

Depression, Negative Symptoms and Neuro-inflammation in Schizophrenia

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Abstract

Symptoms of depression such as low mood and apathy are common in schizophrenia. However, these overlap with negative symptoms such as apathy, poverty of speech and social avoidance and it remains very unclear the extent to which they share underlying pathogenic mechanisms and the degree to which distinct mechanisms converge to produce similar symptoms. Two pathogenic profiles have been proposed for negative symptoms; social amotivation and diminished emotional expressiveness. Recent evidence suggests a pro-inflammatory predisposition to both depression and negative symptoms of schizophrenia. The studies presented in this thesis aim to i) explore the relationship between depression and negative symptoms, ii) investigate whether they are driven by a common pro-inflammatory process, and iii) determine whether this may be a viable target for anti-inflammatory treatments. The analyses are based on data from a placebo-controlled clinical trial of minocycline (an anti-inflammatory antibiotic) to treat negative symptoms and reduce inflammatory markers.

Both in cross-section, and longitudinally over the course of the 12 months, depressive symptoms were associated with the avolition-apathy. Expressive deficits appeared specific to negative symptoms. The expressive deficit domain rather than depression-related avolition-apathy was associated with an immune abnormality throughout the 12-month clinical trial; namely increased TNF-α. Finally, the prescription of minocycline does not lead to a reduction in depressive and negative symptomology, nor inflammatory marker status.

Dedication

This thesis is dedicated to my family. In particular, I would like to dedicate this thesis to my mother, who sadly passed away shortly after submitting this thesis.

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List of Abbreviations

ARMS	At-risk mental state
BMI	Body Mass Index
CDSS	Calgary Depression Scale in Schizophrenia
CI	Chief Investigator
CMHT	Community Mental Health Team
CRF	Case Report Form
CSO	Clinical Studies Officer
DTI	Diffuse Tensor Imaging
DUP	Duration of untreated psychosis
EDTA	Ethylenediamine tetraacetic acid
EIP	Early Intervention in Psychosis
EPSE	Extrapyramidal Side Effects
GAF	Global assessment of functioning
GCP	Good Clinical Practice
HPA axis	Hypothalamic Pituitary Adrenal Axis
MDD	Major Depressive Disorder
MDT	Multi-Disciplinary Team
M.I.N.I.	MINI International Neuropsychiatric Interview
MRI	Magnetic Resonance Imaging
NMDA	N-methyl-D-aspartate
PANSS	Positive and Negative Syndrome Scale
PET	Positron Emission Tomography
PI	Principle investigator
PIL	Participant information leaflet
RA	Research Assistant/Associate
R&D	Research and Development
RMO	Responsible Medical Officer
SAS	Simpson Angus Scale

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1. Introduction

1.1 Schizophrenia: negative symptoms and depression overview

The clinical features of schizophrenia highlight its diverse psychopathology (Owen et al., 2016); these include positive, negative and cognitive symptom domains. Positive symptoms are phenomena that are outside of normal experience, typically hearing voices in the absence of a stimulus (hallucinations) or holding beliefs that are not true (delusions). Negative symptoms in contrast refer to losses or a lack of normal behaviours and motivation such as diminished expression of emotions, social isolation and lack of self-care (Barnes, 1993). In more detail, they include anhedonia, alogia, blunted affect, apathy and amotivation (Andreasen, 1982). Cognitive dysfunction (including deficits in memory, attention and executive functioning) (van Os and Kapur, 2009) is one of the prominent characteristics of the illness. Prior to onset of frank psychotic symptoms, some individuals may experience sub-threshold symptoms, that would confer risk for psychosis (Yung et al., 2003).

Depression is also a common clinical feature in established schizophrenia. Depression includes a group of mental health symptoms, with the defining characteristic being a loss of positive affect. Other symptoms include anhedonia (Beck, 1967), lack of libido, fatigue, tearfulness, reduced sleep and diminished appetite, social withdrawal, feelings of guilt, shame and worthlessness, reduced self-esteem, feelings of hopelessness and suicidal ideation (National Collaborating Centre for Mental Health, 2010). Depression

is a significant risk factor for suicide in schizophrenia, as evidenced in a review by (Upthegrove et al., 2016) and mood instability is associated with paranoid ideation and hallucinations (Marwaha et al., 2014). These findings indicate that depression represents a significant clinical challenge in those with schizophrenia and demonstrates the importance of understanding this aspect of a patient's presentation.

Positive symptoms typically respond to antipsychotic medication such as risperidone, quetiapine and clozapine. However, there are no medications that unequivocally treat negative symptoms, which is a key predictor of poor social and occupational outcome. The lack of pharmacological treatment strategies may be due to there being no clear pathogenic mechanisms and therefore no satisfactory molecular targets for developing new treatments. Some negative symptoms for example apathy and loss of motivation may share underlying molecular and cognitive mechanisms such as loss of sensitivity to rewards, with similar abnormalities in depression. There has been much interest in the evidence that schizophrenia and depression may involve abnormal activation of immune mechanisms. This thesis tested the idea that abnormal inflammatory responses may specifically underpin apathy and anhedonia both in depression and in schizophrenia whereas other aspects of the negative symptoms such as loss of emotional responsiveness do not.

1.2 **Epidemiology**

A review by Kirkbride et al. (2012) found that the annual incidence of schizophrenia in the UK is 15 per 100,000. Estimates of the prevalence of schizophrenia

are around 4.6 per 1000 (Bhugra, 2005) with a morbid risk of 7.2 per 1000 (McGrath et al., 2008). Schizophrenia typically occurs in individuals aged between fifteen and thirty (Hafner, 2014), and occurs in both men and women, with a slight predominance for men (McGrath et al., 2008, Kirkbride et al., 2006) and minority ethnic groups (Leff et al., 1992, Fearon et al., 2006, Bhugra et al., 1997).

Schizophrenia does not only impact on mental health. The Schizophrenia Commission (2012) concluded that people with a diagnosis of schizophrenia die between fifteen and twenty years before the average population, and this mortality gap has increased over recent years (Saha et al., 2007), possibly through poor diet, smoking cigarettes, consuming illicit substances, the metabolic side effects of antipsychotic medication, or diminished activity (Connolly and Kelly, 2005). Beyond the clinical manifestations of the disease, schizophrenia also presents an enormous economic burden, which has been highlighted by the findings of The Schizophrenia Commission (2012), who estimate a total societal cost of schizophrenia and psychosis is around £12 billion per year in the UK.

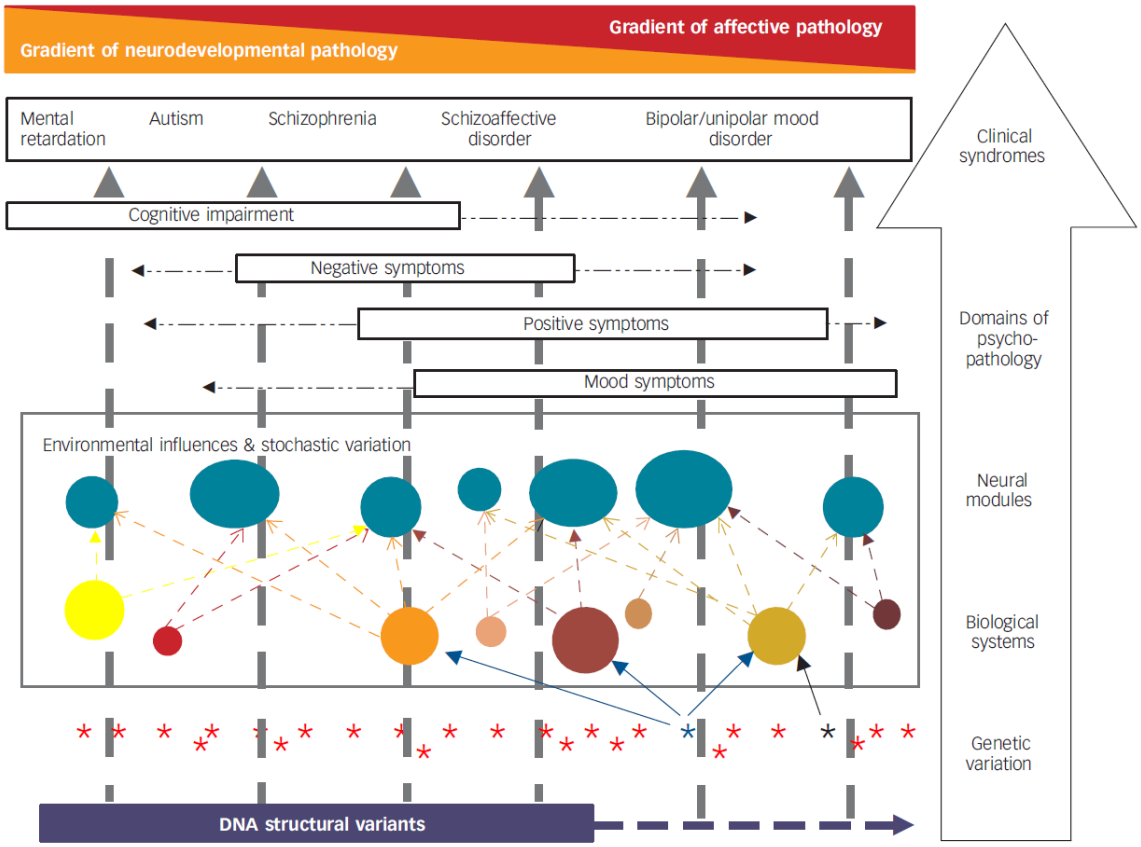
1.3 Continuum representation of psychosis between schizophrenia and affective/bipolar disorder

Current conceptions of schizophrenia as laid out by the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2013) and International Statistical Classification of Diseases and Health Related Problems (ICD) (WHO, 1993) adopt the Kreaplinian dichotomy which broadly divides major mood and

psychotic illness into schizophrenia and bipolar disorder (American Psychiatric Association, 2013, WHO, 1993). This approach places less emphasis on the clinical and neurobiological overlap between these two, and other mental illnesses such as autism spectrum disorder (ASD) (Vorstman et al., 2013, Chisholm et al., 2015, Cheung et al., 2010, Craddock and Owen, 2010, Craddock et al., 2006, Owen, 2014). However, understanding schizophrenia as an exclusively categorical disease seems implausible. There is an overlap in symptomology and co-occurrence between illnesses such as autism (cognitive impairment), bipolar disorder (mood symptoms and positive symptoms) and schizophrenia (Lewis and Lieberman, 2000, Fusar-Poli et al., 2014).

There is also evidence to suggest the neurobiological underpinnings of schizophrenia may also confer risk for other mental illnesses. Twin studies have demonstrated a genetic susceptibility for both schizophrenia, bipolar disorder and schizoaffective disorder (McGuffin et al., 1982) as well as ASD (Vorstman et al., 2013, Moreno-De-Luca et al., 2010). Craddock and Owen (2010) propose a continuum model of psychosis, which suggests that genetic variation can influence multiple biological systems, which are also influenced by the environment and other genetic factors. These factors then influence neural mechanisms which may lead to the domain of psychopathology and clinical syndrome experienced as shown in figure one.

Figure 1: The adapted continuum model as proposed by (Craddock and Owen, 2010), which shows the relationship between schizophrenia and other mental health illnesses.



1.4 What are the negative symptoms of schizophrenia?

1.4.1 Definition and description of negative symptoms

Negative symptoms of schizophrenia present as a range of difficulties that result in the lack or absence of elements within the normal repertoire of human behaviour and function. These include avolition, asociality, anhedonia, blunted affect and alogia (Barnes, 1993).Avolition: Avolition is defined as a lack of drive, energy and interest (Andreasen, 1982), which can impact on areas such as work, school and self-care (Kaiser et al., 2016).

It has been strongly associated with subjective distress (Selten et al., 2000). Bleuler (1950) described individuals exhibiting this negative symptom as being "lazy and negligent because they no longer have the urge to do anything either of their own initiative or at the bidding of another" (p. 70). Kraepelin (1919) attributed a central role of avolition in dementia praecox, assuming that this dictated the gradual decline observed in patients with chronic illness (Foussias and Remington, 2010).

Asociality: Asociality is the withdrawal from social contacts that is the consequence of a lack of desire to maintain or make social contact, or indifference for social contacts (Kirkpatrick et al., 2011b). In children, asociality is a risk factor for schizophrenia (Matheson et al., 2013) and a significant predictor of poor longer-term psychosocial functioning (Marchesi et al., 2015).

Anhedonia: Anhedonia is defined as the diminished capacity to experience pleasure and pleasant emotion (Horan et al., 2006, Kring and Germans, 2000, Andreasen, 1982). Anhedonia has been the subject of debate for many years given its close relationship with depression. Klein's (1984) distinction between anticipatory pleasure (ability to experience pleasure for future activities) and consummatory pleasure (experience normal levels of pleasure when engaging in enjoyable activities) has led some to conclude that anhedonia may present partially in schizophrenia; specifically anticipatory anhedonia, and not consummatory anhedonia may form part of the negative domain in schizophrenia (Gard et al., 2007).

Blunted affect: Blunted affect (and in its extreme form, flattened affect) is defined as a decrease in outward expression of emotion, characterised by a decrease in spontaneous movements, poor eye contact and a reduction of facial and vocal expression, and paucity of expressive gestures (Kilian et al., 2015). This negative symptom forms a significant component of the expressive deficits sub-domain of negative symptoms, along with alogia (as discussed in section 1.2.4.1).

Alogia: Horan et al. (2011) define alogia as a reduction in outward expression of speech. Previous definitions of alogia incorporated cognitive aspects of speech expression, which was termed poverty in content of speech. However, poverty in content of speech also relates to formal thought disorder (Palacios-Araus et al., 1995).

1.4.2 Primary versus secondary negative symptoms

Efforts have been made to distinguish primary from secondary negative symptoms (Carpenter, 1991, Carpenter et al., 1988). Primary negative symptoms represent a domain of symptoms that are intrinsic to the schizophrenia disease process itself (Carpenter et al., 1985). These symptoms do not appear transient or episodic (Earnst and Kring, 1997). It is clinically difficult to distinguish primary from secondary negative symptoms at the level of phenomenology: the underlying cause is needed for this (Peralta et al., 2000, Kirschner et al., 2016a). Secondary negative symptoms are transient in nature and respond to treatment via treating the cause of the negative symptom. Secondary negative symptoms are held to account for over 50% of negative symptoms observed in schizophrenia (Lyne et al., 2015) and are the result of;

- *Side effects from medication*: Side effects from antipsychotic medication has been suggested to mimic some of the negative symptoms by causing sedation (Miller, 2004), the akinesia/bradykinesia component of parkinsonianism (Barnes and McPhillips, 1995) and amotivation (Carpenter et al., 1985).
- *Depression*: Depression that is misattributed as a negative symptom. Depressive symptoms are common in all phases of schizophrenia (Upthegrove et al., 2013, Barnes et al., 1989a, Hafner et al., 1999, Sands and Harrow, 1999). However, the interplay between depressive and negative symptoms is complex, and may be dependent on the stage of the schizophrenia illness (Chang et al., 2015). Furthermore, depressive symptoms may only contribute to some of the secondary negative symptoms, specifically negative symptoms that are conceptualised as forming the avolition-apathy sub-domain (such as avolition, apathy and anhedonia) (Kulhara et al., 1989, Siris, 2000a).
- *Substance abuse:* Over-use of elicit substances, such as cannabis, have been linked with apathy and amotivation (Schmits and Quertemont, 2013) and may therefore present as a negative symptom.
- *Positive symptoms:* Social withdrawal may be secondary to abject paranoia or hallucination (Kirkpatrick, 2014, Buchanan, 2007, Carpenter et al., 1985), which may present as negative symptoms. In support of this, Kelley et al. (1999) found that a reduction in positive symptoms correlated with a reduction in symptoms that make up the avolition-apathy sub-domain but not with the expressive deficits sub-domain.

Irrespective of the cause, negative symptoms significantly impact on the lives of those experiencing them, especially through an increased recovery time (Erhart et al., 2006) and are held to account for much of the poorer social and occupational functioning (Hofer et al., 2006).

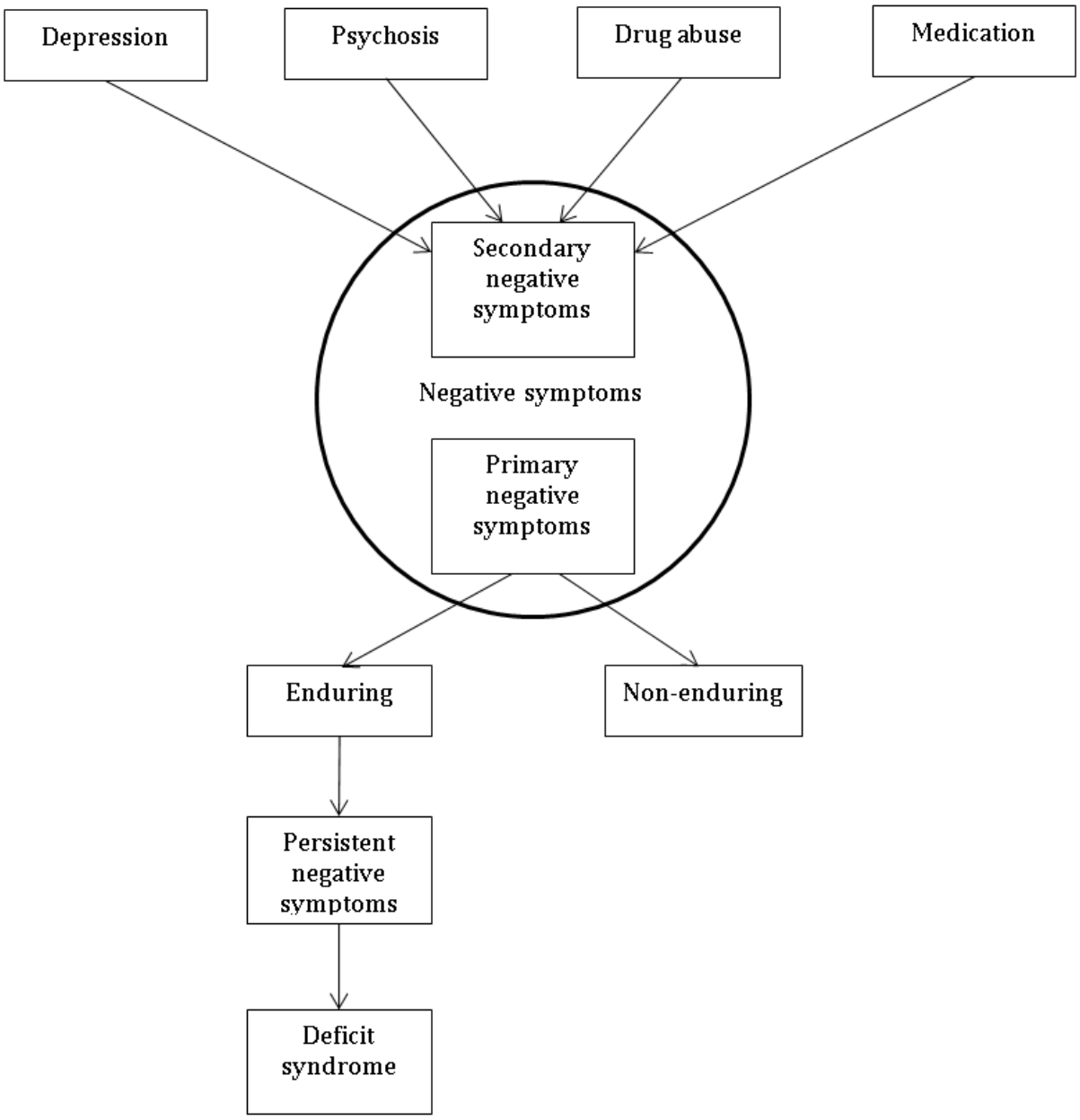
1.4.2.1 **Persistent negative symptoms and the deficit syndrome**

Primary negative symptoms can be further distinguished into enduring (also called deficit symptoms) and non-enduring negative symptoms, with enduring negative symptoms being stable for at least 1 year (Buchanan, 2007, Kirkpatrick and Galderisi, 2008). The deficit state is defined by the following criteria; two or more *primary* negative symptoms (low affect, diminished emotional range, poverty in speech, reduction of interests, diminished sense of purpose or lack of social drive), present for twelve months even when clinically stable (Kirkpatrick and Galderisi, 2008). Deficit and non-deficit state schizophrenia can be differentiated clinically, in terms of treatment response. Kopelowicz et al. (2000) treated thirty-nine deficit and non-deficit schizophrenia patients with olanzapine. They found that those with non-deficit schizophrenia showed an improvement in positive, negative and extrapyramidal symptoms, whereas those with deficit schizophrenia showed only an improvement in extrapyramidal side effects, suggesting that olanzapine reduces secondary negative symptoms but has no effect on primary negative symptoms. These findings also show a different treatment response between deficit and non-deficit schizophrenia. Differences were also observed in terms of neuropsychology (Kirkpatrick and Galderisi, 2008). A meta-analysis of thirteen longitudinal studies by Cohen et al. (2007) found that those with deficit schizophrenia

were more neuropsychologically impaired in terms of social cognition and language than non-deficit schizophrenia patients.

Alternatively, persistent negative symptoms endure at moderate severity and in the absence of many positive, depressive or extrapyramidal side effects (Buchanan, 2007), which are present for a period of six consecutive months following stabilisation of a first episode (Hovington et al., 2012). The deficit state, however, is diagnosed if primary negative symptoms persist for twelve months. Persistent negative symptoms can be identified in the early stages of the schizophrenia illness, and indeed in those who are considered at ultra-high risk for schizophrenia (Yung et al., 2019). The stability of persistent negative symptoms and the deficit state however, have been challenged by Kalisz and Cechnicki (2016). They found that, over a twenty year period, the diagnosis of deficit syndrome and persistent negative syndrome fluctuated and changed. See figure two for a schematic of the relationship between primary and secondary negative symptoms, the causes of secondary negative symptoms and the potential consequences and classifications of primary negative symptoms. This schematic also depicts the relative position of persistent negative symptoms.

Figure 2: The relationship between primary and secondary negative symptoms, along with factors that cause the secondary negative symptoms and the consequences of primary negative symptoms.



1.4.2.2 **Are negative symptoms stable over time?**

The stability of negative symptoms have been the subject of debate for many years and perceptions have changed. Early descriptions by both Bleuler (1950) and Kraepelin

(1919) conceptualised negative symptoms as both worsening over time and remaining more stable than the "accessory" positive symptoms, and permanent due to irreparable damage to the brain (Crow et al., 1980, Crow, 1985). Carpenter et al. (1985) has suggested that secondary negative symptoms may fluctuate over time depending on their cause, whereas primary negative symptoms were proposed to be more stable.

