional blood sample (about a teaspoon of blood) to test for the COVID virus at the end of the study. There are no additional questions or procedures.

With your permission, we would like to store the sample you have given us at the end of the study for future use. The sample will be labelled with a unique study identification number to ensure it is non-identifiable. The blood samples will be stored at Queen’s University Belfast and/or the University of Southampton and processed at the University of Southampton. Storage of these samples will enable us to undertake more research if new knowledge and technology becomes available. We do not know for sure what these future studies will be but they may involve genetic analysis, sharing samples with collaborators abroad or research in collaboration with partners such as commercial companies.

Samples will be stored under the RVH HTA Research Licence 12059 pending appropriate ethical approval for any future research use. Although it is not considered a tissue bank, the University’s HTA licence allows for samples to be stored pending future use.

How long will my participation last?
If you wish to take part in this additional part of the study your additional involvement will last approximately 5 minutes. We will repeat the additional study visit procedure in visits already planned in the RESIST study with no additional visits. These will all take part in your home at a time convenient to you.

You may choose not to attend this research appointment by advising the researcher in advance. Withdrawal will not have any adverse impact on your clinical treatment. There are no restrictions or requirements as part of this study.
Is there any treatment in this study?
This study does not involve any treatment or tests that are harmful. You will continue on your prescribed RA treatment as planned. There is a small risk of bruising and fainting associated with providing a blood sample. A fully trained individual will take the blood samples to ensure that any discomfort is kept to a minimum.

What are the possible benefits of taking part?
At the end of the study, but not before, the clinical team will inform you by letter whether you had been exposed to COVID-19 virus with a contact number should you have any further questions.

What do I do if I feel I have been harmed?
It is very unlikely you will have any problems during this project but if you have any concerns please contact the supervisors whose contact details are listed below.

Will my taking part in this study be kept confidential?
All personal information collected about you during the course of the research will be kept strictly confidential. We will assign you a unique study identity number. The unique study ID will be used in the database that will be created with the information obtained from the study. This database will be kept on encrypted computers along with copies of your signed consent form and will be securely stored at Queen’s University Belfast and the University of Southampton. With your consent, we will inform your GP if you require a medical follow-up of any of the tests undertaken as part of this study.

What will happen to the results of the research study?
In any publications/reports that arise from this study, the role that volunteers played will be acknowledged. No individual will be identified in any of these releases.
How will we use information about you?
In this research study we will use information from you and your medical records for this research project. This information will include your:

- NHS number
- Date of Birth
- Name
- Contact Details

People will use this information to do the research or to check your records to make sure that the research is being done properly. People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. We will keep all information about you safe and secure. Once we have finished the study, we will keep some of the data so we can check the results and for future research. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information is used?

- You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.
- We need to manage your records in specific ways for the research to be reliable. This means that we won’t be able to let you see or change the data we hold about you.

Where can you find out more about how your information is used?
You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- our leaflet available from https://www.qub.ac.uk/privacynotice/Research/ListofResearchPrivacyNotices/PrivacyNoticeforResearchParticipants.html
- by asking one of the research team
- by sending an email to b.mcguinness@qub.ac.uk
Complaints: If you have a concern about any aspect of this study, you can speak with the Chief Investigator, Dr Bernadette McGuinness, whose contact details are listed below. If you remain unhappy and wish to make a formal complaint you may do so by contacting the Belfast Heath and Social Care Trust’s Complaints Department. You can contact them at Complaints Department, Musgrave Park Hospital, McKinney House, 6th Floor, Stockman’s Lane, Belfast BT9 7JB. Telephone: (028) 9504 8000, Email: complaints@belfasttrust.hscni.net.

Dr Bernadette McGuinness, Dr Chris Cardwell, Centre for Public Health, Queen’s University Belfast, Grosvenor Road, Belfast, BT12 6BA

b.mcguinness@qub.ac.uk

Prof Clive Holmes, Clinical and Experimental Sciences, University of Southampton, Southampton, SO17 1BJ
c.holmes@soton.ac.uk

Prof Chris Edwards, University of Southampton, Southampton, SO17 1BJ
c.edwards@soton.ac.uk

Dr Gary Meenagh, Antrim Area Hospital, 45 Bush Road, Antrim, BT41 2RL
Gary.Meenagh@northerntrust.hscni.net

The researchers would like to thank you for taking part in this study.
Appendix 2.18 – RESIST: Participant consent form for COVID-19

Patient Consent Form for COVID-19

1. I confirm I have read and understand the information sheet dated 15 June 2020, Version 2 for the above study.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. I will get a signed copy of this form for my records.

3. I voluntarily agree to allow study staff to collect, use and share my health data. I understand that I am not giving up any of my legal rights by signing this form. I understand the study is being conducted by researchers from Queen’s University Belfast and University of Southampton, that my personal information will be held securely on University (or Trust) premises and handled in accordance with the provisions of the Data Protection Act 1998.

4. I understand that the storage of blood samples for the COVID-19 test is entirely optional.

5. I agree that the blood samples I have given and the information gathered about me can be stored by Queen’s University Belfast and the University of Southampton for possible use in future research projects.

6. I agree to the COVID-19 questionnaire and the use of results for the research study.

7. I agree for my study partner to provide information about my medical history.

PATIENT

Print name: _____________________________

Signature: _____________________________

Date: _____________________________
INVESTIGATOR

- I have carefully explained to both the patient and the study partner the nature and purpose of the above study.

- There has been an opportunity for both the patient and the study partner to ask questions about this research study.

- I have answered all questions that the patient and study partner have about this study.

Print name: ____________________________________________

Signature: ____________________________________________

Date: ________________________________________________
Appendix 2.19 – RESIST: COVID-19 Symptom Severity Scale

<table>
<thead>
<tr>
<th>COVID-19 Symptom Severity Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant ID:</td>
</tr>
</tbody>
</table>

Participant to be asked as whether these symptoms have been present in the previous six months. Symptoms rated 0 to 4 points.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>✓</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms of disease</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Uncomplicated Mild disease. To include either mild fever, dry cough, sore throat, nasal congestion, persistent headache, muscle pain or loss of smell. No shortness of breath.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>As 1, but also persistent cough and shortness of breath.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>As 2, but severe shortness of breath.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Acute respiratory distress requiring ventilation or death.</td>
<td></td>
</tr>
</tbody>
</table>