The conclusion that negative symptoms worsen over time is supported by Pfohl and Winokur (1983). In their study, Pfohl and Winokur (1983) followed-up fifty-two patients with schizophrenia over twenty-five years and found that negative symptoms such as avolition, social withdrawal and blunted affect increased over time. These findings however have not been replicated in a longitudinal study of thirty-nine patients with schizophrenia over five years by Pogue-Geile and Harrow (1985), who found that negative symptoms remained stable. Others have suggested that negative symptoms can improve over time. Johnstone et al. (1987) followed up ninety-two patients with schizophrenia over four years and found that negative symptoms were more stable than positive symptoms, but the deficits were not irreversible. A more recent study by Whitty et al. (2008) supported these findings. Finally, a meta-analysis of 5944 participants across forty-one studies by Savill et al. (2015) found negative symptoms reduced over time suggesting that negative symptoms may not be as stable as previously conceptualised. Some have suggested that certain negative symptoms may be more stable than others; Dollfus and Petit (1995b) and Johnstone et al. (1987) both reported that blunted affect and alogia were more stable than other negative symptoms.

1.4.2.3 **Do negative symptoms constitute a single syndrome?**

Factor analytic approaches on the different negative symptom scales have been used to offer a solution to the difficulties in measuring and conceptualising negative symptoms (Messinger et al., 2011). Factor analysis has yielded several sub-domains of negative symptoms (table 1).

Table 1: A summary of the sub-domains of negative symptoms generated from factor analysis studies.

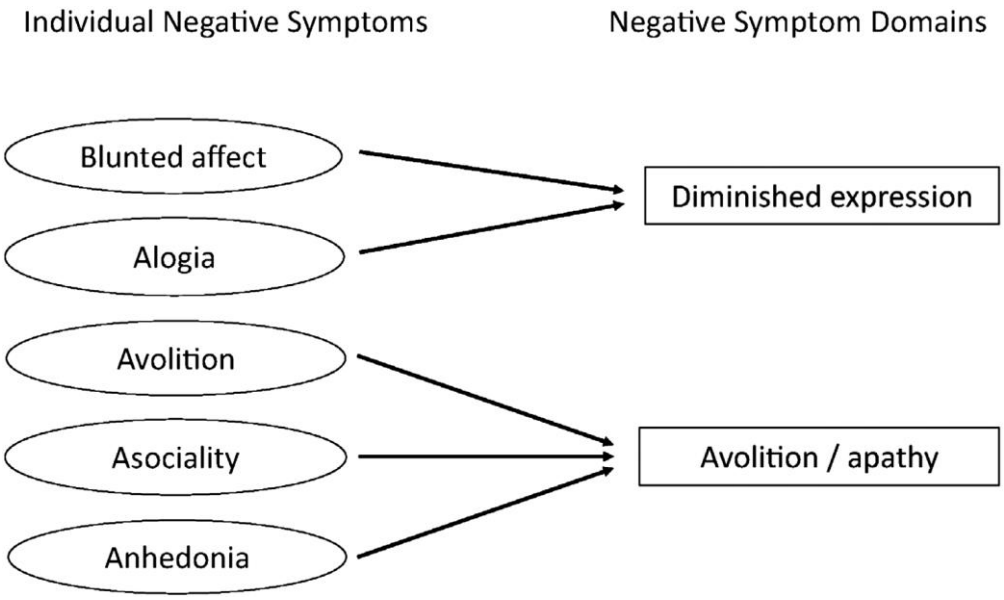
Author	Sub-domain of negative symptoms
Alphs et al. (1989)	1. Affect 2. External involvement 3. Retardation 4. Personal presentation 5. Thinking 6. Interpersonal interest 7. Blocking
Keefe et al. (1992)	1. Diminished-expression 2. Social dysfunction 3. Disorganisation
Mueser et al. (1994)	1. Affective flattening/blunting. 2. Avolition/apathy and anhedonia/asociality 3. Alogia and inattention
Axelrod et al. (1994)	1. Communication 2. Affect 3. Social involvement 4. Motivation 5. Gross cognitive 6. Retardation
Minas et al. (1994)	1. Negative signs (affective flattening and poverty of speech) 2. Psychosocial dysfunction (avolition-apathy and anhedonia-asociality)

Peralta and Cuesta (1995)	1. Affective flattening 2. Alogia 3. Avolition-apathy 4. Anhedonia-asociality 5. Attention
Sayers et al. (1996)	1. Diminished-expression 2. Inattention-alogia 3. Social amotivation
Toomey et al. (1997)	1. Diminished-expression 2. Disordered relating
Kelley et al. (1999)	1. Affective flattening 2. Diminished motivation
Peralta and Cuesta (1999)	1. Poverty of affect/speech 2. Social dysfunction 3. Attention
Emsley et al. (2001)	1. Diminished-expression 2. Disordered relating
Malla et al. (2002)	1. Affective flattening/alogia (excluding poverty of content of speech) 2. Avolition/anhedonia 3. Attention
Kimhy et al. (2006)	1. Avolition 2. Emotional expression
Nakaya and Ohmori (2008)	1. Avolition 2. Poor emotional expression
Tremeau et al. (2008)	1. Motor-affective 2. Motor social
Liemburg et al. (2013)	1. Expressive deficits 2. Social amotivation

Most of these analyses include symptoms that may also relate to cognitive dysfunction (such as poverty in speech and impaired attention) (Foussias and Remington, 2010). A recent factor analysis and review suggest that two major sub-domains; avolition-apathy sub-domain (comprising avolition, anhedonia and asociality) and the expressive

deficit (consisting of blunted affect and alogia) (Messinger et al., 2011, Liemburg et al., 2013) (see figure 3). These sub-domains have not only been shown to remain stable over time (Norman et al., 2015), but are also consistent with item ten on the areas of agreement within the National Institute of Mental Health (NIMH) Measurement and Treatment Research to improve Cognition in Schizophrenia (MATRICS) on negative symptoms Consensus Statement (Kirkpatrick et al., 2006), thus suggesting that these two sub-domains may represent separate therapeutic targets and may have separate neurobiological substrates. These sub-domains have been found in both first episode (Malla et al., 2002), chronic schizophrenia (Keefe et al., 1992), in those medicated (Sayers et al., 1996) and medication naïve (Kelley et al., 1999).

Figure 3: Negative symptoms separated into avolition-apathy and diminished-expression sub-domains (Kaiser et al., 2011).



1.4.2.4 **External validity of the avolition-apathy syndrome**

1.4.2.4.1 Clinical features and correlates of the avolition-apathy syndrome

Avolition-apathy is comprised of avolition, anhedonia and asociality. Individuals experiencing avolition-apathy are 1) more likely to have a family member who has experienced a psychiatric problem with hospitalisation as a result, 2) less likely to be employed or achieve high-quality work, 3) are more disorganised, 4) display poorer social functioning, 5) experience greater social and cognitive deficits, 6) have a more gradual onset of psychosis, and 7) more likely to be male than those with expressive deficits (Strauss et al., 2013). These findings suggest that avolition-apathy can be separated clinically, may be the product of differing underlying psychopathology and may be more severe than expressive deficits (Strauss et al., 2013). Avolition-apathy may be more prevalent than expressive deficit (Lyne et al., 2015) and appears strongly associated with the duration of untreated psychosis (DUP) (Malla et al., 2002), which itself conveys a risk for poorer prognosis (Penttilä et al., 2014). Moreover, the avolition-apathy sub-domain has also been associated with reduced compliance with medication (Tattan and Creed, 2001).

Evidence for the distinction between the avolition-apathy and expressive deficits sub-domains can be observed through differing treatment responses. Barnes et al. (2016) found that those administered citalopram showed a reduction in symptoms that correspond to the avolition-apathy sub-domains but it had no effect on symptoms that indicate the expressive deficits sub-domain.

1.4.2.4.2 Biological correlates of the avolition-apathy syndrome

Neuroimaging studies have suggested a distinct neuropathological marker for the avolition-apathy sub-domain. Ohtani et al. (2014) examined twenty-seven patients with chronic schizophrenia and twenty-six healthy controls using diffusion tensor imaging (DTI). They found white matter abnormalities in connections between the medial orbitofrontal cortex and rostral anterior cingulate cortex which corresponded with anhedonia-asociality and avolition-apathy. These findings implicate a specific neuroanatomical association with avolition-apathy that may be distinct from expressive deficits. Asami et al. (2014) used DTI and fractional anisotropy (FA) and identified structural white matter abnormalities in the left frontal lobe that correlated with the avolition-apathy sub-domain. Juckel et al. (2006) have shown, using functional MRI (fMRI), ventral striatal hypoactivation associated with negative symptoms during an incentive monetary delay task suggesting dysfunctional reward systems may contribute to negative symptoms such as anhedonia and apathy. Recent findings have suggested that this association may be via the avolition-apathy sub-domain, and not the expressive deficits domain (Kirschner et al., 2016b), thus providing further evidence for dissociable sub-domains within the negative symptoms.

There may also be neural specificity for each symptom within the avolition-apathy sub-domain. The dorsal caudate and dorsolateral prefrontal cortex (dlPFC) is associated with reward anticipation and decision making. Mucci et al. (2015) used functional MRI to study brain regions activated during the monetary incentive delay task in twenty-eight patients with schizophrenia and twenty-two healthy controls. They found lower dorsal

caudate activation in those with high avolition scores. Also, dorsal caudate activity during reward anticipation was associated with avolition but not anhedonia. This suggests that specific striatal regions may be responsible for the generation of certain symptoms within this sub-domain.

1.4.2.5 **Expressive deficits**

1.4.2.5.1 Clinical features and correlates of expressive deficits

Expressive deficit consists of blunted affect and alogia. Individuals with expressive deficits are 1) more likely to have an abrupt onset of psychosis, 2) more likely to be hospitalised for longer periods of time (Strauss et al., 2013) and 3) more likely to experience greater neurocognitive dysfunction including that in working memory, processing speed, reasoning/problem solving and verbal learning (Sevy et al., 2018). In a prospective study of sixty-three patients with at least moderate severity of flattened affect and ninety-nine patients without flattened affect, Gur et al. (2006) found those with flattened affect performed worse on an emotion recognition task than those without flattened affect. These findings suggested that deficits in emotional perception result in an inability to reciprocate social interactions and apparent blunting of affect.

To explain alogia, the cognitive resource limitation model has been postulated. This model suggests that when cognitive resources are depleted (such as when in social situations that require speech), verbal expression becomes diminished. This is because cognitive processes are placed under stress which impacts on speech production and results in a reduction of speech (Cohen et al., 2012, Cohen et al., 2014). Cognitive

resources may become depleted more quickly in schizophrenia because of the widespread effects of neuroanatomical abnormalities and functional inefficiency (Cohen et al., 2012). Other explanations include extrapyramidal side effects and medication (Sarkar et al., 2015).

1.4.2.5.2 Biological correlates of the expressive deficits syndrome

Many studies have investigated emotion processing using fMRI to follow brain responses to viewing standardised images of faces expressing emotions such as fear, happiness or sadness. Others have used images of objects or scenes of varying intensity of positive, negative or neutral intensity (IAPTS images). A number of emotion processing studies in schizophrenia have reported that blunted affect is associated with decreased responsiveness in the left amygdala, parahippocampal gyrus to emotional images (Lepage et al., 2011, Rahm et al., 2015, Taylor et al., 2012, Dowd and Barch, 2010) and ventrolateral prefrontal cortex (Stip et al., 2005).

Expressive deficits have been associated with hypoactivation of the fronto-thalamic circuitry (consisting of thalamic, prefrontal, precentral, parietal and temporal brain areas). In an fMRI study, Dlabac-de Lange et al. (2018) tested twenty participants with schizophrenia and distinguished between those with and without expressive deficits. They presented participants with the 'wall of faces task' and found that those with high expressive deficits showed hypoactivation of the fronto-thalamic circuitry during ambiguous social appraisal. These findings suggest that there may be a specific brain area that is responsible for expressive deficits in schizophrenia.

The findings presented by Dlabac-de Lange et al. (2018) support the presence of two distinct and dissociable sub-domains within the negative symptoms of schizophrenia; avolition-apathy and expressive deficits. However, it remains unclear how these sub-domains interact with one another, and how they interact with other symptom dimensions in schizophrenia.

1.4.3 Assessing negative symptoms

The assessment of negative symptoms has proved challenging both for clinicians and also researchers, which can be particularly difficult when assessing expressive deficits and distinguishing symptoms from poor premorbid functioning. However, the main challenges in assessing negative symptoms are largely related to distinguishing primary negative symptoms from secondary negative symptoms, including those driven by depressive features (Barnes, 1993).

Global assessments of negative symptoms include the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). Specific scales designed to assess negative symptoms include the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1989), the Brief Negative Symptom Scale (BNSS) (Kirkpatrick et al., 2011b) and the Clinical Assessment Interview for Negative Symptoms (CAINS) (Horan et al., 2011). The SANS (Andreasen, 1989) has been considered the 'gold standard' for the assessment of negative symptoms and explores all symptoms which make up the negative domain, however the SANS does not distinguish between sub-domains of avolition-apathy and expressive deficits. Other,

more recently developed negative symptom assessments (such as the Clinical Assessment Interview for Negative symptoms - CAINS) (Horan et al., 2011) adequately separate expressive deficits from avolition-apathy, thus reflecting changes in conceptions of negative symptoms. Scales that focus on specific sub-domains include the Rating Scale for Emotional Blunting (EBS) (Abrams and Taylor, 1978), the Apathy Evaluation Scale (AES) (Marin et al., 1991) and the Specific Loss of Interest and Pleasure Scale (SLIPS) (Winer et al., 2014). Assessments of negative symptoms can also be categorised as being 'observer rated', such as the PANSS and SANS or 'self-assessed' such as the Subjective Deficit Syndrome Scale. See table two for a list of measures used to assess negative symptoms, which demonstrates a comprehensive overview of assessments used to measure negative symptoms and details which assessments provide a sub-domain, global or specific focus and whether the assessment is self-rated or observer rated.

Table 2: List of assessments used to measure negative symptoms separating method of assessment and approach to negative symptoms.

Assessment	Global, specific or sub-domain	Symptoms/construct	Self-rated/ observer rated
Affective Flattening Scale (AFS) (Andreasen, 1979)	Sub-domain	Affective flattening	Observer rated
Anticipatory and Consummatory Interpersonal Pleasure Scale (ACIPS) (Gooding and Pflum, 2014)	Sub-domain	Anticipatory social pleasure, consummatory social pleasure	Self-rated
Apathy Evaluation Scale (AES) (Marin et al., 1991)	Sub-domain	Apathy	Observer rated
Brief Negative Symptom Scale (BNSS) (Kirkpatrick et al., 2011b)	Specific	Blunted affect, alogia, asociality, anhedonia, avolition	Observer rated

Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962)	Global	Apathy, anergia and withdrawal	Observer rated
Clinical Assessment Interview for Negative Symptoms (CAINS) (Horan et al., 2011)	Specific	Affective flattening, alogia asociality, avolition and anhedonia	Observer rated
Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002)	Global	Asociality, amotivation, anhedonia	Self-rated
High Royds Evaluation of Negativity Scale (HEN) (Mortimer et al., 1989)	Specific	Appearance, behaviour, speech, thought, affect, functioning	Observer rated
Krawiecka–Manchester Scale (KMS) (Krawiecka et al., 1977)	Global	Poverty of speech, flattened incongruous affect, psychomotor retardation	Observer rated
Motivation and Pleasure Scale—Self-Report (MAP-SR) (Llerena et al., 2013)	Specific	Social and non-social activities, consummatory and anticipatory pleasure in social, recreation and work	Self-rated
Motor Affective Social Scale (MASS) (Tremeau et al., 2008)	Sub-domain	Alogia, unit-behaviour-score	Observer rated
Negative Symptom Assessment (NSA-16/4) (Alphs et al., 1989), (Alphs et al., 2010)	Specific	Communication, emotion, motivation, sociality, retardation	Observer rated
Negative Symptoms Behaviour Rating Scales (NSRS)	Specific	Poverty of speech, flat affect, psychomotor retardation	Observer rated
Negative Symptom Rating Scale (NSRS) (Iager et al., 1985)	Specific	Thought-processes, cognition, volition/motivation, affect	Observer rated
Physical and Social Anhedonia Scale (PAS) (Chapman et al., 1976)	Sub-domain	Anhedonia, decreased pleasure for physical stimuli	Self-rated
Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987)	Global	Blunted affect, emotional withdrawal, poor-rapport, passive/apathetic social withdrawal, difficulty in abstract thinking, lack of spontaneity, flow of conversation and stereotyped thinking	Observer rated

Rating Scale for Emotional Blunting (EBS) (Abrams and Taylor, 1978)	Sub-domain	Affect, thought content, behaviour	Observer rated
Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1989)	Specific	Affective flattening, alogia, avolition, anhedonia, attentional impairment	Observer rated
Schedule for Affective Disorders and Schizophrenia — Negative Symptom Scale (SADS-NSS) (Lewine et al., 1983)	Specific	Loss of interest, blunted affect, slowed speech and body movements, fatigue, depressed appearance, inappropriate affect, thought disorder, poverty of content	Observer rated
Schedule for the Deficit Syndrome (SDS) (Kirkpatrick et al., 1989)	Specific	Restricted affect, diminished emotional range, poverty of speech, curbing of interests, diminished sense of purpose, diminished social drive	Observer rated
Self-Assessment Anhedonia Scale (SAAS) (Olivares et al., 2005)	Sub-domain	Anhedonia	Self-rated
Self-evaluation of Negative Symptoms (SNS) (Dollfus et al., 2016)	Specific	Social withdrawal, diminished emotional range, avolition, anhedonia, alogia	Self-rated
Short Scale for Rating Activity-Withdrawal in Schizophrenics (SSRAWS) (Venables, 1957)	Sub-domain	Activity-withdrawal (including social and speech)	Observer rated
Snaith–Hamilton-Pleasure-Scale (SHAPS) (Snaith et al., 1995)	Sub-domain	Anhedonia	Self-rated
Social Anhedonia Scale (SAS) (Eckblad et al., 1982)	Sub-domain	Anhedonia, decreased pleasure, associability	Self-rated
Specific Loss of Interest and Pleasure Scale (SLIPS) (Winer et al., 2014)	Sub-domain	Anhedonia	Observer rated
Subjective Deficit Syndrome Scale (SDSS) (Jaeger et al., 1990)	Global	Amotivation, anhedonia, loss of emotions	Self-rated
Subjective Experience of Deficits in Schizophrenia (SEDS) (Liddle and Barnes, 1988)	Global	Reduced emotion, anhedonia, inability to feel intimacy, drive/energy, lack of motivation, lack of interests, lack of energy	Self-rated
Subjective Experience of Negative Symptoms (SENS) (Selten et al., 1993)	Specific	Awareness, causal attribution and distress	Self-rated

Temporal Experience of Pleasure Scale (TEPS) (Gard et al., 2006)	Sub-domain	Trait anticipatory pleasure and consummatory pleasure	Self-rated

1.4.4 Treatment of negative symptoms

For most individuals experiencing schizophrenia, improvement of negative symptoms correlates with improvement of positive symptoms and depression. The treatment of extrapyramidal side effects with anticholinergic medication can also improve globally rated secondary negative symptoms (Buchanan, 2007). However, for those who do not respond (which account for between 15 and 20% of individuals) (Bobes et al., 2010), negative symptoms are enduring, and antipsychotic medication is of limited benefit (Barnes and Paton, 2011).

1.4.4.1 Pharmacological treatment strategies

There are currently no pharmacological treatments available that are specifically designed for negative symptoms in schizophrenia (Aleman et al., 2016) and currently available treatments offer only a modest benefit at best (Barnes and Paton, 2011). The distinction between primary and secondary negative symptoms is important. Primary negative symptoms are symptoms that are assumed to be core to the neurobiology of schizophrenia, whereas secondary negative symptoms can be the result of several factors (such as positive symptomology, depression, medication side effects or illicit substance misuse). Second generation antipsychotic medication were reported to have improved treatment efficacy than first generation antipsychotics (Barnes, 2011). While clozapine has been found to be effective in treating negative symptoms in treatment resistant

schizophrenia (Wahlbeck et al., 1999, Kane et al., 1988), there is little evidence that second generation antipsychotic medication has any benefit over first generation antipsychotic medication in treating negative symptoms (Jones et al., 2006, Lieberman et al., 2005a). Indeed, Leucht et al. (2009) conducted a meta-analysis of 21533 patients across 150 RCTs comparing first generation and second generation antipsychotics. They found second generation antipsychotics showed greater efficacy in reducing negative symptoms than first generation antipsychotics, however the magnitude of this difference was small.

Antidepressant medication is the most common class used as augmentation with antipsychotic medication to treat negative symptoms. The most commonly used are selective serotonin reuptake inhibitors (SSRIs) such as mirtazapine and fluoxetine, with fluoxetine being suggested to be the most effective addition in a meta-analysis of twenty three trials using 819 patients by Singh et al. (2010). A Cochrane review concluded combining antidepressant and antipsychotic medication leads to a reduction in some negative symptoms compared with antipsychotic monotherapy (Rummel et al., 2006). Furthermore, a systematic review of eighty-two trials examining the efficacy of antidepressant treatment adjunctive to antipsychotic medication to treat negative symptoms in chronic schizophrenia concluded that antipsychotic plus antidepressant dual therapy was more effective in treating negative symptoms than antipsychotics alone (Helfer et al., 2016). Many of the studies included did not differentiate primary from secondary negative symptoms, thus the findings may be considered contaminated with differing and varying negative symptoms and therefore may not offer a true representation when treating negative symptoms.

Much of the previous research has examined negative symptoms as a unitary concept and assessed whether treatment improves the global negative symptom domain, however, this approach is limited. Indeed some previous studies have reported a differential treatment response when examining the two negative symptom sub-domains (avolition-apathy and expressive deficits) (Barnes et al., 2016, Shafti et al., 2005). Barnes et al. (2016) reported no significant differences between treatment and placebo groups in reducing negative symptoms; however, when examining the two sub-domains of negative symptoms separately, those treated with citalopram displayed lower levels of avolition-apathy. This not only provides a hopeful treatment strategy for tackling some negative symptoms, but also suggests that there may be differing underlying neurobiology that underpins these two sub-domains. It is also possible that the partial effect of citalopram on avolition-apathy was mediated through treatment of depressive symptoms.

1.4.4.2 **Psychological interventions**

Several treatment strategies based on psychological principles have been employed to successfully treat negative symptoms (Lutgens et al., 2017). Psychological models of negative symptoms suggest that the negative symptoms are the defensive position that is adapted following acute stress (Bleuler, 1950). The diathesis-stress model suggests that the negative symptoms are the result of genetic and environmental factors which results in some individuals being more susceptible to negative symptoms than others (Rector et al., 2005). They go on to suggest that the negative symptoms in schizophrenia are driven by disengagement as a response to delusional beliefs, social

threat and the belief that the individual will perform poorly in tasks and social activities (Rector et al., 2005). The most established form of psychological therapy is cognitive behavioural therapy (CBT) (Beck, 2011) which suggests that our interpretation of events is a consequence of our feelings and behaviour, which create and maintain unhelpful responses (Elis et al., 2013), therefore, negative symptoms are generated through thoughts, interpretations and expectations about events (Velthorst et al., 2015). CBT may be effective in reducing negative symptoms, and this treatment effect was also observed at follow-up (Borras et al., 2009, Klingberg et al., 2011). However, two meta-analyses have suggested that CBT may not be effective in negative symptom reduction in its current form (Velthorst et al., 2015, Jauhar et al., 2014), but adapted versions of CBT may have therapeutic benefit (Jauhar et al., 2014).

1.5 Depression in schizophrenia

Depressive symptoms have been reported to occur in cross-sectional studies in around 30-40% of individuals with schizophrenia (Conley et al., 2007, Majadas et al., 2012, Schennach et al., 2015), with around 80% of individuals experiencing a clinically significant depressive episode once or more in the early phase of schizophrenia (Upthegrove et al., 2010). Furthermore, Hafner et al. (2005b) found that attenuated depressive symptoms in schizophrenia appeared around four and a half years prior to first admission, and predated all other symptom domains in schizophrenia (Krabbendam et al., 2005). Identification of affective symptoms in schizophrenia also feature in the early descriptions by both Kraepelin (1919) and Bleuler (1950). Depressive symptoms are

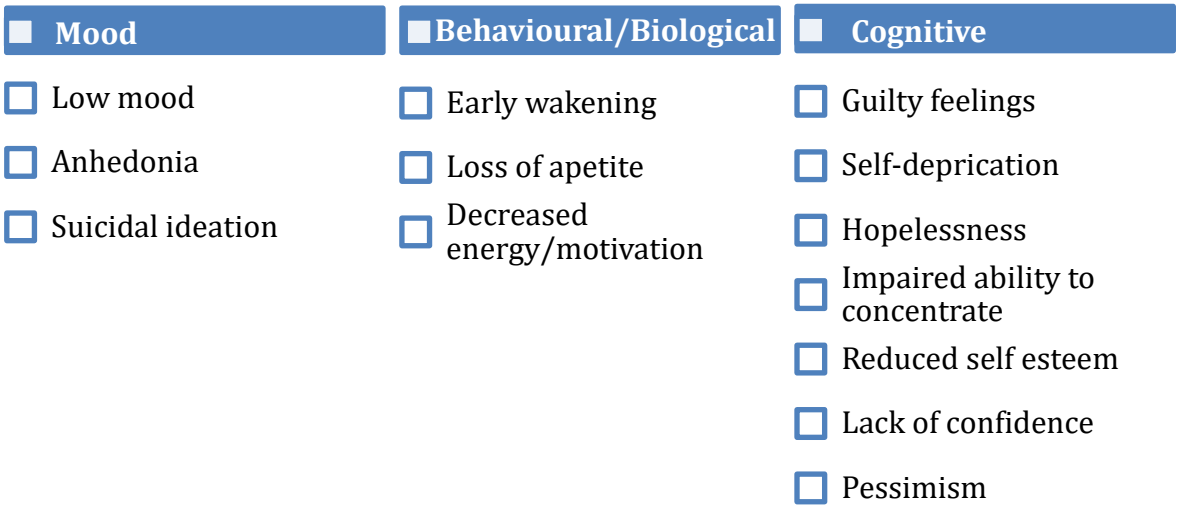
found in the prodromal phase of schizophrenia prior to full development of psychosis (Yung and McGorry, 1996).

The presence of depression in early psychosis was previously considered a positive prognostic indicator; possibly signalling that the individual's illness was on 'the bipolar side' of a continuum of affective/psychotic illness, as opposed to the 'autistic end' of the continuum (Upthegrove et al., 2016). However, more recent evidence suggests an association with poorer outcomes, lower psychological well-being (Strauss et al., 2012) and diminished quality of life (van Rooijen et al., 2019). The occurrence of depression in early psychosis has been associated with future depressive episodes, suicide and increased likelihood to command hallucinations (Upthegrove et al., 2013, Dutta et al., 2011, Sandhu et al., 2013), thus emphasising the importance of depression in the course of early psychosis and prognosis.

1.5.1 How does depression present in schizophrenia?

Depression in schizophrenia is characterised by core symptoms including depressed mood, hopelessness and anhedonia (Schennach et al., 2015, Chemerinski et al., 2008). Other symptoms include guilty feelings, self-deprecation, suicidal ideation, impaired ability to concentrate, early wakening, loss of appetite (National Collaborating Centre for Mental Health, 2010) and mood instability (Balbuena et al., 2016). These symptoms can be categorised into three main groups; mood, cognitive and behavioural/biological symptoms (see figure 4).

Figure 4: Depressive symptoms in schizophrenia categorised by their defining feature.



This distinction between depressive and negative symptoms has been the subject of much debate, particularly since the 1980s. Early research concluded that depressive and negative symptoms could not be discerned (Pogue-Geile, 1989, Siris et al., 1988). However, subsequent factor analyses have concluded that depressive and negative symptoms can be distinguished (Klaassen et al., 2011, McGorry et al., 1998, Muller et al., 2001) and have implicated depressive features as a separate symptom domain in schizophrenia. The development of the Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al., 1993) has showed that depression can be examined separately from negative symptoms. Addington et al. (1993) administered the CDSS and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) to 100 outpatients and fifty in-patients with schizophrenia. They found a weak correlation between the CDSS and PANSS negative subscale in inpatients but not outpatients. Confirmatory factor analysis showed that the model hypothesising specificity of depression and negative symptoms, with a

goodness fit of .89 (Addington et al., 1993). This supports the view that depression and negative symptoms represent separate symptom domains.

Depressive symptoms in schizophrenia have been attributed to antipsychotic medication which may present as retarded depression (Johnson, 1981, Leff et al., 1988). These findings have not been supported by Baynes et al. (2000) who found no significant association between depressive symptoms and akathisia or extrapyramidal side effects. Moreover, given that depression is seen in the prodromal phase (prior to antipsychotic medication administration) and fluctuates in severity through the course of illness, it appears that extrapyramidal side effects from antipsychotic medication may not be the only cause of depressive symptoms in schizophrenia.

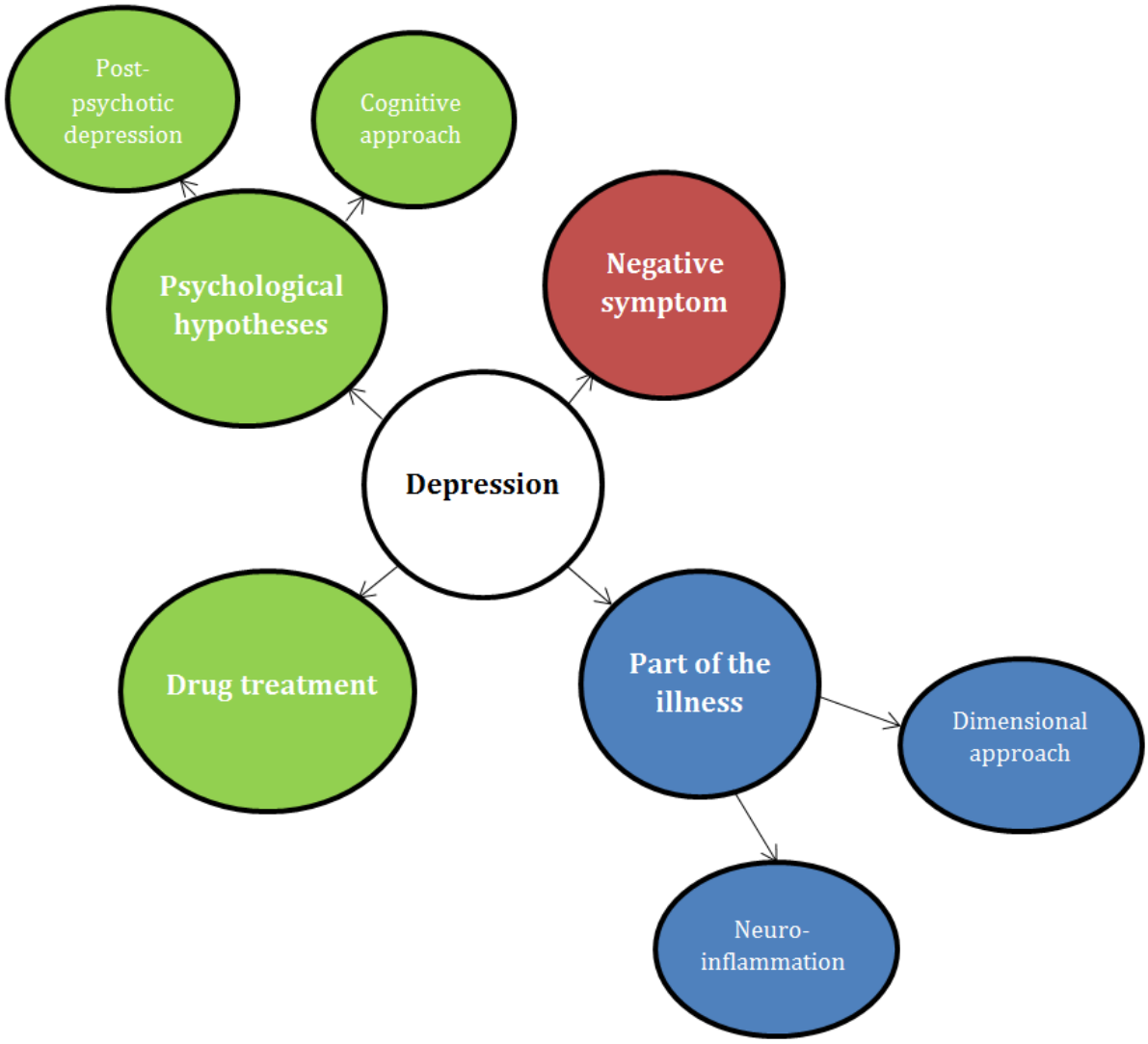
1.5.2 Why does depression occur in schizophrenia?

The diagnosis of depression is a syndrome - a group of symptoms that occur together including, low mood, hopelessness, anhedonia, pessimism and self-deprecation (Beck, 1967). The syndrome is commonly seen (is co-morbid with) many other medical and psychiatric disorders as well as schizophrenia. In some cases, depression may be triggered by suffering associated with the primary disorder. In others, a shared underlying pathogenesis may contribute to co-morbidity, for example impaired dopamine function in Parkinson's disease likely underlies vulnerability to depression. Psychosocial adversity is common in schizophrenia and this may also contribute to the co-morbidity (Birchwood et al., 2005). Depression in schizophrenia may describe the 'mood' aspect of depression, without the cognitive and behavioural/biological categories of depression.

These may be episodic and reflect a state, rather than a trait, aspect of illness (Chiappelli et al., 2014). This study examined ninety eight individuals with psychosis and 115 healthy controls, and found that trait depression was prominent in schizophrenia, and distinct from negative symptoms (Chiappelli et al., 2014). This may be accompanied by other symptoms that make up the diagnostic criteria of depression (Siris, 2000b).

Several biological and psychological mechanisms have been proposed to account for the occurrence of depressive symptoms in schizophrenia. Indeed, it seems likely that these mechanisms may co-exist to varying degrees in different patients. (See figure 5).

Figure 5: The various explanations of depressive symptoms in schizophrenia (N.B. depression and neuro-inflammation will be explained later in the introductory chapters). Items in blue indicate hypotheses of depression being intrinsic to schizophrenia; items in green indicate hypotheses of depression being a reaction to schizophrenia and the item in red indicates a hypothesis of depression being co-morbid to schizophrenia.



1.5.2.1 **Depression as intrinsic to schizophrenia**

1.5.2.1.1 Dimensional approach

Depressive symptoms in schizophrenia have been viewed as overlapping phenomenologically with the negative symptoms with areas of commonality being asociality, amotivation, anhedonia, and apathy (Andreasen and Olsen, 1982, Carpenter et al., 1985, Crow, 1980, Romney and Candido, 2001, Lindenmayer and Kay, 1989). However an alternative approach suggested by McGorry et al. (1998) postulates a dimensional approach to psychoses which generates a model of overlapping domains, or clusters of symptoms with distinct presentations. Mood symptoms have been included in five factor models of psychosis; positive symptoms, negative symptoms, depression, mania and cognitive (van Os, 2008). These models of psychoses allow for co-morbidity in more than one domain, with an individual patient presenting with varying degrees of symptom severity in each domain.

1.5.2.1.2 Biological mechanisms

Similar changes in brain structure have been observed in early schizophrenia and psychotic depression (Lee et al., 2016) with insular grey matter loss being more pronounced in schizophrenia.

One explanation for grey matter loss is inflammation. Noto et al. (2015b) found that increases in cytokine concentrations (Il-6, IL-4, IL-10 and TNF-α) in patients with

schizophrenia and depression (the role in inflammation in depression and schizophrenia is discussed in section 1.7).

Kumari et al. (2016) presented sixty-three participants (and 20 healthy controls) facial emotional recognition task during fMRI. Both patients with affective psychosis and schizophrenia show an enhanced response when presented with fearful face, particularly in the thalamus. However, the enhanced emotional response of depressed patients with schizophrenia may be due to paranoia, but these findings were not reported.

It has been suggested that that depressive symptoms are a consequence of antipsychotic medication, leading to symptoms that mimic retarded depression (such as parkinsonism, sedation, akinesia, bradykinesia or loss of spontaneity) (Barnes et al., 1989b). However, this 'pharmacogenic' hypothesis has not received support from early work examining depressive symptoms in schizophrenia. Johnson (1981) found that depression was present in thirty-seven untreated new and acute patients with schizophrenia. Moreover, Leff et al. (1988) found 45% of anti-psychotic drug free patients experienced depression implying that drug-induced depression in schizophrenia cannot account for all cases of depression in schizophrenia. Finally, Hafner et al. (2005a) found that depression is seen in those who were antipsychotic medication naïve in the prodromal phase who subsequently develop schizophrenia suggesting that depression represents a core feature of schizophrenia and is not a result of drug treatment.

1.5.2.2 Psychological origins of depression in schizophrenia

Cognitive model of depression: Depressive symptoms observed in schizophrenia have also been attributed to the individuals' psychological response to their illness. Birchwood et al. (2005) proposed a cognitive account of depressive symptoms in early schizophrenia. They found over 60% of individuals who experienced auditory hallucinations also met criteria for moderate depression, which was related to the individual's social appraisal, social status, shame and interpersonal beliefs. Furthermore, the subjective experiences of psychological deficits may be an important factor in the occurrence of depressive symptoms (Liddle et al., 1993).

Post-psychotic depression: Post-psychotic depression may account for the occurrence of depression in schizophrenia. This theory suggests that depression is revealed following successful treatment of an individual's psychosis, and therefore depressive symptoms are generated by the individual's appraisal of their diagnosis and its implications for their social role and status (Birchwood et al., 2000). This may be exacerbated by an individual's awareness of their illness and the realisation that they have been ill, and their experiences and beliefs were not real. However, post-psychotic depression may only account for some of the occurrences of depression in schizophrenia. Given that depressive symptoms are frequent in the early stages of schizophrenia (Upthegrove et al., 2013) and in the prodromal phase (Hafner et al., 2005b), depressive symptoms that emerge following psychosis cannot explain the high prevalence of depressive symptoms in schizophrenia.

1.5.3 Summary

In summary depressive symptoms in schizophrenia may not be a consequence of one cause, but the result of several aetiological mechanisms that may differ from individual to individual. These separate causes of depressive symptoms in schizophrenia may account for different pathways leading to depression. These range from depression as a manifestation of the negative syndrome, or due to drug treatment, or psychological pathways, or intrinsic to schizophrenia and may differ between individuals.

1.5.4 Treatment of depression in schizophrenia

The primary neurobiological systems that are thought to underlie the pathogenesis of depression are serotonin, noradrenaline and dopamine. Several classes of pharmacological therapies have been developed, the earliest being monoamine oxidase inhibitors (MAOIs), such as phenelzine (Youdim and Bakhle, 2006) and tricyclic antidepressants (TCAs) (Klerman and Cole, 1965), such as amitriptyline. TCAs aim to exert an antidepressant effect by inhibiting the reuptake of monoamine neurotransmission thus enhancing noradrenaline (NA) and 5-hydroxytryptymine (5HT) transmission (National Collaborating Centre for Mental Health, 2010). MAOIs bind with monoamine oxidase, which is an enzyme responsible for breaking down monoamine neurotransmitters, such as serotonin and noradrenaline.

Another class of antidepressant therapy that also aims to enhance levels of serotonin is selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline

and citalopram, the most widely used being citalopram (National Collaborating Centre for Mental Health, 2010). SSRIs reduce depressive symptoms by increasing serotonin neurotransmission. This is achieved by inhibiting the reuptake of serotonin in the presynaptic neurone.

Pharmacological and psychological therapeutic options for depression in schizophrenia are poor, resulting in depressive symptoms being inadequately treated (Lako et al., 2012). Despite the use of antidepressant medication, the prevalence of depression in schizophrenia remains high, as do the rates of suicide. The evidence for the use of antidepressant medication in schizophrenia is low with only a few sufficiently powered randomised controlled trials being carried out in schizophrenia. Helfer et al. (2016) conducted a meta-analysis of 3608 patients across eighty-two studies and found that depressive and negative symptoms slightly reduced following administration of antidepressant medication. A systematic review and meta-analysis by Gregory et al. (2017) of twenty-six studies found that antidepressant use in schizophrenia may be effective but the evidence of their effectiveness is mixed. The mixed findings of antidepressant use in schizophrenia may suggest that depression is qualitatively different to unipolar depression. Previous findings have suggested an increased role of shame, anhedonia and hopelessness in depression in schizophrenia (Upthegrove et al., 2014b). Therefore, more targeted therapeutic options may be needed to treat depression in schizophrenia.

Furthermore, a Cochrane review of twenty trials assessing CBT versus other psychological treatments concluded that CBT was no more effective in treating depressive

symptoms in schizophrenia than other psychological therapies (Jones et al., 2012). However, many of the studies included in the review were small and of limited quality, suggesting that larger studies of greater quality are needed to understand the benefit of psychological therapies in schizophrenia. It is clear from this evidence that depression remains pervasive in early schizophrenia and is inadequately treated.

1.6 Neurobiology of schizophrenia

1.6.1 Dopamine hypothesis and negative symptoms

Dopamine (DA) has been implicated in the maintenance of negative symptoms. A review by Seeman and Van Tol (1994) and Davis et al. (1991) found that negative symptoms were associated with low dopaminergic activity in the prefrontal cortex. This is supported by several lines of evidence; firstly, a Cochrane review concluded that amphetamines (dopamine releasing agents) ameliorate negative symptoms (Nolte et al., 2004). Secondly, Okubo et al. (1997) used positron emission tomography (PET) to image drug-naïve and drug-free patients with schizophrenia and found that irregular availability of cortical dopamine receptors in the prefrontal cortex is related to the severity of negative symptoms (such as emotional withdrawal) and performance on the Wisconsin Card Sorting Task. Finally, there is a positive association between negative symptoms and iodebenzamide (a dopamine antagonist), suggesting dopaminergic hypofunctioning in negative symptoms (Pogarell et al., 2012). These findings suggest that DA is important in understanding the neurobiology of negative symptoms in schizophrenia.

The dopamine hypothesis of schizophrenia does not provide a complete account of psychotic symptoms because a considerable proportion of patients continue to experience positive and negative symptoms despite adequate treatment with dopamine receptor blocking antipsychotic drugs. One example is treatment resistant schizophrenia, of which a considerable proportion of individuals with schizophrenia fall into this category and are prescribed clozapine (Mortimer et al., 2010). Furthermore, only positive

symptoms correlate strongly with increased dopamine release in PET studies (Laruelle et al., 1999). In light of these disadvantages with the dopamine hypothesis, Howes and Kapur (2014) suggest that schizophrenia is a consequence of two differing neurobiological processes, one type of schizophrenia which is a consequence of hyperdopaminergia, and the other type occurs despite normal levels of dopamine.

1.6.2 Glutamate hypothesis

The administration of high dose phencyclidine (PCP) or ketamine has been shown to produce a schizophrenia-like state, characterised by behavioural withdrawal and apathy (Chen and Weston, 1960). These symptoms form part of the negative symptom domain in schizophrenia, as well as symptoms akin to neurocognitive dysfunction in schizophrenia (Javitt, 2010, Luby et al., 1959). Furthermore, use of ketamine has been shown to produce symptoms similar to psychosis in both individuals with schizophrenia (Jentsch and Roth, 1999, Lahti et al., 1995), and healthy individuals (Krystal et al., 1994). Both PCP and ketamine act as an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist (Javitt, 2010); leading to the hypothesis that dysfunction located at the NMDA receptor may be implicated in the neurobiology of schizophrenia. Indeed, evidence from magnetic resonance (MR) spectroscopy studies suggest that abnormally increased levels of glutamate are present in the frontal cortex in early stages of the disease; in the at-risk mental state (ARMS) for schizophrenia, which was also related to grey matter volume loss (Stone et al., 2009). This suggests that glutamatergic dysfunction may be implicated in the development and maintenance of schizophrenia even in the early phases of the illness.

1.6.3 Structural and functional changes in schizophrenia associated with negative symptoms and depression

Schizophrenia has been associated with structural brain alterations. Grey matter loss is mainly found bilaterally in the frontal and temporal gyrus, anterior cingulate gyrus, amygdala and hippocampus, and the insular (Cahn et al., 2006, Radua et al., 2012, Wible et al., 2001, Anderson et al., 2002, Cascella et al., 2010). Indeed, subtle grey matter loss occurs in the transition to psychosis and in the early course of schizophrenia (Cahn et al., 2006, van Haren et al., 2008, Lieberman et al., 2005b). Negative symptoms of schizophrenia have been associated with volumetric reductions in the temporal cortex (Hazlett et al., 2008, Koutsouleris et al., 2008), and bilateral caudate (Young et al., 1991a). A systematic review of twelve studies by Li et al. (2017) found significant grey matter reduction related to negative symptoms in the left caudate nucleus, left precentral region, bilateral medial frontal gyrus, thalamus and insula, compared to controls. Areas of the brain that is involved in reward includes the basal ganglia, caudate nucleus and thalamus (Graybiel, 2005). These findings suggest that abnormal reward processing seen in negative symptoms may be driven by grey matter volume loss in these areas.

Despite there being no abnormality associated with a single area of the brain responsible for depression, several morphological changes have been observed in those who are depressed (Oakes et al., 2017). Untreated patients with depression show decreases in hippocampal volume compared to those treated (Sheline et al., 2003), particularly in the left hippocampus (Frodl et al., 2008). Moreover, there has also been volumetric loss in the left anterior and subgenu part of the cingulate cortex medial

prefrontal cortex and amygdala (Price and Drevets, 2010, Frodl et al., 2008, Hasler, 2010, Botteron et al., 2002), as well as the orbitofrontal cortex, ventrolateral prefrontal cortex, parahippocampal gyrus and temporopolar cortex (Bowen et al., 1989, Lyoo et al., 2004, Oakes et al., 2017). One review by Drevets et al. (2008) found that grey matter volume in the hippocampus, posterior cingulate, subgenual anterior cingulate and pregenual anterior cingulate was reduced. Given the overlap of areas affected by depression and negative symptoms (such as the cingulate gyrus and hippocampus), these areas may constitute the neural basis of depression and negative symptoms.

1.7 Immune system in schizophrenia and depression

1.7.1 What is inflammation?

Inflammation is the body's response to harmful stimuli (such as pathogens). Immunity is defined as protection from infection (Ferrero-Miliani et al., 2007). The immune system is the primary biological system that provides the line of defence against infection and is considered highly organised and complex. It is made up of several cells that help co-ordinate and maintain it. The immune system has traditionally been conceptualised as being composed of two arms; the innate immune system and the adaptive immune system (Parkin and Cohen, 2001). The response of the immune system to infection or other threats gives rise to inflammation.

1.7.1.1 Innate immune system

The innate immune system is the older and less sophisticated (in terms of its ability to adapt to pathogens) of the two arms of the immune system and makes up the first line of defence against pathogens (Upthegrove and Barnes, 2014). The cellular component of the innate system includes the following:

- *Dendritic cells*: these bridge the innate and adaptive immune system arms.
- *Natural killer cells:* Activation of the 'killer activation receptor' initiates the production of cytokine molecules from the cell. Activation of the 'killer inhibition receptor' inhibits this action.
- *Granulocytes:* These can be further broken down into:
    I. *Neutrophils:* These are mobilised by several stimuli (such as cytokines) and contribute to inflammation.
    II. *Macrophages:* These play a role in phagocytosis and initiate the production of cytokines through the stimulation of lymphocytes and other immune cells. Microglia are macrophages that reside in the brain (Frick et al., 2013). This group of cells can be differentiated into two functional states:
        i. M1 – Macrophages that produce pro-inflammatory cytokines (such as IL-1, IL-6, IL-12 and TNF-α).
        ii. M2 – Macrophages that inhibit pro-inflammatory cytokines by producing immune-regulatory cytokines (such as IL-10, TGF-β).

III. *Basophils and Mast cells:* these exert their effects on the immune response by binding with components of the compliment system and producing pro-inflammatory cytokines.

In addition to the cellular component of the innate immune system, there is a humoral component consisting of:

- *Acute phase proteins:* These are a class of proteins that respond to infection. An example of this is the C-reactive protein (CRP). CRP is an opsosnin (a molecule that enhances phagocytosis by marking an antigen) that is also a protein that is used to measure acute and chronic inflammation. An acute phase response occurs as a result of increases in TNF-α and IL-6, which stimulates the liver to produce more CRP (Pepys and Hirschfield, 2003).
- *The compliment cascade:* this is a process whereby pathogens are destroyed by phagocytic cells.
- *Cytokines:* Cytokines are low molecular weight proteins (Shachar and Karin, 2012) that sub serve cellular communications and are integral in the controlling of both arms of the immune system. Cytokines are particularly important in the stimulation, differentiation, proliferation and function of immune cells (reviewed in Upthegrove and Barnes (2014), as well as regulation of the blood brain barrier (reviewed in Muller and Ackenheil (1998). Cytokines are responsible for the activation of microglia in brain and are also released from microglia in the brain with a variety of downstream effects. They hold a vital role in controlling inflammation. Cytokines are involved in both producing inflammation, via pro-

inflammatory cytokines (such as IL-1β, IL-2, IL-6, TNF-α, and IFN-γ) and reducing inflammation via anti-inflammatory cytokines (such as IL-4, IL-5 and IL-10) (Najjar et al., 2013). The activation of pro-inflammatory cytokines indicates macrophage (M1) activation (Miller et al., 2011). Cytokines can be categorised in accordance to their cell of origin; interleukins (IL): these influence leucocytes and lymphocytes. These are produced by lymphocytes, and m*onocytes* which are produced by activated mononuclear phagocytes.

A summary of the cytokines altered in schizophrenia and depression can be found below (see table 3).

Table 3: Some of the relevant cytokines to schizophrenia, their function and their action (pro- or anti-inflammatory). Adapted from Upthegrove et al. (2014a)

Cytokine	Function	Pro/anti-inflammatory
Interleukin-1 (IL-1)	Acts on several cell types such as T-cells, B-cells, and monocytes. Stimulates neutrophil production.	Pro-inflammatory
Interleukin-2 (IL-2)	Stimulates proliferation and differentiation of T and B-cells and activates NK cells.	Anti-inflammatory
Interleukin-4 (IL-4)	Promote T-cell differentiation; growth and differentiation of B-cells.	Anti-inflammatory
Interleukin-6 (IL-6)	Regulates B and T-cell function; induces inflammation and the acute response.	Pro-inflammatory
Interleukin-8 (IL-8)	Activates neutrophils and attract basophils and some lymphocytes.	Pro-inflammatory
Interleukin-10 (IL-10)	Enhances proliferation of B-cells and mast cells. Antagonises generation of the T_H1 cells.	Anti-inflammatory
Interleukin-12 (IL-12)	Induces differentiation of T_H1 subset of helper T-cells, enhances NK and cytotoxic T-cell activity.	Pro-inflammatory
Transforming growth factor (TGF-β)	Inhibits other cytokines such as IL-12.	Anti-inflammatory
Tumour necrosis factor (TNF-α)	Regulates immune cells and is a trait inflammatory cytokine.	Pro-inflammatory

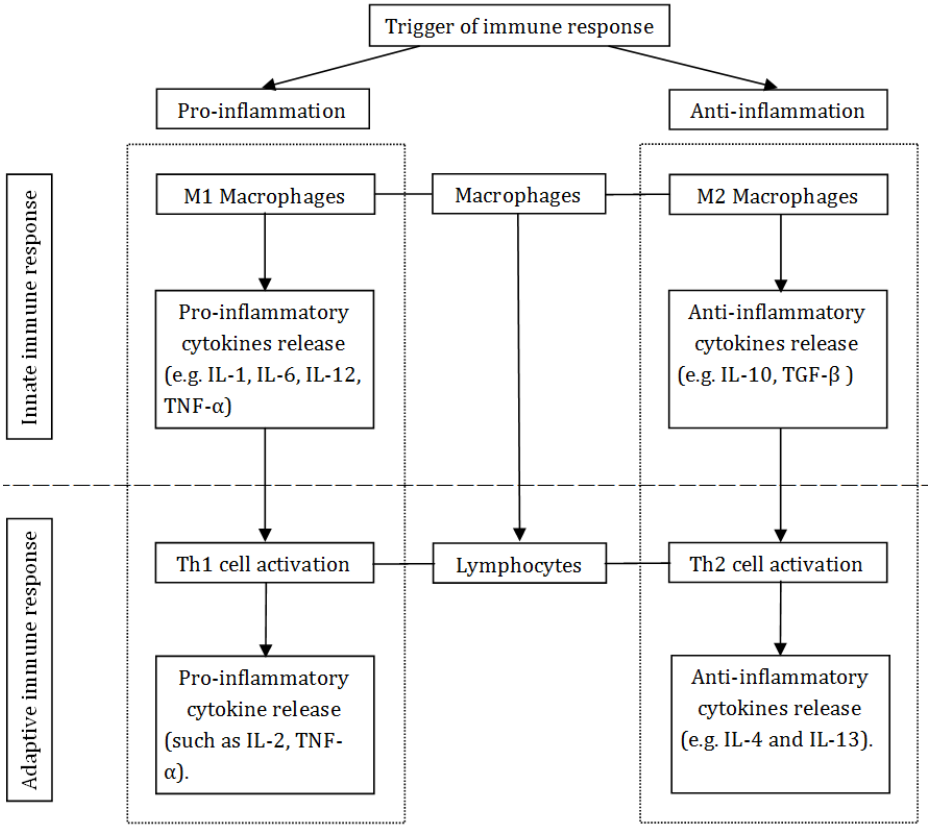
1.7.1.2 **Adaptive immune system**

This arm of the immune system involves immunological memory and re-exposure to previous immunological insults (such as from pathogens - reviewed in Upthegrove and Barnes (2014) and Khandaker et al. (2015) and are mediated by antibodies. Antibodies are a protein that are produced to counteract a specific antigen and are produced by B-lymphocytes. Any responses from this arm are specific to the pathogen and increase with intensity following re-exposure. However, activation of this arm is usually following a period of innate activation. There are two main components that form this arm; the

humoral and cellular components. The humoral components of the immune systems' adaptive arm consist of cytokines and antibodies. The cellular component is made up of lymphocytes, of which there are two kinds; B-lymphocytes (B-cells) and T-lymphocytes (T-cells). T-cells can be further broken down into:

I. *T-helper 1 (T_h1):* These stimulate pro-inflammation by activating pro-inflammatory cytokines (such as IL-2 and TNF-α).
II. *T-regulatory (Treg):* Treg cells regulate the immune system and have an immunosuppressive action on the immune system.
III. *T-helper 2 (T_h2):* These produce anti-inflammatory cytokines (e.g. IL-4 and IL-13).

Figure 6: The flow of activation of macrophages in the immune response relevant to schizophrenia, and the relationship between the macrophages and lymphocytes in the innate and adaptive immune system.



1.7.2 The immune system, schizophrenia and depression

1.7.2.1 What is the evidence for immune activation in schizophrenia?

1.7.2.1.1 Epidemiological studies

Associations between the immune system and the pathology of schizophrenia have been well documented (Reus et al., 2015, Leza et al., 2015) for nearly 100 years. In 1926, Menninger described 200 cases of post-influenza psychosis, some of which were similar to dementia praecox (Menninger, 1926). Smith and Maes (1995) suggested that

schizophrenia may be the result of initiated immune pathways via activated macrophages and T-lymphocytes, which triggers the release of pro-inflammatory cytokines, thus producing a neurotoxic effect. This theory has received considerable support (Brown, 2011), from several lines of evidence. Infection exposure during foetal development has been identified as a risk factor for schizophrenia. Canetta et al. (2014) found elevated gestational CRP concentrations associated with offspring schizophrenia. A review by Torrey et al. (1997) and meta-analysis by Davies et al. (2003) concluded that children born at a time of heightened risk of infection (winter/early spring), and influenza during pregnancy, have been associated with a risk for schizophrenia (Bradbury and Miller, 1985, Brown et al., 2004, Fineberg and Ellman, 2013). However, a meta-analysis of eight studies (n = 20,017) by McGrath and Welham (1999) was unable to replicate these findings in southern hemisphere countries, suggesting other factors (such as low vitamin D exposure) may influence a risk for schizophrenia. Finally, individuals with an autoimmune condition have been found to have a higher risk of schizophrenia (Benros et al., 2011, Eaton et al., 2006). These findings suggest that the immune system may be implicated in the pathology of schizophrenia.

1.7.2.1.2 Circulating cytokines

Activation of pro-inflammatory cytokines occurs as part of the immune response (Na et al., 2014) and is important in co-ordinating this response (Upthegrove and Barnes, 2014). The finding of elevated pro-inflammatory cytokines, such as IL-1β IL-6, IL-8 and TNF-α, and acute phase proteins (such as CRP), which aid neuro-inflammation, have been replicated in schizophrenia (Potvin et al., 2008, Lin et al., 1998, Zanardini et al., 2003,

Laskaris et al., 2016, Wium-Andersen et al., 2014, Miller et al., 2014). Furthermore, a reduction of anti-inflammatory cytokines is also related to schizophrenia symptomology (Khandaker and Dantzer, 2016, Noto et al., 2015a). Moreover, childhood IL-6 concentrations is associated with a two-fold increased dose-response risk of future schizophrenia (Khandaker et al., 2014).

Pro-inflammatory markers (such as IL-6, TNF-α and CRP) appear higher in antipsychotic-naïve patients (Upthegrove et al., 2014a) and patients in acute relapse (Miller et al., 2011). Mondelli et al. (2015a) have demonstrated that treatment-resistant first episode psychosis patients show higher levels of IL-6 and IFN-γ compared to those who responded to treatment. They also found that these cytokines remained raised after three months of treatment in the treatment-resistant group, compared to those who responded to antipsychotic medication.

Indeed, antipsychotic and antidepressant medication has been shown to decrease levels of cytokines (such as IL-6 and TNF-α) (Miller et al., 2011) suggesting that medication has some influence on the immune system (Saunders and Muchmore, 1964). Moreover, body mass index, smoking status, and illicit substance use have all been shown to exert some effect on the immune system and can influence cytokine concentrations (Koola, 2016).

1.7.2.1.3 Auto-immunity

The role of the N-methyl-D-aspartate (NMDA) receptor in schizophrenia has been discussed in the glutamate hypothesis of schizophrenia. Lennox et al. (2017) examined a

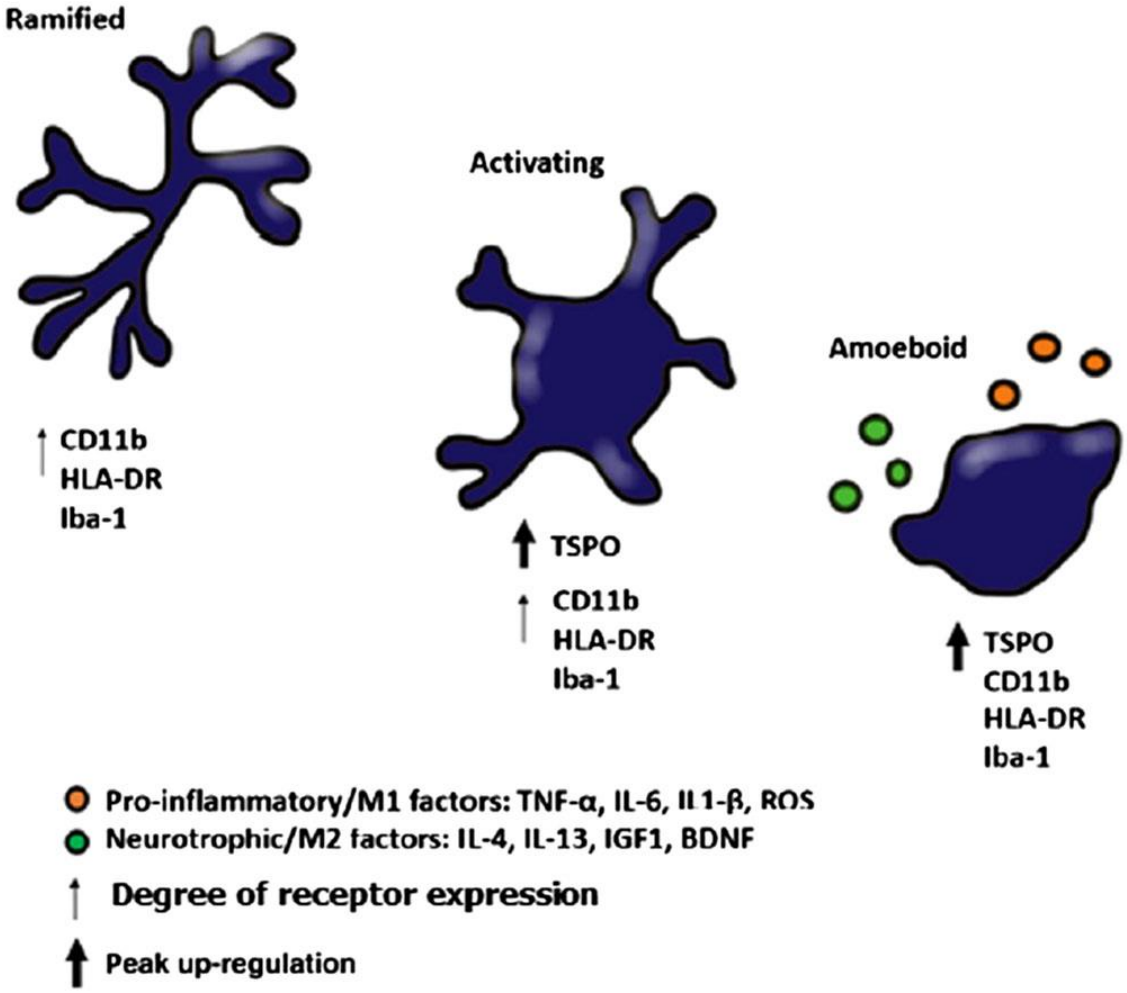
series of neuronal cell surface antibodies (anti-NMDAR, anti-LGI1, anti-CASPR2, anti-$GABA_AR$ and anti-AMPAR) within the serum of 228 first episode psychosis patients, and of these patients, 9% had one or more of these antibodies with NMDAR being exclusively present in schizophrenia patients and not in controls. Anti-bodies against the NMDA receptor have been reported in some cases of first episode psychosis and schizophrenia, as reviewed by Deakin et al. (2014). Indeed, some of these anti-bodies have been associated with NMDA-receptor encephalitis which may initially present with psychotic symptoms. Confirmation of the role of antibodies against the NMDA receptor comes through removal of these antibodies, which leads to clinical improvement. Mohn et al. (1999) found that mice expressing abnormal levels of the NMDAR subunit displayed behaviour similar to schizophrenia (e.g. deficits in social and sexual behaviour), that was ameliorated by treatment with antipsychotic medication.

1.7.2.1.4 Activated microglia

Microglial cells represent the first line of defence in the immune system (Kettenmann et al., 2011) and are present in the brain in two states: ramified and amoeboid (see figure 7). Under healthy conditions, ramified microglia scan the brain for pathogens (Nimmerjahn et al., 2005), where upon discovery of a pathogen, change shape and function to its activated state. When activated, the cell body of the microglia swells up to enhance migration to a pathogen site. Once activated, microglial cells phagocytose cellular debris and trigger the production of cytokines contributing to inflammation causing secondary neuronal degeneration and white matter loss, decreased neurogenesis and synaptic dysfunction (Laskaris et al., 2016). This is particularly prominent in the

frontal and temporal lobes (Wierzba-Bobrowicz et al., 2005), potentially implicating dysfunction of both M1 and M2 pathways.

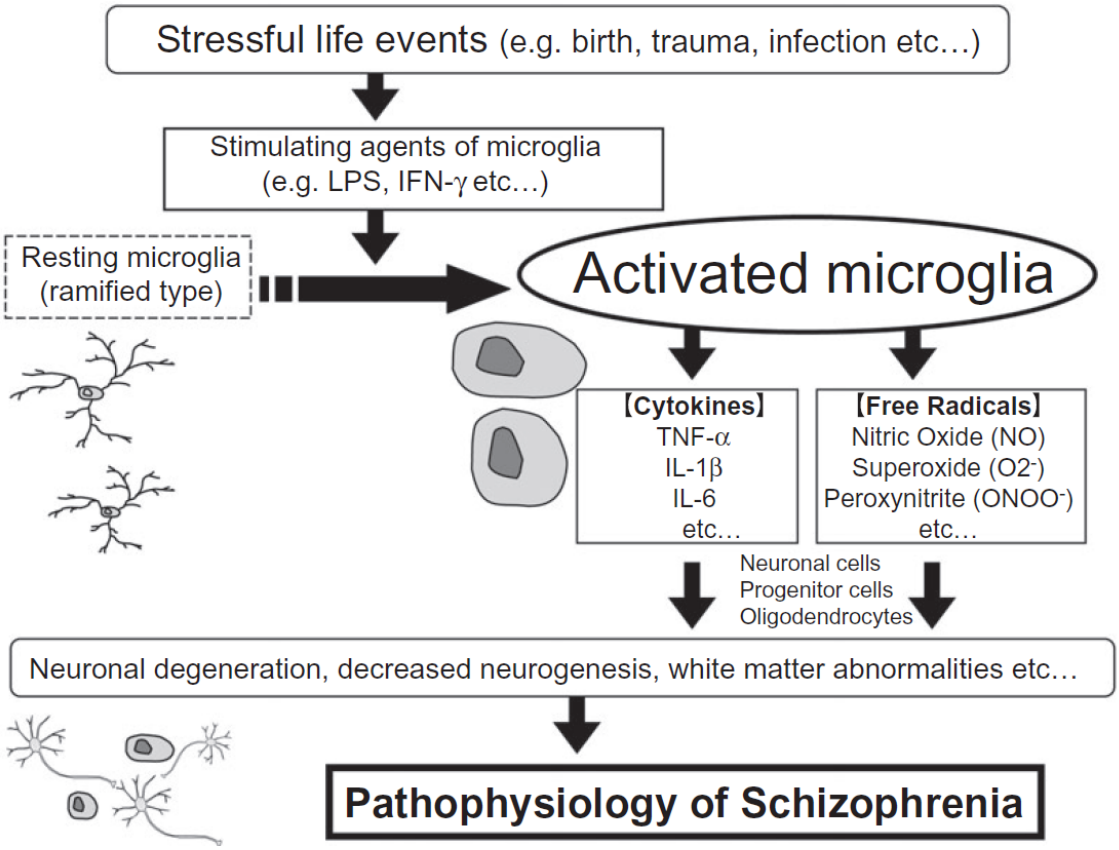
Figure 7: The transitioning state of microglial activation from ramified state to amoeboid (Kettenmann et al., 2011).



Abnormal microglial functioning has been suggested to be the result of the abnormal neural pruning, prenatal complications leading to an immune reaction following ischemic brain trauma, or the result of immune dysfunction associated with schizophrenia (Munn, 2000). The microglial hypothesis for schizophrenia (Monji et al.,

2009, Munn, 2000) postulates that pro-inflammatory cytokines are released or elevated following a stressful life event (such as trauma or prenatal infection) and activate microglia. Activation of microglia then triggers the further release of several pro-inflammatory cytokines (such as IL-6, IL-1β and TNF-α) and free radicals (such as nitric oxide, superoxide and peroxynitrate). The release of these inflammatory agents causes neuronal degeneration, decreased neurogenesis and white matter abnormalities, which in turn leads to the pathophysiology of schizophrenia (see figure 8) (Monji et al., 2009).

Figure 8: The cascade of events as suggested in the microglial hypothesis (Monji et al., 2009).



Activated microglia also leads to chronic neuro-inflammation resulting in a disturbance of neurotransmitters such as γ-aminobutyric acid (GABA), dopamine and

glutamate (Monji et al., 2009). Sustained increase in pro-inflammatory cytokines raises kynurenic acid concentration (Erhardt et al., 2007, Schwieler et al., 2015), which acts as an NMDA receptor antagonist that along with glutamatergic hypofunction (Erhardt et al., 2007), may be the cause of cognitive dysfunction and positive symptomology in schizophrenia. Furthermore, chronic neuro-inflammation has also been linked with dopamine dysregulation, particularly the mesolimbic dopaminergic neurons (Meyer and Feldon, 2009). These all may contribute to the neurobiology associated with schizophrenia.

There have been several lines of evidence to support the microglial hypothesis. Post-mortem studies have found aberrant activation of microglial cells in a subset of individuals with schizophrenia (Monji et al., 2009), however, others have failed to replicate these findings (Kahn and Sommer, 2015). Bloomfield et al. (2016) found, using PET imaging analysis, elevated microglial activity in individuals with schizophrenia and those in the ultra-high-risk category of schizophrenia, indicating microglial activation prior to onset of illness. Furthermore, a meta-analysis by Najjar and Pearlman (2015) concluded that neuro-inflammation was associated with white matter pathology in those with schizophrenia, suggesting that activated microglia may be held to account for some cases of schizophrenia, but not all cases.

1.7.2.1.5 Post-mortem brain changes in schizophrenia

Post-mortem brain changes in schizophrenia have suggested inflammation in schizophrenia, specifically microglial activation in white matter regions, as shown by an

animal model by Bauman et al. (2014). One systematic review by van Kesteren et al. (2017) of forty-one articles with a sample size of 783 participants with schizophrenia and 762 controls, found of the papers measuring microglial activation (n = 11), there was an increase in microglia in those with schizophrenia. These studies add to the growing evidence base for inflammation in schizophrenia, specifically microglial activation.

1.7.2.2 **What is the evidence for immune activation in depression?**

1.7.2.2.1 Circulating cytokines

Behaviours such as loss of interest, disturbed sleep, low mood and cognitive dysfunction are observed in depression. These behaviours can be mediated by aberrant cytokine activity and have been termed cytokine-induced sickness (Dantzer et al., 2008). The cytokine hypothesis (Maes, 1995) suggests depression is a consequence of altered cytokine levels causing neuro-inflammation (Vidrih et al., 2012). Findings have consistently shown an increase in levels of pro-inflammatory cytokines and acute phase proteins in major depressive disorder (MDD). These cytokines include sIL-2R, IL-6, TNF-α and CRP, which are elevated in acute depression, and normalise upon recovery (Young et al., 2014, Valkanova et al., 2013, Haapakoski et al., 2015, Liu et al., 2012, Goldsmith et al., 2016, Howren et al., 2009) which have been replicated in longitudinal studies (Khandaker et al., 2014, Zalli et al., 2016). Moreover, dysregulation of the inflammatory response is related with depressive symptom severity (Zalli et al., 2016). These findings lead to the conclusion that sustained activation of the innate immune system results in an increase in the number of pro-inflammatory cytokines and acute phase proteins, which

cause MDD (Maes, 1995, Smith, 1991). Indeed, Harrison et al. (2009) have demonstrated that healthy controls that have had artificially induced inflammation, experience depressive symptoms and impaired cognitive performance that was associated with increased IL-6. This suggests that occurrence of depressive symptoms may be influenced by the presence of pro-inflammatory cytokines and subsequent neuro-inflammation.

The involvement of the immune system in depression has also been highlighted by treatment response. Agents that exert their effects on the immune system, such as minocycline and celecoxib (Muller et al., 2006) have been found a useful treatment for depression (Soczynska et al., 2012), thus implicating the immune system in the underlying pathology of depression.

Cytokines and serotonin: Cytokines have been found to act on both 5-HT and DA neurotransmitter systems. Antidepressant medication, particularly selective serotonin reuptake inhibitors (SSRI's), contains immunoregulatory properties, thus decreasing production of pro-inflammatory cytokines, and increasing anti-inflammatory cytokines (Maes et al., 1999, Xia et al., 1996, Sutcigil et al., 2007). Inflammatory cytokines have been shown to significantly increase the turnover of 5-HT (Felger and Lotrich, 2013) which result in an increase in depressive-like behaviours (Dunn et al., 2005) in experimental animals. Given that the 5-HT system has been implicated in depression, and SSRI's have been effective in reducing depressive symptoms (Sutcigil et al., 2007), inflammatory markers may be implicated in the 5-HT system dysfunction, thus contributing to depressive symptomology.

Cytokines and the Hypothalamic-pituitary-adrenal axis: The hypothalamic-pituitary-adrenal axis (HPA) activity has been increased in those with depression (Varghese and Brown, 2001). A dysfunctional inhibitory feedback mechanism has been suggested to cause this HPA axis hyperactivity (Young et al., 1991b). Corticosteroids are synthesised in the adrenal cortex. Corticosteroids, particularly cortisol, regulate the immune response and cytokine production (Vidrih et al., 2012). The HPA axis is activated by pro-inflammatory cytokines, inhibiting the negative feedback corticosteroids in the HPA axis. Both corticosteroidal dysregulation and pro-inflammatory cytokine production result in HPA axis dysregulation (Sapolsky et al., 1987, Miller et al., 1999). Prolonged activation of corticosteroids could result in decreased sensitivity of the hypothalamus and pituitary causing a reduction of negative feedback (Miller et al., 1999). However, given that between 30 and 50% of patients with MDD do not show HPA axis hyperactivity (de Beaurepaire, 2002), cytokine induced dysregulation of the HPA axis might not account for all of the neurobiological correlates underpinnings of MDD.

Microglial activation: Over-expressed and under-expressed microglia has been associated with depression through different molecular pathways (Singhal and Baune, 2017). A meta-analysis of twenty-four studies by Dowlati et al. (2010) found that microglial over-expression may trigger the release of several pro-inflammatory cytokines (such as IL-6, and TNF-α). Moreover, under-expression may lead to hippocampal degeneration (Tong et al., 2017). Increased TNF-α brought about by microglial activation, caused hippocampal apoptosis (Cacci et al., 2005), which has been found in unipolar depression (Videbech and Ravnkilde, 2004). Impaired hippocampal genesis has been suggested to underlie some depression (Jacobs et al., 2000), and a review by Sierra et al.

(2014) has shown activated microglia suppresses hippocampal neurogenesis. Therefore, these findings suggest that hippocampal degeneration may be brought about by microglial activation.

Auto-immunity: A meta-analysis of nine studies by Pearlman and Najjar (2014) found individuals with both schizophrenia and depression are three times more likely to have elevated N-Methyl-D-aspartate receptor (NMDAR) antibodies compared to healthy controls. A review by Postal and Appenzeller (2015) highlight the role of antibodies in the pathophysiology of depression. One study by Hirohata et al. (2014) found antibodies reactive with the NMDA receptor NR2 (which is expressed in the hippocampus and cortex) were higher in those with depression. The presence of the NMDA receptor antibodies in depression is supported by treating treatment-resistant depressive patients with ketamine, an NMDA non-competitive antagonist (Sanacora et al., 2008).

1.7.3 The immunological basis of depressive symptoms in schizophrenia

Evidence suggests that there are also commonalities in the markers of neuro-inflammation in both schizophrenia and depression. Concentrations of IL-6, IL-18, sIL-2R and TNF-α have been shown to be significantly higher in patients with depression and schizophrenia compared to healthy controls (Sasayama et al., 2013, Al-Hakeim et al., 2015), with levels highest in the schizophrenia group. Increased concentrations of IL-6, IL-10 and TNF-α were also replicated using a medication naïve group, depression group and healthy controls (Noto et al., 2011). Khandaker et al. (2014) also found that increased childhood concentrations of IL-6 was associated with a risk for developing depression and

psychosis in adulthood, suggesting that there may be a common underlying pathway that leads to depression and schizophrenia.

Furthermore, minocycline has been effective in treating depressive symptoms in those with psychotic depression (Miyaoka et al., 2012). The similarities in neuro-inflammatory markers and treatment response in both depression and schizophrenia imply that there may be a common underlying pathway that leads to these disorders (as well as bipolar disorder) which is based on immune dysfunction (Goldsmith et al., 2016) or that schizophrenia may be immunologically primed for increased depression (Anderson et al., 2013).

Levels of some cytokines may vary depending on the clinical state of those with schizophrenia (Miller et al., 2014, Baumeister et al., 2014, Miller et al., 2011). These maybe conceptualised as state markers of immune dysfunction in schizophrenia inflammation. Concentrations of other cytokines are persistently abnormal regardless of changes in clinical status and maybe trait immune markers of risk for depression *(or trait markers of possible immune processes in depression)* are therefore conceptualised as trait markers of neuro-inflammation (Miller et al., 2014). State-based markers generally consist of IL-6, TGF-β, IL-1β and CRP (Miller et al., 2014, Kirkpatrick and Miller, 2013) and may fluctuate throughout the course of illness with higher concentrations being associated with the acute exacerbations of schizophrenia compared to healthy controls and stable medicated patients (Kirkpatrick and Miller, 2013). Trait markers comprise TNF-α, sIL-2r, and IL-12. Concentrations of these inflammatory markers appear constantly raised in individuals with schizophrenia compared to healthy controls and are independent of clinical status.

Depression has been associated with elevated concentrations of the state markers IL-6 and IL-1β, associated with severity of illness (Howren et al., 2009).

Some negative symptoms may be stable and enduring, particularly when primary. The distinction between depressive and negative symptoms in schizophrenia may lie in the state-trait distinction of inflammatory markers, with depressive symptoms being associated with state-related markers and negative symptoms being linked with trait-related markers. However, many of the previous studies assessed severity of schizophrenia as a global score (e.g. total PANSS score) rather than focusing on negative symptoms such as Noto et al. (2011). Therefore, it cannot be determined if inflammation is associated with any particular group of symptoms within schizophrenia (such as negative symptoms or depression). Moreover, much of the previous research examining inflammatory markers in MDD and schizophrenia have focused on individuals with MDD and individuals with schizophrenia, and not depressive symptoms within schizophrenia, thus limiting any conclusions that may be based on these findings for depressive features in schizophrenia.

1.7.4 What is the evidence against inflammation specific to negative symptoms and depression in schizophrenia?

The previous two sections have outlined the evidence in support of immune activation underpinning depression and schizophrenia. However, inflammation may not be specific to negative symptoms in schizophrenia. Müller et al. (1997) identified a significant relationship between CSF soluble IL-6R and paranoid auditory hallucinations,

suggesting that immune dysfunction may be associated with the positive syndrome, as well as negative and depressive symptoms. Despite these findings, much of the previous research on immune dysfunction in schizophrenia have not focused on specific sets of symptoms, which may imply that inflammation may not be associated with any specific symptom within the diagnostic category of schizophrenia, but indicative of overall illness burden. Moreover, much of the previous work has focused on chronic patients with schizophrenia (Potvin et al., 2008), which may experience a different immunological profile to those in their first episode. Therefore it is unclear whether inflammation is a cause, or a consequence, of pathological changes seen in schizophrenia.

The association between inflammation and other psychiatric disorders has been documented, with evidence suggesting that a link exists between inflammation and anxiety (Rossi et al., 2012), post-traumatic stress disorder (Eraly et al., 2014) and autism (Brown et al., 2014). These findings suggest that inflammation may not be specific to depression and negative symptoms but common across psychiatric disorders. Finally, there are several other lifestyle factors that can impact on inflammation, such as obesity and smoking status, which may impact on immune system functioning.

1.7.5 Studies examining anti-inflammatory medication in schizophrenia

Several anti-inflammatory drugs have been used to treat schizophrenia thus aiming to reduce symptoms via reducing inflammation. Cyclooxygenase – (COX-2) inhibitors, which are a type of non-steroidal anti-inflammatory drugs, have been found to reduce depression which may be through its action on the immune system and its

influence on serotoninergic activity. Muller et al. (2006) found that depression scores were reduced in depressed patients taking reboxetine plus placebo versus depressed patients taking reboxetine plus celecoxib (a type of COX-2 inhibitor). These findings not only add to the growing body of evidence indicating immune dysfunction underpinning depression, but also suggest that the immune system may be a viable target for the treatment of depression.

Celecoxib has also been trialled in schizophrenia. A randomised double-blind controlled trial of celecoxib and risperidone dual therapy versus risperidone monotherapy found a reduction in all three subsets of PANSS scores in both groups but the reduction was greater in the celecoxib group (Muller et al., 2002). Furthermore, a meta-analysis of 626 inpatients across eight RCT's by Zheng et al. (2017) found that celecoxib was more effective in reducing negative symptoms than placebo. These findings suggest that the immune system is a viable target for treatment, and immune dysfunction is not just an epiphenomenon.

Minocycline is a commonly used tetracyclic anti-biotic and anti-inflammatory agent. It has putative neuroprotective and anti-apoptotic properties. The neuroprotective properties of minocycline have been demonstrated in stroke (Fagan et al., 2011) and Parkinson's disease (Du et al., 2001), and although neurodegeneration is not prominent in post mortem studies in schizophrenia, there is still subtle grey matter volume loss (Cahn et al., 2006) that is associated with negative symptoms (McKechanie et al., 2015). Minocycline may help reduce the loss of neural branching and may help prevent the loss of astroglial and oligodendroglial cells which are essential for neuronal function. In

support of the microglial hypothesis of schizophrenia, minocycline has evidence of being effective in reducing microglial activation, as well as offering neuroprotection in humans (for a review, see (Frick et al., 2013, Domercq and Matute, 2004) and animals (for a review, see Domercq and Matute, 2004). However, there is only one report of the effect of minocycline on circlating cytokines. Kloppenburg et al. (1995) administered minocycline to rhumatiod artheritis patients and found a reduction in IL-6 concentrations during six months of treatment with minocycline.

Therapeutic options that modulate microglial function have been found to offer benefit in reducing symptoms of schizophrenia when administered adjunctive to antipsychotic medication (Kelly et al., 2011, Miyaoka et al., 2007, Miyaoka et al., 2008). This finding was found to be particularly pronounced in improving negative and cognitive symptoms in schizophrenia (Levkovitz et al., 2010, Chaudhry et al., 2012), when administered adjunctive to antipsychotic medication. Thus, minocycline may help reduce negative symptoms which could be through its action on activated microglia.

2. The relationship between negative symptoms and depression in schizophrenia – A systematic review

Published article

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CRK and RU carried out the systematic review and tabulated the results. CRK produced the first draft of the manuscript. All authors contributed to revisions of the manuscript and development of ideas. Authors have approved the final manuscript.

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2.1 **Abstract**

Background: Traditionally, depression and schizophrenia have been considered unrelated illnesses with distinct nosology and symptomology. However, in recent years there has been increasing interest in the occurrence of depression in schizophrenia, how depressive and negative symptoms may relate, and proposed dimensional models of psychosis challenging the Kraepelinian dichotomy.

Aims: Provide an update on the evidence base for the nature of the relationship between negative symptoms and depressive features in people with schizophrenia and propose new models that reflect their complex relationship.

Method: A systematic review following PRISMA guidelines. 2210 articles were identified from EMBASE, PsychInfo and Medline and a further 2 articles were hand searched from references. 27 met inclusion criteria and were included in the review.

Results: In schizophrenia, primary evidence suggests symptoms of low mood, suicidal ideation and pessimism have more specificity for depression whereas alogia and blunted affect may have more specificity as negative symptoms. Anhedonia, anergia and avolition may be common to both.

Conclusions: It may be possible to further distinguish depressive features from negative symptoms in schizophrenia when detailed phenomenology is considered. However, in a proposed dimensional model, these two domains continue to share certain phenomena, highlighting their close relationship.

Key words: Depression, schizophrenia

Summations
- Symptoms such as low mood, suicidal ideation and pessimism are cited within a depressive domain, whereas symptoms such as alogia and blunted affect appear to indicate negative symptoms. Anhedonia, anergia and avolition occur across both domains.
- Detailed understanding of underlying phenomenology may provide potential treatment targets as well informing future research assessing underlying aetiological mechanisms.

Considerations
- Research methodologies of included studies were highly variable, leaving interpretation of the results open to bias.
- Future studies should focus on employing appropriate scales to assess depression and consider the negative symptoms as two dissociable sub-domains (avolition-apathy and expressive-deficit).

2.2 Introduction

Schizophrenia has been conceptualised as a neuro-developmentally mediated, cognitive illness marked by a more unfavourable outcome than affective disorders (Kahn and Keefe, 2013). Positive and negative symptoms and cognitive dysfunction are core features of the illness, however there is a phenomenological overlap between schizophrenia and mood disorders (Murray et al., 2005); mood symptoms are common features in prodromal psychosis and established schizophrenia (Upthegrove et al., 2016), while psychotic symptoms frequently occur with severe mood disorders. Such observations, as well as burgeoning biological evidence (Craddock and Owen, 2010, Koutsouleris et al., 2015), challenge the Kraepelinian dichotomy. One area of perhaps neglected phenomenological enquiry is negative symptoms; negative symptoms may present variously as a loss of motivation to act and a lack of the elements that make up the normal repertoire of social and emotional responsiveness (Barnes, 1993), yet these are also commonly seen in depression (Malla, 1995).

Andreasen (1982) characterised negative symptoms as consisting of affective blunting or flattening, alogia, anhedonia/asociality and avolition/apathy and attention; although attentional deficits may indicate cognitive impairment rather than a manifestation of negative symptoms. Much of the earlier research exploring negative symptoms has assumed that they form one discrete syndrome. However, factor analyses have isolated two sub-domains within negative symptoms (Messinger et al., 2011, Liemburg et al., 2013), namely avolition-amotivation and expressive deficits. The avolition-amotivation sub-domain is considered to be formed of the symptoms; avolition,

asociality and anhedonia, whereas the diminished expression sub-domain comprises blunted affect and alogia (Foussias et al., 2014, Kaiser et al., 2011, Kaiser et al., 2016).

Depression is a mood disorder that is characterised by low mood and anhedonia (Beck, 1967). Affective symptoms have been recognised as occurring commonly in non-affective psychosis, in 50% - 80% of patients at some point during their illness (Upthegrove et al., 2010, Koreen et al., 1993). Previously, Siris et al. (1988) found that half of individuals with post-psychotic depression also met criteria for negative symptoms, implying a syndromal overlap between depressive and negative symptoms. However Addington et al. (1990) designed the Calgary Depression Scale for Schizophrenia to assess depression separately from negative symptoms and also from positive symptoms and extrapyramidal symptoms such as parkinsonism.

Previous models have conceptualised depression and negative symptoms as belonging to distinct and separate clinical syndromes (Andreasen and Olsen, 1982, Carpenter et al., 1985, Crow, 1980, Romney and Candido, 2001, Lindenmayer and Kay, 1989). Within a dimensional approach to psychoses, a model of overlapping domains with distinct presentations allows further exploration of the heterogeneity involved. Initially presented as three domains of reality distortion, psychomotor poverty and disorganization syndrome (Liddle, 1987), more recently mood symptoms have been included in five factor models; positive symptoms, negative symptoms, depression, mania and cognitive (van Os, 2008). These models of symptoms of psychoses allow for co-morbidity in more than one domain, with an individual patient presenting with varying degrees of symptom severity in each domain.

The exact nature of the relationship between depression and negative symptoms in a 'non-affective' psychotic illness such as schizophrenia remains uncertain. Identifying depression in schizophrenia and treating this symptom domain is essential given that depression in schizophrenia has been associated with poor prognosis and suicide (Upthegrove et al., 2016, Conley et al., 2007). Understanding this heterogeneity further is potentially important for the elucidation of the underlying aetiological mechanisms and the development of better, targeted, treatments.

2.2.1 Aim of the study

The aim of this systematic review was to provide a novel and up-to-date review of the evidence for the phenomenological co-occurrence of depression and negative symptoms in schizophrenia.

2.3 Methods

A systematic search and review following the PRISMA guidelines (Moher et al., 2009) was conducted. We limited our search to studies examining depression and negative symptoms in schizophrenia and excluded studies looking at other conditions. There were no limits put on the origin or date of a publication.

2.3.1.1 Inclusion criteria

1. Studies that aimed to report the relationship between negative and depressive symptoms in schizophrenia.

2. Studies that were published in English.

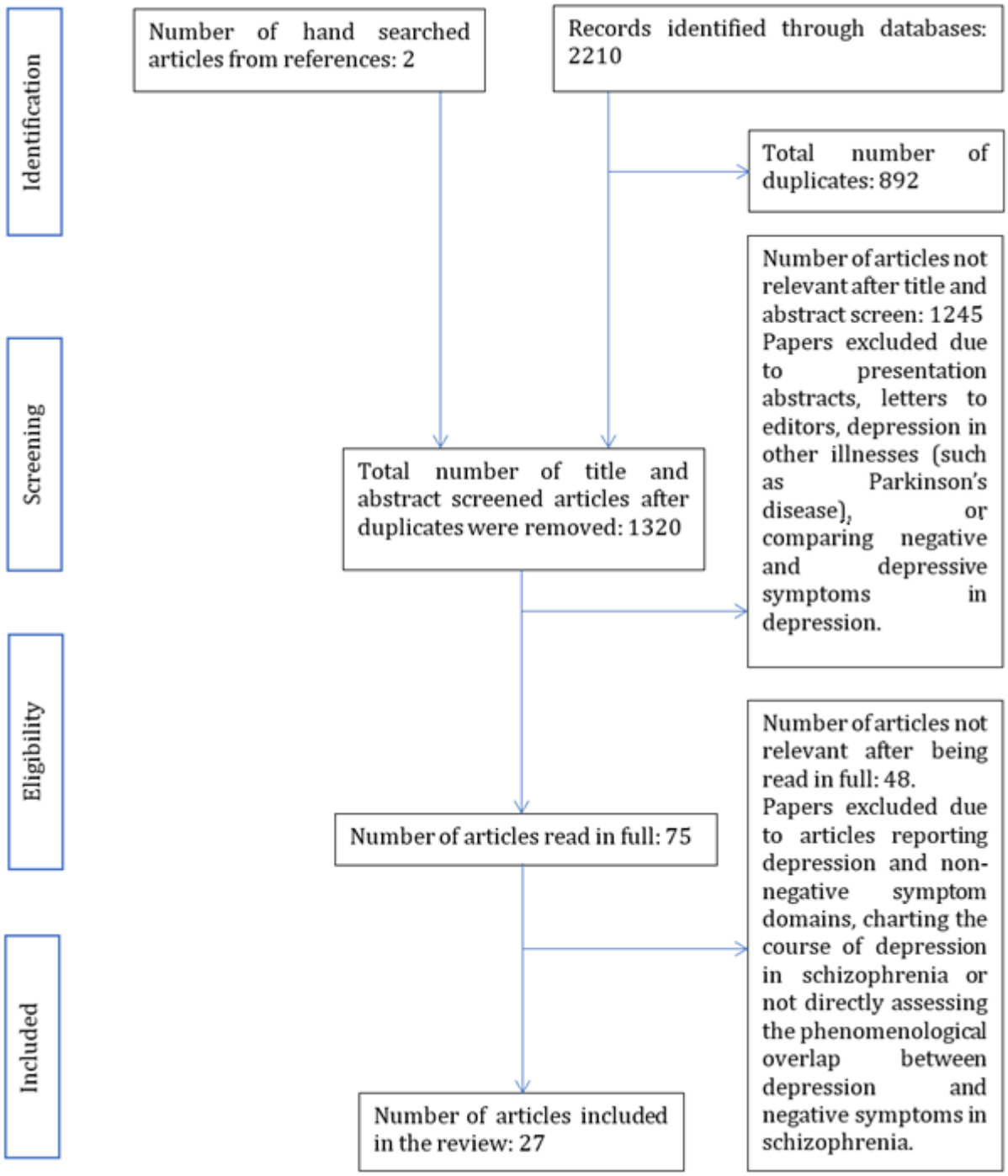
2.3.1.2 **Exclusion criteria**

1. Review articles, letters to editors and presentation abstracts
2. Papers that reported depression and only other (non-negative) symptom domains (e.g. positive symptoms and depression; social functioning, insight)

The following databases were searched on 05/01/2018: MEDLINE, PSYCHINFO, and EMBASE. The following search terms were used: "SCHIZOPHRENIA"; or "SCHIZOPHRENIA, CATACTONIC" or "SCHIZOPHRENIA CHILDHOOD" or "SCHIZOPHRENIA, DISORGANISED" or "SCHIZOPHRENIA, PARANOID" or "PSYCHOTIC DISORDERS" or "SCHIZOPHREN*" or "PSYCHOSIS" or "PSYCHOSES" or "FIRST EPISODE PSYCHOSIS" and "DEPRESSION" or "DEPRESSIVE DISORDER" or "MOOD DISORDERS" or "DYSTHYMIC DISORDER" or "DEPRESSIVE DISORDER, MAJOR" or "TREATMENT RESISTANT" or "DEPRESSION" or "AFFECTIVE DISORDER" and "NEGATIVE SYMPTOM" or "ANHEDONIA" or "APATHY" or "ALOGIA" or "ASOCIALITY" or "AFFECTIVE FLATTENING" or "BLUNTED AFFECT" or "AVOLITION" or "SOCIAL WITHDRAWAL" or "LOSS MOTIVAT*" or "LACK MOTIVAT*" (see appendix 1 for the full search strategy)

Two reviewers (CRK and RU) independently reviewed all the papers screened and agreed on which papers should be included in the review (see appendix 2).

Figure 9: Flow diagram of studies included in the systematic review



Results

2210 articles were identified. Following the removal of 892 duplicates, the titles and abstracts of the remaining 1320 articles were screened, and 75 were found to be potentially relevant. Of those 75 papers, read in full, 25 papers met the inclusion criteria and were eligible for the review. References cited in the papers were hand searched, which yielded two further eligible articles, bringing the total number of studies included to 27 (see Figure nine).

2.3.1.3 Study characteristics

Sampling

Thirteen papers reported on samples from inpatient units (Barnes et al., 1989b, Hafner et al., 2005a, Hafner et al., 2005b, Kibel et al., 1993, Muller. et al., 2002, Nakaya et al., 1998, Nakaya et al., 1997, Newcomer et al., 1990, Sax et al., 1996, Tapp et al., 2001, Koreen et al., 1993, Ragin et al., 1989, Bottlender et al., 2003) while nine of the studies examined patients in community settings (Baynes et al., 2000, Majadas et al., 2012, Pogue-Geile, 1989, Rocca et al., 2005, Whiteford et al., 1987, Upthegrove et al., 2010, Chiappelli et al., 2014, Zisook et al., 1999, Rossi et al., 2017). One study (Romney and Candido, 2001) included participants from both inpatient and outpatient units while the remaining four studies did not provide information on the source of the patient sample (Oosthuizen et al., 2002, Maggini et al., 2004, Kulhara et al., 1989, Tharyan and Kuruvilla, 1994). The study sample sizes ranged from 42 to 921 participants, with a total number of 3062.

2.3.1.4 **Rating scales used**

Depression scales

The majority of studies employed a single scale to assess symptoms of depression, with the most commonly used being either the Hamilton Rating Scale for Depression (Baynes et al., 2000, Koreen et al., 1993, Nakaya et al., 1998, Nakaya et al., 1997, Newcomer et al., 1990, Romney and Candido, 2001, Sax et al., 1996, Tapp et al., 2001, Whiteford et al., 1987, Tharyan and Kuruvilla, 1994). Six studies used the Calgary Depression Scale for Schizophrenia (CDSS) (Maggini et al., 2004, Majadas et al., 2012, Muller. et al., 2002, Rocca et al., 2005, Upthegrove et al., 2010, Rossi et al., 2017), and Five studies used the Beck Depression Inventory (Barnes et al., 1989b, Baynes et al., 2000, Pogue-Geile, 1989, Romney and Candido, 2001).

Negative symptom scales

The majority of studies used either Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1989) or the negative subscale of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) to assess negative symptoms; (Barnes et al., 1989b, Baynes et al., 2000, Kibel et al., 1993, Koreen et al., 1993, Kulhara et al., 1989, Maggini et al., 2004, Majadas et al., 2012, Muller. et al., 2002, Nakaya et al., 1998, Nakaya et al., 1997, Oosthuizen et al., 2002, Pogue-Geile, 1989, Rocca et al., 2005, Romney and Candido, 2001, Sax et al., 1996, Tapp et al., 2001, Upthegrove et al., 2010, Whiteford et al., 1987, Bottlender et al., 2003, Zisook et al., 1999, Tharyan and Kuruvilla, 1994). Five studies (Kulhara et al., 1989, Newcomer et al., 1990, Tapp et al., 2001, Zisook et al., 1999, Rossi et

al., 2017) used the negative subscale of the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). Romney and Candido (2001) rated anhedonia separately using Physical Anhedonia Scale (PAS) (Chapman et al., 1976) and Social Anhedonia Scale (SAS) (Eckblad et al., 1982).

2.3.1.5 **Study design and analysis**

The majority of studies employed a cross-sectional design (Barnes et al., 1989b, Baynes et al., 2000, Chiappelli et al., 2014, Hafner et al., 2005b, Hafner et al., 2005a, Kibel et al., 1993, Kulhara et al., 1989, Maggini et al., 2004, Majadas et al., 2012, Muller. et al., 2002, Pogue-Geile, 1989, Rocca et al., 2005, Romney and Candido, 2001, Sax et al., 1996, Whiteford et al., 1987, Rossi et al., 2017, Zisook et al., 1999). One study adopted a prospective design (Koreen et al., 1993). One study employed a combined prospective and retrospective study design (Upthegrove et al., 2010) and four studies implemented a prospective, longitudinal study design (Nakaya et al., 1997, Nakaya et al., 1998, Ragin et al., 1989, Zisook et al., 1999). One study reported on a cross-sectional and longitudinal arm (Bottlender et al., 2003). An experimental design was used by two studies included in the review; Oosthuizen et al. (2002) carried out an open-labelled trial and Tapp et al. (2001) conducted a double-blind experiment. Newcomer et al. (1990) used a retrospectively-drawn sample. In two studies (Chiappelli et al., 2014, Romney and Candido, 2001), a factor analysis was included as part of the data analysis.

2.3.1.6 **Medication**

Five of the 27 studies reviewed recorded the use of antidepressants, with the prevalence of antidepressant use ranging from 3 to 14% (Chiappelli et al., 2014, Koreen et al., 1993, Muller. et al., 2002, Oosthuizen et al., 2002, Muller et al., 2001). Fourteen reported antipsychotic use (Koreen et al., 1993, Chiappelli et al., 2014, Majadas et al., 2012, Muller. et al., 2002, Nakaya et al., 1998, Nakaya et al., 1997, Rocca et al., 2005, Oosthuizen et al., 2002, Tapp et al., 2001, Romney and Candido, 2001, Upthegrove et al., 2010, Ragin et al., 1989, Zisook et al., 1999, Tharyan and Kuruvilla, 1994). Majadas et al. (2012) excluded patients who were taking antidepressant medication, while Newcomer et al. (1990) sampled patients who were medication naïve. Tharyan and Kuruvilla (1994) sampled both medicated and unmedicated patients.

2.3.1.7 **Prevalence and reporting of negative symptoms and depression**

The prevalence of depression in the studies reviewed ranged from 30% to 80%, depending on the criteria for the presence of depression (Hafner et al., 2005a, Tharyan and Kuruvilla, 1994) or depressive symptoms (Oosthuizen et al., 2002, Koreen et al., 1993, Hafner et al., 2005b, Maggini et al., 2004, Majadas et al., 2012, Tapp et al., 2001, Upthegrove et al., 2010, Zisook et al., 1999) that were used.

Factor analysis was reported by 2 studies, which described a depression domain independent from negative symptoms and positive symptoms (Chiappelli et al., 2014, Romney and Candido, 2001). Symptoms such as low mood, pessimism, suicidal ideation and impaired tolerance to stress were considered depressive features (Kibel et al., 1993,

Kulhara et al., 1989). The negative symptom domain included alogia, poor attention and concentration, blunted affect and social withdrawal (Whiteford et al., 1987). Anhedonia, emotional blunting, anergia, amotivation, asociality and avolition occurred in both the negative and depressive domains (Romney and Candido, 2001, Sax et al., 1996, Whiteford et al., 1987).

Results from all of the studies reviewed were tabulated into symptoms that were reported as distinct depressive features, symptoms that were distinct negative symptoms and symptoms that overlapped the two symptom domains (see Table 4).

Table 4: Association of symptoms within the depressive and negative symptom domains

Symptom	Depressive symptoms	Depressive and negative symptoms	Negative symptoms
Anhedonia		X	
Emotional blunting		X	
Anergia		X	
Amotivation		X	
Asociality		X	
Avolition		X	
Low mood	X		
Pessimism	X		
Suicidal ideation	X		
Observed sadness			X
Alogia			X
Poor attention and concentration			X
Blunted affect			X
Social withdrawal			X

2.4 Discussion

The findings of the studies reviewed suggest whilst considerable heterogeneity is present, some depressive features such as low mood, suicidal ideation, and pessimism are distinct from a negative symptom domain characterised by alogia and blunted affect in schizophrenia. For example Liemburg et al. (2013) and Messinger et al. (2011) found that while depressed mood and suicidal intent reflect a depressive domain, blunted affect and alogia belong to the 'expressive deficit' negative symptom domain. Symptoms such as avolition, anergia and amotivation, considered to constitute 'social amotivation', appear to occur in both the depressive and negative domains. The findings presented are consistent with those of Whiteford et al. (1987), who emphasised that while symptoms such as alogia and poor attention were distinctly negative symptoms, loss of energy and anhedonia were present in both the depressive and negative domains. Observed sadness together with inability to feel was reported as a negative symptom by Kibel et al in (1993) as distinguished from subjective sadness. However, this was not replicated by other studies. One explanation for this finding is that there can be a misattribution of blunted affect or lack of facial expression as observed sadness.

Sax et al. (1996), found the co-occurrence of depressive and negative symptoms to be confined to poor motivation, anhedonia and social withdrawal, but this may be artefactual; specifically, the product of similarly worded items on different rating scales. The findings relating to whether anhedonia should be considered as part of the negative symptom or depressive symptom domains are inconsistent, and our results suggest anhedonia occurs in both. Evidence from factor analysis studies suggests that anhedonia

may be more closely aligned with depression rather than schizophrenia (Maggini et al., 2004, Romney and Candido, 2001). Alternatively, it has been considered as a negative symptom of schizophrenia because of its close relationship with blunted affect, emotional blunting and social withdrawal (Romney and Candido, 2001).

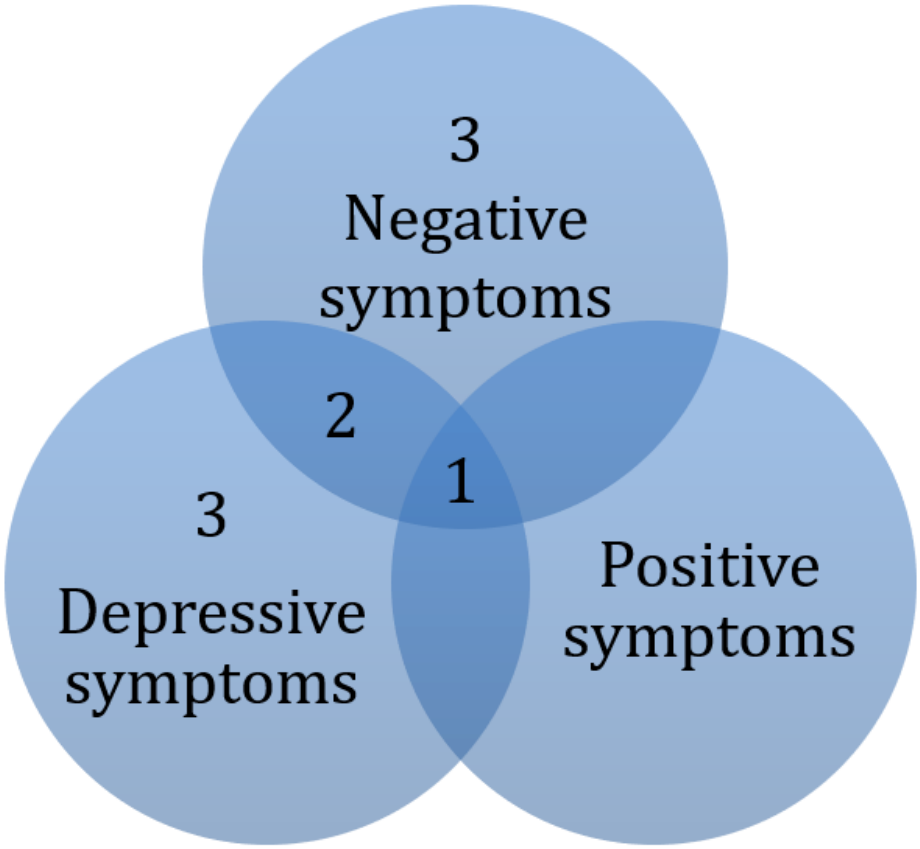
The inconsistency of classification of anhedonia may partly reflect that anhedonia may be a feature of negative and depressive domains in schizophrenia in different forms, differentiated by distinguishing between anticipatory and consummatory anhedonia. Thus, anhedonia as part of depression may reflect a loss of interest in previously rewarding or enjoyable activity because of the inability to experience pleasure. However, individuals with schizophrenia may experience normal levels of pleasure when engaging in enjoyable activities (consummatory pleasure) but lack the ability to experience pleasure for future activities (anticipatory pleasure), and therefore have an anticipatory anhedonia but not a consummatory anhedonia (Gard et al., 2007). These may reflect differing underlying processes; abnormal psychological and dysfunctional behavioural processes that are responsible for differing manifestations of anhedonia (Dowd and Barch, 2010). However, when Li et al. (2015) observed people with moderate depressive disorder and individuals with schizophrenia, they found anticipatory and consummatory anhedonia were present in both conditions. Furthermore, Buck and Lysaker (2013) observed strong correlations between scores of consummatory and anticipatory anhedonia in schizophrenia, suggesting that the explanatory value of this distinction regarding anhedonia as a negative or depressive symptom may be limited (Buck and Lysaker, 2013). However, the notion that anhedonia as a negative symptom has a different pathophysiology from its presentation as a depressive symptom is speculative and further

research is needed to address this. Furthermore, the findings prompting such a notion may simply reflect a limitation in the scales used to assess the change of hedonic experiences in patients with schizophrenia.

2.4.1 A dimensional model within domains

If we consider negative symptoms as being formed of a diminished-expression domain and avolition-amotivation sub-domain (Messinger et al., 2011), the presented findings suggest that the relationship is primarily between depression and avolition-amotivation phenomena. The relationship between depressive and negative symptoms may be represented in a dimensional hierarchical model (see figure 10), with symptoms denoting general, attenuated non-specific symptoms; those that span both depressive and negative symptoms (such as anergia and poverty in speech) and those that represent specific symptoms of depression (such as consummatory anhedonia, hopelessness) and negative symptoms (such as anticipatory anhedonia, blunted affect). Levels within a dimensional hierarchy have often been implied in a positive domain e.g. social anxiety/subtle self-referential thinking building to brief attenuated symptoms followed by frank delusional beliefs and hallucinations (see Mishara and Fusar-Poli (2013).

Figure 10: Dimensional model of negative, depressive and positive symptoms in schizophrenia. 1= General, attenuated non-specific symptoms; 2. Symptoms that span both depressive and negative symptoms; and 3. Symptoms specific to the depressive or negative symptoms domains



In our model, symptoms that form the expressive deficits sub-domain appear to be specific to negative symptoms and are represented by '3' in the negative symptom domain of our dimensional model. Symptoms such as low mood, suicidal ideation and pessimism are considered core features of depression and are denoted by '3' in the depression domain. Symptoms that form the avolition-amotivation sub-domain of negative

symptoms appear to consist of features that span both depressive and negative syndromes and are represented by '2' in our dimensional model.

However, only one of the studies (Chiappelli et al., 2014) employed a scale that allows for the distinction between the two negative symptom sub-domains (the Brief Negative Symptom Scale) (Kirkpatrick et al., 2011a). This highlights the importance of future studies using scales able to assess the distinction between the sub-domains that form negative symptoms, such as the Clinical Assessment Interview for Negative Symptoms (CAINS: (Kring et al., 2013). This would have particular implications for studies assessing the effectiveness of domain specific interventions.

2.4.2 Limitations

Studies included were markedly heterogeneous; both medicated and unmedicated patients were included in the study samples as well as broad diagnostic groups including schizophrenia and first-episode psychosis. There was also a lack of consistency regarding the methods used to assess depression and negative symptoms. It can be argued that the SANS (Andreasen, 1989) provides the most comprehensive and valid coverage of negative symptom domains in schizophrenia and is highly tuned to detecting differences between positive and negative symptoms in schizophrenia, yet it was not universally used. However, the use of the SANS has its limitations not least the item assessing attention, which may be considered a manifestation of cognitive dysfunction and not form part of the negative syndrome. Nevertheless, no phenomenological measure has been developed that is able to differentiate between primary and secondary negative symptoms, and the

subjective experiences related to negative symptoms are not elicited (Lincoln et al., 2016, Liddle et al., 1993, Liddle and Barnes, 1988). Also, to measure depression, some studies used the HRSD (Romney and Candido, 2001), others used the CDSS (Upthegrove et al., 2010) and some others used the BDI (Baynes et al., 2000). Finally, symptoms such as weight loss, loss of appetite and anxiety were not examined in the included articles. These symptoms may be conceptualised as being associated with depression and negative symptoms. This may represent a limitation with the studies included in the review.

2.4.3 Implications

The DSM RDoC criteria and NIMH objectives recommend that research studies should focus increasingly on specific indicative symptoms to ascertain treatment effects (Whitehead et al., 2002, Insel et al., 2010). Our review has suggested a putative model (see Figure 10) which could be used as a starting point for the investigation underlying biological processes and targeted treatment within a dimensional psychosis model (Noto et al., 2015b, Kumari et al., 2016, Chuang et al., 2014). Being able to clinically distinguish between depressive and negative symptoms in schizophrenia would not only allow for early, targeted treatment, but may also provide some indication of a patient's symptom burden and possibly have implications for prognosis, which can then be used to help inform a tailored treatment plan. Discriminating between anticipatory and consummatory anhedonia may prove to be a useful distinction in relation to association with either the depressive or negative domain which would suggest that the two domains within anhedonia (consummatory and anticipatory) may be products of differing aetiology.

Distinguishing between these two domains may provide clinical benefit through targeted therapy, which can be directed at either anticipatory or consummatory anhedonia and has been shown to be effective when applying psychological therapy (Favrod et al., 2010). To date, there have been relatively few studies assessing the use of antidepressants to treat depression in schizophrenia and those that have been reported yielded mixed results, possibly the result of poor understanding of the core symptoms of depression therein (Goode and Manning, 1983, Kirli and Caliskan, 1998, Mulholland et al., 2003, Sandhu et al., 2013, Barnes et al., 2016) Furthermore, to understand the relationship between depression and negative symptoms in schizophrenia, highly sensitive measures and thorough longitudinal studies, which chart symptom development and course, are required.

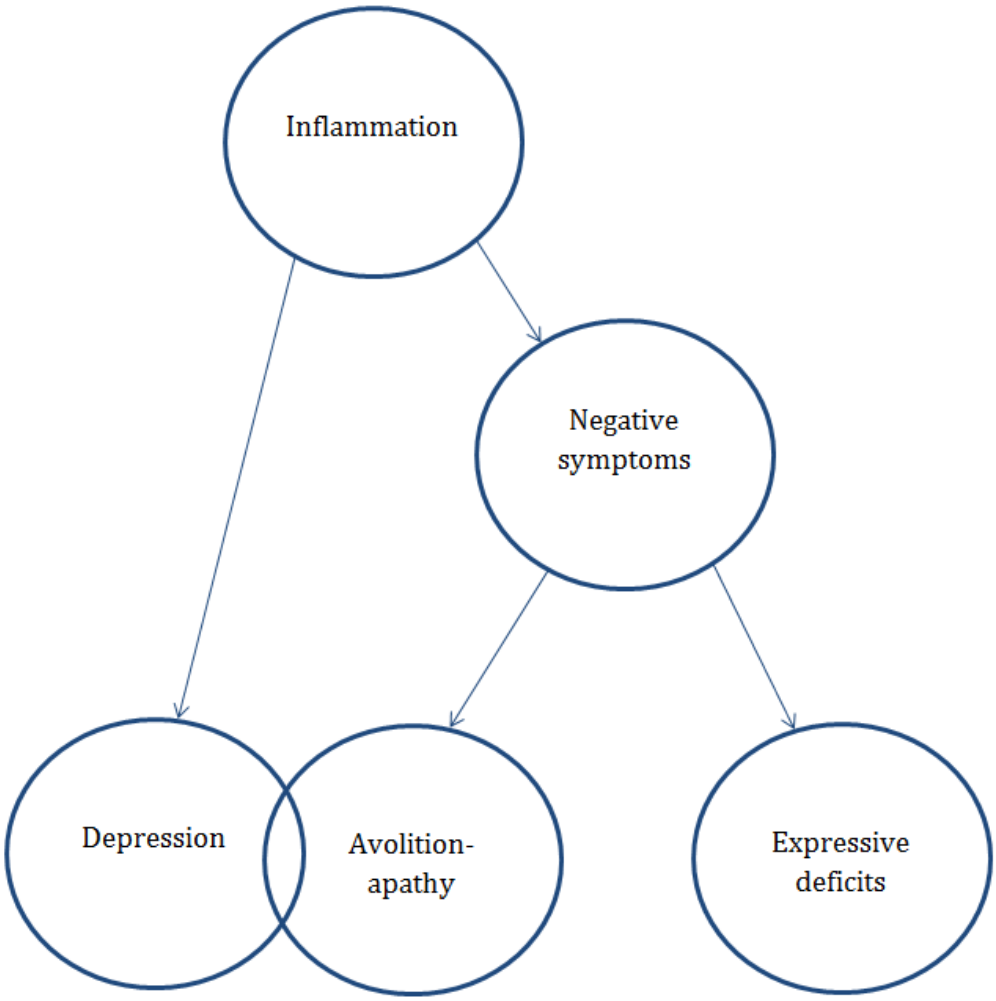
3. Summary, hypotheses, aims and predictions

3.1.1 Summary

Evidence presented in chapter 1.4.2.3 suggests that there are two sub-domains that underpin the negative symptoms; expressive deficits and avolition-apathy (Messinger et al., 2011, Kirkpatrick, 2014, Liemburg et al., 2013). These two sub-domains may be the product of differing underlying neurobiology including hypoactivation of the frontothalamic circuitry and activation of reward processing areas such as the dorsal caudate and striatum (Ohtani et al., 2014 & Lepage et al., 2011), and have differential treatment responses (Barnes et al., 2016, Aleman et al., 2016). There is also evidence to suggest that there is an overlap of depressive and negative symptomology, which may be via symptoms that correspond to the avolition-apathy sub-domain (such as apathy, asociality and anhedonia). Expressive-deficits however appear independent of depressive symptoms in schizophrenia (Barnes et al., 2016).

Negative symptoms in schizophrenia may emerge following a sustained period of low grade neuro-inflammation (Hafner et al., 2005b, Barnes et al., 2016) (see figure 11). Previous findings have suggested an elevation in pro-inflammatory markers in untreated schizophrenia (Upthegrove et al., 2014a); some being trait markers (e.g. TNF-α), stable throughout the course of illness together with state (e.g. IL-6 and CRP) markers that fluctuate depending on clinical presentation and symptoms (Baumeister et al., 2014, Kirkpatrick and Miller, 2013, Anderson et al., 2013).

Figure 11: Inflammation as the common aetiological process that drives depressive and negative symptoms dimensions in schizophrenia. A common underlying neuro-inflammatory profile may explain the symptom co-occurrence seen in depression and the avolition-apathy domain of negative symptoms.



Chronic neuro-inflammation acting through activated microglia may lead to grey matter loss (Cahn et al., 2006) in patients with schizophrenia, particularly in frontal and temporal areas, and this is associated with severity of negative symptoms and poor functional outcome (McKechanie et al., 2015, Haijma et al., 2013).

Minocycline has neuroprotective properties and has been shown to have some effect in treating negative and depressive symptoms (Miyaoka et al., 2012, Levkovitz et al., 2010, Chaudhry et al., 2012).

3.1.2 Aims

This research aimed to examine the relationship between depressive and negative symptoms in early psychosis. Firstly, this research focused on the syndromal overlap between these symptom domains, examined both in cross-section and longitudinally. Then, this research examined potential biological mechanisms underpinning this relationship, specifically focusing on inflammatory processes. Finally, this research assessed whether the addition of minocycline will lead to a reduction in negative or depressive symptomology.

Aims of thesis:

1. Explore the co-occurrence of depressive and negative symptoms in schizophrenia both in cross-section and longitudinally.
2. Determine the influence of inflammation on depressive and negative symptoms in schizophrenia.
3. Assess the effect of minocycline on negative and depressive symptomology.

3.1.3 Hypotheses

1. Depression and the avolition-apathy sub-domain of negative symptoms co-occur and will be associated with each other longitudinally. The expressive deficit sub-domain is independent of depression in schizophrenia.
2. Depression and the avolition-apathy sub-domain of negative symptoms will be associated with state-based inflammatory markers (IL-6 and CRP). The expressive deficit sub-domain of negative symptoms will be associated with stable, trait cytokine (TNF-α).
3. Administration of minocycline will lead to a reduction of neuro-inflammation, as measured by state markers (IL-6 and CRP), but will have no impact on trait markers (TNF-α).
4. A reduction in neuro-inflammation will be associated with an improvement of depressive and the avolition-apathy sub-domain of negative symptoms.

3.1.4 Predictions

Table 5: Specific aims and predictions pertaining to each study.

Study	Aim	Hypothesis
Longitudinal relationship between depressive	• Explore the longitudinal relationship between depression and negative symptoms in early schizophrenia.	1. Depression and the avolition-apathy sub-domain of negative symptoms correlate and are associated with each other both in cross-section and longitudinally.

and negative symptoms	• Explore the longitudinal relationship between avolition-apathy and expressive deficits domains of negative symptoms and depression.	2. The expressive deficits sub-domain is independent of depression in schizophrenia.
Immune dysfunction and schizophrenia	• To define the pro-inflammatory markers that are associated with depression and negative symptom domains in schizophrenia.	1. Depression and the avolition-apathy sub-domain of negative symptoms will be associated with state pro-inflammatory markers (IL-6 and CRP). 2. The expressive deficits sub-domain of negative symptoms will be associated with stable, trait markers (TNF-α).
Treatment of Immune dysfunction in negative and depressive symptoms.	• To assess the effect of minocycline on depressive and negative symptoms	1. Administration of minocycline will lead to a reduction of pro-inflammation as measured by state markers but will have no impact on trait markers. 2. A reduction in neuro-inflammation will lead to an improvement of depressive and the avolition-apathy sub-domain of negative symptoms but will not reduce expressive deficits.

4. Methodology

This chapter will describe the design of studies, participant recruitment, inclusion/exclusion criteria, and assessments used in the studies described in this thesis. The processes of ethical approval and potential ethical issues that may have arisen through this research have also been acknowledged. This will be followed by an overview of the statistical analysis adopted.

4.1 Design

Data for this thesis was taken as part of a large study assessing the effectiveness of minocycline to treat negative symptoms in psychosis (BeneMin) (Chief investigator: Professor Bill Deakin), trial Registration: Current Controlled Trials ISRCTN49141214, NIHR EME grant number 2010-022463-35. An experimental design was employed to assess participants for twelve months, longitudinally, using a variety of validated questionnaires, clinical interviews and physical examinations (see section 4.7 for a full description of the measures used in the following studies, as well as a rationale for using these assessments).

4.2 Recruitment

Participant recruitment took place between April 2013 and March 2015 at the University of Manchester, University of Cambridge, King's College, London, University

College, London, and Lancashire NHS trust. Recruitment at the University of Birmingham began in March 2014 and at Cheshire and Wirral NHS partnership in June 2014. BeneMin was a double blind, randomised placebo controlled efficacy and mechanistic study that used minocycline adjunctive to standard anti-psychotic drug treatment to improve, or lessen the development of, negative symptoms in schizophrenia.

At the discretion of the responsible medical officer (who assessed eligibility in accordance with the strict eligibility criteria – see below), potential participants were identified. A member of the care team (community psychiatric nurse - CPN or consultant) would then discuss participation of the study with the patient when appropriate and, if the patient was interested, then consent to contact was given. Once consent to contact was obtained, potential participants were then contacted to outline the objectives and requirements of the study and to discuss the Patient Information Leaflet (PIL) (see appendix 3), which lasted ten minutes. This detailed what was required of the participant and any commonly asked questions. The following day a member of the study team contacted the patient to see if they would be interested to attending a screening assessment and to answer any questions that the patient had. The screening assessment was essential to carry out an in-depth assessment of the potential participants' eligibility and to obtain informed consent (see appendix 4). Once recruited, the participants GP and care team were informed. The PhD student at Birmingham was responsible for nineteen of the 207 participants randomised onto the study. These participants were recruited in one year from March 2014. Recruitment ended in March 2015 with the final follow up assessment being completed in April 2016.

4.3 Data management and cleaning

In addition to recruitment, all baseline and follow up clinical assessments using standardised scales, support in MRI scanning and site file management in Birmingham were carried out by the PhD student. The role of this PhD student also included preparation, storage and organisation of the transportation of all cytokine samples to Kings College London (KCL) and ensuring GCP standards were upheld at the Birmingham site. The PhD student was also responsible for much of the data cleaning as well as data entry at other sites (the PhD student completed the comprehensive data cleaning at UCL, Cambridge, Manchester, Lancashire, Birmingham and Edinburgh). This study employed a systematic approach to data cleaning (see appendix 5 for the data cleaning pro-forma) whereby the PhD student checked that the participant ID, date and visit was detailed on every page of the case report form (CRF). Following this, the information detailed on the CRF was then cross-matched with the information stored on OpenCDMS (www.opencdms.org) to ensure that the data had been entered correctly. Any queries were then raised with the study management team (consisting of the project manager, deputy project manager and chief investigator) to ensure an appropriate and standardised resolution.

4.4 Setting and Sampling

All participants were recruited through early intervention in psychosis service (EIP) in eight sites. EIP is a dedicated service model adopting an assertive outreach approach that employs an intense case management approach to treatment to ensure

maximum potential in aiding recovery for individuals with schizophrenia. Indeed, EIP has been found to reduce hospital admissions, relapse rates and reduce symptom severity (Bird et al., 2010, Marwaha et al., 2016). Patients are typically referred to EIP through GP's, Community Mental Health Teams (CMHT's) and Home Treatment Teams and normally stay with the EIP for the critical first three years before being discharged.

Eligible participants were identified through caseload screening either by the patients' responsible medical officer (RMO) or study clinical studies officer's (CSO). Once eligible participants had been identified and approached to take part in the study, any new referrals were screened for participation.

Several different recruitment strategies were employed to suit the care team. These included attending the weekly multi-disciplinary team (MDT) meeting and presenting at planning meetings. This ensured that any new referrals were considered to take part in the research. In addition to this, posters and leaflets were also distributed amongst the care teams to ensure maximum recruitment potential.

4.5 Inclusion Criteria/Exclusion Criteria

Participants included in the research adhered to the strict inclusion/exclusion criteria set out below in table six.

Table 6: The criteria for inclusion and exclusion onto the study.

Inclusion criteria	Exclusion criteria
Be male or female aged 16 to 40	Not currently abuse illicit substances
Meet DSMIV criteria for schizophrenia, schizophreniform or schizo-affective psychosis	Not pose a serious suicidal or violent risk
Be in a current episode as defined by the presence of positive symptoms (a score >2 on P1, 2, 3 or 6 on PANSS).	Not have taken an investigational medicinal product within 30 days of baseline assessments.
Be within 5 years of symptom onset	
Be fluent in English	
Have capacity to provide informed consent	

4.6 **Planned intervention**

The tetracycline antibiotic minocycline was used to assess the main aim of the BeneMin trial, which was to determine the influence of inflammation on negative symptoms in schizophrenia. Minocycline was used to test the final objectives of this thesis, which was to assess the effect of minocycline on negative and depressive symptomology. To assess whether depression and negative symptoms were associated with inflammation, either minocycline or a matching placebo was administered, which was taken as 100mg capsules twice daily for two weeks and then increased to 300mg daily for the remainder of the twelve-month study period. Participants were randomly allocated to either the treatment (minocycline) arm or placebo arm of the study, which was operated by the online software, OpenCDMS.

4.7 **Materials**

The following sub-section describes the relevant clinical and physical assessments of the 207 participants from the BeneMin trial. Assessments were conducted largely in the participants' homes but also in the hospital clinic where appropriate. Assessments varied in length but typically lasted between 1.5 and 2.5 hours depending on the nature of the assessments (i.e. fewer assessments were completed at the two-month assessment compared to the twelve-month assessment) (Figure 12 shows the assessments that were carried out at each time point over the twelve months of participation). Data was then rated (if required by the measure used) and entered onto the specialist software OpenCDMS. This software organised the collection of data onto a password protected, secure database anonymously whilst allowing for quality control and randomisation of treatment allocation. This also ensured that data was collected and organised, tailored to the design of BeneMin. Data was then downloaded from OpenCDMS and transferred to an SPSS file for analysis. Further assessments included the social functioning scale (Birchwood et al., 1990) and the Wechsler Abbreviated Scale Intelligence (Wechsler, 2001, Wechsler, 1999), however these were not relevant in this thesis and therefore were not included in analysis.

Data was obtained on a variety of demographic and baseline variables. The patients' date of birth, gender and ethnicity were obtained at baseline. These are important variables to consider when analysing the data to assess whether these variables impact on the findings. The participant's full medication history was taken at baseline and was charted throughout their participation in the study because both anti-

psychotic and anti-depressant medication may have an impact on inflammatory markers. The patients' height and weight were also recorded to calculate BMI at baseline and twelve months of participation as BMI and treatment with antipsychotic and antidepressants are known to impact on circulating cytokines.

To establish an accurate and current diagnosis at the time of randomisation, selected sections of the M.I.N.I. International Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997) was administered. Relevant sections carried out were sections A. Major Depressive Episode (including Major Depressive Episode with Melancholic Features); D. (Hypo) Manic Episode; and L. Psychotic Disorders (part 1 and 2 – differential diagnosis between psychotic and mood disorders). Data from this structured interview yielded enough information in which to make a diagnosis using diagnostic algorithms.

The Duration of Untreated Psychosis (DUP) was calculated using data from the participants' medical records. DUP was defined in accordance with the definition set out by Larsen et al. (1996) which is a commonly used definition that describes DUP as the duration between 'onset of psychosis' to the 'onset of treatment'. A description of the definition used by Larsen et al. (1996) to describe 'onset of psychosis' and 'onset of treatment' is detailed below:

a) Onset of psychosis – was defined as the presence of at least one positive symptoms (with a PANSS - Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) score of four or above) which is present amongst other psychotic symptoms or a cluster of positive symptoms from the PANSS interview (delusions, conceptual

disorganisation or hallucination) that equate to a total score of seven (excluding absent ratings); which must be present for two weeks unless remission due to treatment (Brunet et al., 2007).

b) Onset of treatment – was defined as a patient requiring antipsychotic treatment, as set by the dosage levels recommended by the British National Formulary (BNF), regularly for one month or until there was a significant subsidence of symptoms (Brunet et al., 2007).

4.7.1 Measures to assess negative symptoms

4.7.1.1 Positive and Negative Syndrome Scale

The Structured Clinical Interview for Positive and Negative Syndrome Scale (Kay et al., 1987) (PANSS) was used to assess positive, negative and general symptoms (including depression) in schizophrenia for the preceding seven days, through the use of a semi-structured interview (see appendix 6). Results from this semi-structured interview are then rated by the interviewer on a scale of one to seven (with one being absent and seven being extreme) using a strict criterion to rate the thirty symptoms that make up the PANSS (seven being identified within the positive symptom domain, seven being identified within the negative symptom domain and sixteen being identified as general symptoms).

Developed by Kay et al. in 1987 from the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and the Psychopathology Rating Schedule (PRS) (Singh and Kay, 1975), PANSS is a widely used and internationally renowned for its reliability and

validity and has been shown to be highly sensitive in measuring symptom changes in schizophrenia (Kay et al., 1988, Edgar et al., 2014).

The PANSS interview was conducted at every assessment during the twelve months of participation.

4.7.1.1.1 PANSS Inter-rater reliability

Extensive training on all clinical assessments and regular PANSS inter-rater reliability sessions were held between interviewers at the eight sites took place to ensure reliability. A total of ten inter-rater reliability sessions were held and were subject to analysis to assess internal consistency (see table 7)

Table 7: The results for internal consistency using Cronbach alpha for each of the PANSS inter-rater reliability sessions

Session	Cronbach alpha score
1	.747
2	.896
3	.834
4	.865
5	.619
6	.362
7	.696
8	.605
9	.596
10	.530

These results indicate acceptable internal consistency (as determined by a Cronbach alpha coefficient of above .6) for the majority of PANSS inter-rater reliability sessions.

4.7.2 Measures to assess depression

4.7.2.1 Calgary Depression Scale in Schizophrenia

The Calgary Depression Scale for Schizophrenia (Addington et al., 1990) is a short observer rated semi-structured interview which aims to assess depression in schizophrenia (see appendix 7). The CDSS assesses depression using nine questions (one of which is based on the interviewers' observation) relating to the participants symptoms of the preceding two weeks which contains questions relating to the following domains: depression, hopelessness, self-deprecation, guilty ideas of reference, pathological guilt, morning depression, early wakening, suicide and a clinician observed depression score. Scores are rated on a four-point Likert scale with a maximum score of twenty-seven. A score of four or five indicates mild depression and a total score of six or seven represents severe depression (Bressan et al., 1998). Originally designed to address the overlap between depression and negative symptoms in schizophrenia, the CDSS has been shown to have little overlap with positive and negative symptoms and has little correlation with extrapyramidal symptoms (Addington et al., 2014). Furthermore, the CDSS has been translated into thirty six different languages and has high specificity and sensitivity, as well as high inter-rater reliability (Lançon et al., 2000, Müller et al., 1999, Addington et al., 1992, Addington et al., 2014). It has also been proven superior relative to other commonly used measures of depression such as the Hamilton Depression Rating Scale in separating depression, negative symptoms and extrapyramidal side effects (Collins et al., 1996, Lançon et al., 2000) in schizophrenia. The CDSS was used at all assessments during the twelve months of participation.

4.7.3 Side effects

4.7.3.1 Simpson-Angus Scale

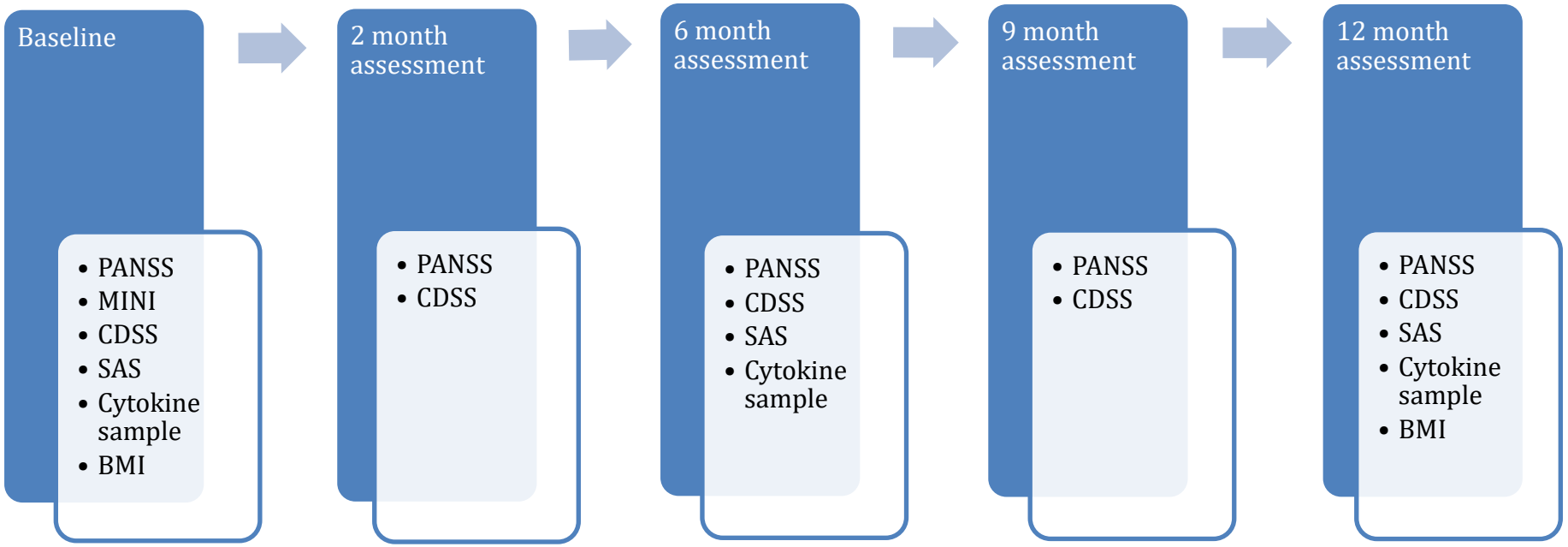
The Simpson-Angus scale (SAS) (Simpson and Angus, 1970) is a ten point observer-rated scale completed in conjunction with a physical examination (see appendix 8). The scale examines a range of extrapyramidal side effects such as rigidity, tremor and hypersalivation. Each item is rated on a scale ranging from zero (representing absent) to four (indicating the most severe of the corresponding extrapyramidal side effect). This scale has been extensively used and demonstrates good internal reliability and consistency (Knol et al., 2009). The SAS examination was carried out at baseline, six months and twelve months.

4.7.4 Inflammatory-marker analysis

Blood samples were taken at baseline, six months, and twelve months of participation. Samples were taken in a 9ml ethylenediamine tetraacetic acid (EDTA) tube to prevent coagulation. The EDTA tube was then placed in a centrifuge for fifteen minutes at 2000g. Following centrifugation, the plasma supernatant was extracted and pipetted into six identical aliquots and equally divided into two boxes. The samples were stored locally in a -80^{o}C freezer before being transported to King's College, London for analysis. The samples were then processed by Meso Scale Discovery Piezoelectric Plate, with high sensitivity immunoassays at King's College London. Multiplex-assays included IL-6, TNF-α, IFN-gamma, IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-13, GM-CSF, IL-1alpha, IL-5, IL-7,

IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF, and CRP. This provided a level of inflammatory markers to assess the participant's level of inflammation, longitudinally.

Figure 12: Flow chart showing the relevant assessments for the studies included in this thesis that were completed at each time point longitudinally over twelve months



4.8 **Ethical Approval and Issues**

Ethical approval for BeneMin was sought and received by the North West - Greater Manchester Central UK National Health Service (NHS) Research Ethics Committee (NRES) reference: 11/NW/0218. Following this, local Trust Research and Development (R&D) approval was then granted for the University of Manchester; University of Edinburgh; University of Cambridge; King's College, London; University College, London and Lancashire Care NHS Foundation Trust. Local R&D approval was then sought and obtained for the University of Birmingham and Cheshire and Wirral Partnership NHS trust to ensure maximum recruitment potential.

Patients with schizophrenia have been identified as potentially vulnerable. Extra measures were put in place to ensure that potential vulnerable participants did not feel pressured to take part in the study. This was achieved by the patient's CPN approaching them and seeking consent from them for a member of the research team to approach them to discuss the research objective, outcomes and participation requirements. Participants' rights of withdrawal were emphasised to ensure that participants did not feel pressured to take part and participants were made aware that this would not affect their treatment. This was also re-iterated at each assessment point throughout the twelve months of participation.

The participants' capacity to understand and decide whether to participate without feeling under pressure is always an ethical issue in clinical research. Capacity was determined by the clinical team. This was overcome by ensuring that verbal and written

consent was received prior to screening for eligibility by the research team. Furthermore, only patients who were deemed to have capacity by their RMO were approached to take part. Following the initial introduction of the study to the patients, the research team would allow the patient to reflect on whether to take part in the study and contact them the following day before recruiting them.

Additional training opportunities were also provided to ensure that these potentially vulnerable participants were recruited sensitively and in accordance with ethical guidelines.

4.9 Statistical analysis

A database using Statistical Package for the Social Sciences (SPSS v.22) was created to manage most of the statistical analyses. This database was then transported to STATA (v.12) for more complex analyses. Initial power calculations were established such that 170 participants will be required (85 per group) to achieve 90% power to detect a significant difference between depression and negative symptom with a standard effect size of .5. All analyses carried out were of two-tailed significance unless otherwise stated and the Alpha level was set at .05. A detailed description of each statistical analysis can be found in the relevant studies; however, a brief overview can be found below.

1. **Longitudinal relationship between depressive and negative symptoms:** Firstly, Spearman's correlation was carried out between primary outcome variables (depressive and negative symptoms scores). Then a regression analysis

was carried out to explore the relationship between these variables and including other predictor variables (such as sex and extrapyramidal side effects). To assess the longitudinal relationship between the primary outcome variables (depressive and domains of negative symptom scores – avolition-apathy and expressive deficits), a linear mixed effects model was constructed, controlling for other variables (such as sex and extrapyramidal side effects).

2. **Immune dysfunction and schizophrenia:** A Spearman's correlation was used to explore the association between depression and negative symptom severity and longitudinal key cytokines. Finally, a longitudinal analysis using linear mixed effects modelling was used to explore the relationship between each clinical measure of symptom severity and cytokines**.**
3. **Treatment effect of minocycline on immune dysfunction in negative and depressive symptoms:** Finally, to assess the effect of minocycline on depressive and negative symptoms, a longitudinal analysis using linear mixed effects modelling was employed. This explored the relationship between each clinical measure of symptom severity (CDSS and PANSS negative scores separated into the avolition-apathy sub-domain and expressive deficits sub-domain) and key inflammatory markers (Il-6, CRP and TNF-α), while controlling for clinical measures (such as extrapyramidal side effects) and treatment arm, and using data at all time periods.

4.9.1 Rationale for linear mixed effects modelling

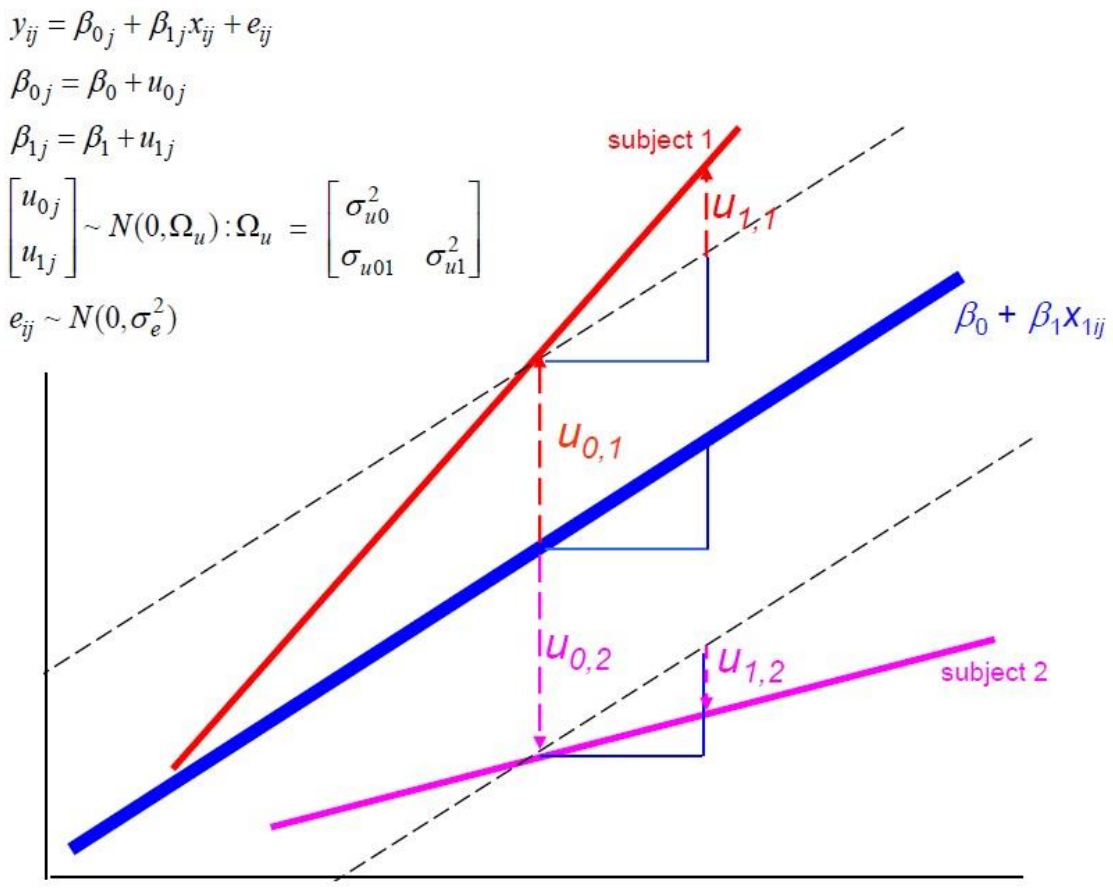
Linear mixed effects modelling is a statistical technique that extends the general linear model to incorporate random effects; it is often used for data that has a natural hierarchical structure (e.g. patients within centres, or repeated measurements within patients over time), and where there is an assumed linear relationship between the independent and dependant variables. Crucial to linear mixed effects modelling in comparison to standard linear regression models, is that they are able to describe data when observations are not independent. When observations are not independent the assumptions of linear regression are violated (i.e. independent residuals). Mixed effects models allow for this dependence by modelling and estimating the covariance structure of the data, which describes how observations are correlated. Standard linear regression models treat all parameters as 'fixed' effects, meaning that their interpretation is restricted to the data at hand. For example, if we wish to allow for the doctor treating patients, and have five doctors in our study, then a fixed effect for doctor would provide the mean effect on outcome for these five doctors. Conversely, using mixed models we can additionally incorporate 'random' effects on particular parameters of interest where the levels of a factor represented in our study are only a sample from a larger set of levels. For example, in our example which includes five doctors we are not interested in the differences between these five doctors but rather the population of doctors that they represent. These random effects assume a distribution of possible effect (coefficient) values for the variable of interest, representing the variation between the groups considered, allowing their interpretation to be applied to a wider population of doctors similar to those seen in the current sample.

There are two main ways in which random effects can be parametrised to account for the hierarchical nature of the dataset; random intercepts, and random slopes (see figure 13). Fitting a random intercept within a mixed model estimates the mean response for all levels of the variable (e.g. individuals), and additionally the variation around this mean response across levels of the variable (e.g. individuals). For example, allowing for a random intercept on a centre variable would allow for variation in response across centres, which may be appropriate due to different clinical practice or expertise in particular centres. Furthermore, random slopes can be allowed within the model, estimating the rate of change of response given levels of a variable of interest (e.g. PANSS scores or cytokine levels), and estimate the variation around the average rate of change across levels. An example of a random slope may be allowing for within-individual variation in rate of change of response, and the correlation between individuals. There are a number of restrictions that can be placed on the model when estimating the relationship (correlation) between levels of a variable in the model, but the most flexible approach is to allow the model to estimate an 'unstructured' covariance matrix, meaning that all parameters describing the relationships between variable levels (e.g. individuals, observations within individuals, or centres) in the data can be allowed for and estimated during model fitting.

In the present analysis, the study design involved clustering effects (so the visit was nested within participants, so there were five observations of each of the dependant variables for each participant), and the dependant variable was the variable of interest (such as longitudinal depression score). Predictor variables were included to assess their

relationship with the dependant variable (e.g. inflammatory markers such as CRP, IL-6 or TNF-α).

Figure 13: Random intercepts and slopes model whereby both intercepts and slopes can vary by group. Here the blue line represents the fixed effect element of the model, while the red and pink lines represent particular individuals whose response differs from the average as described by the random intercept ($U_{0,j}$) and random slope ($U_{1,j}$).



There are several advantages to using linear mixed effects modelling. Firstly, Linear mixed effects modelling is a longitudinal analysis whereby longitudinal data can be analysed using a single statistical technique, rather than a series of cross-sectional linear regression analyses, thus assessing the relationship between variables over time

allowing for the correlation between repeated measurements from the same patient over time, giving better estimates of precision for effects of interest. Also, linear mixed effects modelling is able to handle unbalanced and missing within-subject data. Repeated measures ANOVA will use listwise deletion, which can cause bias and reduce statistical power. Compared to mediation models (such as structural equation models), evidence suggests linear mixed effects modelling performs equally well with respect to power, bias and performance (Blood et al., 2010). Given the advantages of linear mixed effects modelling outlined above, this is the most appropriate statistical analysis for the data set used in this thesis.

5. Longitudinal relationship between depression and negative symptoms

5.1 Introduction

Negative symptoms are a characteristic feature of schizophrenia, reflected in its earliest conceptualisations. Andreasen (1982) classified negative symptoms into affective blunting, alogia, anhedonia/asociality and avolition/apathy. This classification was largely followed by the National Institute of Mental Health (NIMH) Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) consensus statement on negative symptoms (Kirkpatrick et al., 2006), with the exclusion of poverty of content of speech which was differentiated from alogia, and considered a symptom of thought disorder. Negative symptoms not only impact on patient functioning (Rabinowitz et al., 2012), but are also present in the prodromal phase of illness for many (Hafner et al., 2005a) and remain stable throughout the course of illness (Ventura et al., 2015).

The biological distinction between positive and negative symptoms of schizophrenia is chiefly validated by the lack of response of negative symptoms to antipsychotic treatments (Barnes and Paton, 2011). Negative symptoms have been viewed as a unitary concept, implying a single underlying neurobiological pathology. However, factor analyses by Liemburg et al. (2013) and Messinger et al. (2011) have generated an avolition-apathy and expressive deficits sub-domain underpinning negative symptoms. The avolition-apathy factor has positive loadings of avolition, asociality, and

anhedonia ratings. Distinguishing between two sub-domains not only has value in our understanding of negative symptoms, but also have differing clinical predictors and course of illness with avolition-apathy being associated with poorer outcomes than expressive deficits in a cluster analysis of nearly 370 participants (Strauss et al., 2013). Neuroimaging studies show avolition-apathy is associated with reduced white matter volume (Ohtani et al., 2014), and reduced ventral-striatal activation in anticipation of rewards (Juckel et al., 2006). In contrast, the expressive deficits dimension correlates with blunted affect and alogia in several studies (Foussias et al., 2014, Kaiser et al., 2011, Strauss et al., 2013, Messinger et al., 2011, Liemburg et al., 2013) and its neurobiological correlates include hypoactivation of the ventrolateral prefrontal cortex to emotion recognition stimuli (Stip et al., 2005). Some evidence suggests the domains respond differently to treatment. For example Barnes et al. (2016) found that citalopram reduced depression and avolition-apathy, but had no beneficial effect on reducing total negative symptoms or expressive-deficits.

Depression includes a myriad of symptoms, defined by an absence of positive affect and/or loss of pleasure in most activities (NICE, 2009). Other symptoms include anhedonia (Beck, 1967), as well as cognitive (such as feelings of guilt, hopelessness, shame and worthlessness, reduced self-esteem, and suicidal ideation), biological (such as diminished appetite and disturbed sleep) and physical symptoms (such as lack of libido, fatigue, tearfulness, and social withdrawal). Depression often emerges in the prodromal phase of psychosis, pre-dating all other symptom clusters (Hafner et al., 2005a, Krabbendam et al., 2005). Upthegrove et al. (2010) have demonstrated that depression occurs in up to 80% of patients in their first episode of psychosis. Moreover, Koreen et al.

(1993) have shown that between 30% and 50% of patients will experience depression at some point in their psychosis, highlighting the importance of this syndrome in psychosis.

Depression has typically been conceptualised as 'waxing and waning' throughout the course of schizophrenia (Upthegrove et al., 2010) whereas negative symptoms are perceived as stable (Pogue-Geile and Harrow, 1985). Dollfus and Petit (1995a) assessed fifty-seven chronic schizophrenia patients and found that negative symptoms (such as affective flattening and alogia) did not improve following remission of an acute psychotic episode. Thus, exploration of the longitudinal associations for depression and negative symptom sub-domains is likely to be informative. A previous systematic review of twenty-seven studies by Krynicki et al. (2018) found some evidence to suggest a relationship between negative and depressive syndromes in cross-section. Specifically, symptoms corresponding with the avolition-apathy-sub-domain of negative symptoms also occur in depression.

The evidence suggests that depression and negative symptoms are associated with one-another, via the avolition-apathy sub-domain. It is therefore predicted that depression will be associated with avolition-apathy longitudinally but will not be associated with expressive deficits longitudinally. Also, given that depression and avolition-apathy are associated and depression has been conceptualised as 'waxing and waning', it is predicted that depression and avolition apathy will change over time. Expressive-deficits, however, will remain constant and will not change over the course of illness.

5.1.1 Aim

The systematic review presented in the introductory chapters of this thesis by Krynicki et al. (2018) identified that certain symptoms (such as low mood, suicidal ideation and pessimism) had more specificity for depression, whereas symptoms corresponding to the expressive deficits sub-domain (alogia and blunted affect) had more specificity as negative symptoms. Symptoms corresponding to the avolition-apathy sub-domain (anhedonia, anergia and avolition) were common in both. This study aimed to test the hypothesis that the avolition–apathy sub-domain of negative symptoms is related to co-morbid depression in schizophrenia while expressive-deficits are independent of depression in schizophrenia, both in cross-section and longitudinally.

5.2 Methodology

5.2.1 Design

This study used data collected from participants in the BeneMin study in an experimental design to assess the longitudinal relationship between depressive and negative symptoms in early psychosis, over twelve months.

5.2.2 Sample

Two hundred and seven participants experiencing psychosis recruited between April 2013 and March 2015 from Early Intervention in Psychosis (EIP) services across all

eight of the participating BeneMin sites and were followed-up at two months, six months, nine months and twelve months from baseline. Participants adhered to a strict inclusion/exclusion criterion (see table 8).

Table 8: The criteria for inclusion and exclusion onto the study.

Inclusion criteria	Exclusion criteria
Be male or female aged 16 to 40	Not currently abuse illicit substances
Meet DSMIV criteria for schizophrenia, schizophreniform or schizo-affective psychosis	Not pose a serious suicidal or violent risk
Be in a current episode as defined by the presence of positive symptoms (a score >2 on P1, 2, 3 or 6 on PANSS).	Not have taken an investigational medicinal product within 30 days of baseline assessments.
Be within 5 years of symptom onset	
Be fluent in English	
Have capacity to provide informed consent	

5.2.3 Materials

Demographic information such as age, gender and ethnicity and Duration of Untreated Psychosis were obtained. Duration of untreated psychosis (DUP was also calculated using patient records and clinical assessment) was defined in accordance with the Larson et al.'s (1996) definition (see section 4.7 for Larson et al.'s definition).

Negative symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), which is a semi-structured clinical interview assessing patient symptomology for the preceding seven days. This was then scored against strict criteria and rated on separate domains using a Likert scale from one to seven. The avolition-apathy sub-domain of negative symptoms was made up of PANSS items G13 (disturbance

of volition), N2 (emotional withdrawal) and N4 (passive/apathetic social withdrawal), and the expressive deficits sub-domain of negative symptoms was made up of PANSS items N1 (blunted affect), N3 (poor rapport) and N6 (Lack of spontaneity and flow of conversation). This method of differentiating between the avolition-apathy and expressive deficits sub-domains of negative symptoms appears to provide an adequate reflection of these sub-domains, and this was based on factor analysis used in previous research (Barnes et al., 2016, Liemburg et al., 2013). The PANSS was administered at each time point over the twelve months of study participation (baseline, and then two months, six months, nine months, and twelve months from baseline). The PANSS has been widely used and has been shown to be a reliable and valid measure of symptom change in schizophrenia (Edgar et al., 2014).

Depression was assessed by the total score of the Calgary Depression Scale in Schizophrenia (CDSS), which is composed of nine items, eight of which are questions that explore distinct aspects of depression and are scored on a four-point Likert scale. The ninth item is a score based on observer rating. This scale has been shown to assess depressive symptoms independent of negative symptoms and extrapyramidal side effects (Addington et al., 2014). The CDSS was administered at each time point over the twelve months of study participation (baseline, and then two months, six months, nine months, and twelve months from baseline).

Extrapyramidal side effects (EPSE) were assessed using the Simpson-Angus scale (SAS) (Simpson and Angus, 1970). EPSE may occur because of anti-psychotic medication. This is a ten-point observer rated scale that is completed in conjunction with a physical

examination, which aims to establish tremor, rigidity, and hypersalivation. Controlling for extrapyramidal side effects is essential not least because it could account for symptoms that may otherwise be miss-attributed as being negative or depressive symptoms; therefore, controlling for these symptoms will reflect the patient's true symptomology, independent of symptoms being the result of medication side effects. The SAS has been widely used and demonstrates good internal reliability and consistency (Knol et al., 2009).

The SAS was administered at baseline and then six months, and twelve months from baseline.

5.2.4 Statistical analysis

All data were analysed using Statistical Package for the Social Sciences (SPSS v.22) and STATA (v.10). All data was treated as interval data and analyses were carried out was of one tailed significance unless otherwise stated and the alpha level was set at .05. Post hoc power calculations were carried out to determine whether sufficient power would be achieved by the sample size already collected. Power calculations were based on the mean and standard deviation of negative symptom scores from Upthegrove et al. (2010), and the mean sample size from this study. This revealed that a sample of 204 is sufficient to achieve statistical power, with an alpha set at .05 to assess the association between depression and negative symptoms.

Firstly, a Spearman's correlation was used to assess the correlation between each of the predictor variables (depression and negative symptoms) at each time point.

Three linear regression models were constructed at each time point. The first linear regression model aimed to establish the relationship between depression and total negative symptoms. The second regression model aimed to establish the relationship between avolition-apathy and depression. The final linear regression model aimed to establish the relationship between expressive deficits and depression (see appendix 9). Significantly correlated variables with any of the dependant variables at a given time point were included as independent variables in the regression models to control for them.

Finally, to assess the longitudinal relationship between the primary outcome variables (depressive and domains of negative symptom scores – avolition-apathy and expressive deficits), four linear mixed effects model were constructed. The dependant variable in the first model was total negative symptoms, in the second model was avolition-apathy, in the third model was expressive deficits and in the final model was depression, controlling for other variables that were significant in the linear regression model.

5.3 **Results**

5.3.1 Descriptive statistics

5.3.1.1 **Descriptive statistics and baseline clinical characteristics**

Two hundred and four participants were included in the analysis due to incomplete data sets for three participants at baseline. There was a predominance for

male participants (n = 149, 72%), and ages ranged from 17 to 40 (M = 25.76, *SD* = 5.21). Over half of the participants identified themselves as White British (53.6%). (See table 9 for a breakdown of participants in accordance with their ethnic origin).

Table 9: The sample descriptive statistics and clinical characteristics.

Variable		Unit		
		Range:	Mean (M)	Standard deviation (*SD*)
	Age	17 - 40	25.76	5.21
		N		%
Gender	Male	149		73
	Female	56		27
Ethnic group	White British	111		53.6
	White Irish	1		.5
	White other	6		2.9
	Asian or Asian British – Indian	4		1.9
	Asian or Asian British – Pakistani	4		1.9
	Asian or Asian British – other Asian background	11		5.3
	Chinese	2		1.0
	Black or Black British	5		2.4
	Black or Black British – Caribbean	7		3.4
	Mixed – Asian and White	1		.5
	Mixed Black Caribbean and White	4		1.9
	Mixed Black African and White	4		1.9
	Mixed – other mixed background	2		1.0

	Other ethnic background	2	1.0
	Not known	43	20.8
Diagnosis	Schizophrenia	181	87.4
	Schizoaffective disorder	6	2.8
	Schizophreniform	4	1.9
	Mood disorder with psychotic features	1	0.5
	Delusional disorder	1	0.5
	Psychotic disorder not otherwise stated	10	4.8
	Not known	4	1.9

Participants were recruited from all eight of the BeneMin sites, across the United Kingdom. These can be further broken down into twelve NHS prescribing sites. Most participants were recruited from Manchester and Cheshire and Wirral Partnership (CWP) (n = 62, 29.9%). A breakdown of recruitment by site and by prescribing NHS site can be found in table ten.

Table 10: The breakdown of recruitment by University and NHS trust and NHS prescribing site.

Centre	Number (n)	Percentage (%)	NHS prescribing site	Number (n)	Percentage (%)
Birmingham	19	9.2	Birmingham and Solihull	19	9.2
Cambridge	28	13.5	Fulbourn Hospital	19	9.2
			Cavell Centre	9	4.3
Manchester (Inc. Cheshire and Wirral Partnership)	62	29.9	Meadowbrook	17	8.2
			Royal Manchester Children's Hospital	45	21.7
Lancashire	48	23.2	Royal Blackburn Hospital	48	23.2

Edinburgh	15	7.2	Royal Edinburgh Hospital	15	7.2
King's College London	13	6.3	Maudsley Hospital	13	6.3
University College London	22	10.6	St Bernard's Hospital	9	4.3
			St Charles Hospital	8	3.9
			Whittington Health	3	1.4
			St Anne's Hospital	2	1.0

Preliminary analyses revealed that the data was not normally distributed, therefore non-parametric tests were used. Table eleven shows the Kolmogorov-Smirnov test for normality for the variables entered into analysis.

Table 11: test of normality for the key predictor variables entered into analysis at baseline.

	Statistic	Significance
CDSS depression	.13	.00
PANSS positive symptoms	.09	.00
PANSS negative symptoms	.11	.00
Avolition-apathy	.10	.00
Expressive deficits	.14	.00
SAS	.25	.00
Gender	.45	.00
Ethnicity	.48	.00
DUP	.31	.00

A Mann-Whitney U Test was used to assess gender differences between depression scores, positive symptoms, negative symptoms, avolition-apathy sub-domain and expressive deficits sub-domain (see table 12).

Table 12: Mann-Whitney U test for gender differences in key predictor variables at baseline.

	Males		Females		*U*	*z*	*p*	*r*
	n	*Md*	*n*	*Md*				
Positive symptoms	149	16	56	17	4080	-2.00	.80	-.01
Negative symptoms	149	17	56	15	3411	-2.00	.04	-.01
Avolition-apathy	149	8	56	7	3452	-1.91	.05	-.13
Expressive deficits	149	6	56	6	3878	-.78	.43	-.05
Depression	148	4	56	5	3975	-.45	.65	-.03

This revealed no significant gender difference in positive symptoms, expressive deficits and depression. There were however significant differences in total negative symptoms between males and females, with males experiencing more negative symptoms than females. Also, there were significant differences in the avolition-apathy sub-domain between males and females, with males experiencing more avolition-apathy.

A Kruskal-Wallis Test was used to assess differences between four ethnic groups (White, Black, Asian and Mixed) and PANSS positive scores; PANSS negative scores; expressive deficits; Avolition-apathy and CDSS depression scores. This revealed that there were no significant differences between ethnic groups and PANSS negative scores, x^2 (3, n =162) = 3.48, p = .32; expressive deficits scores, x^2 (3, n =162) = 1.77, p =.62; avolition-apathy, x^2 (3, n =162) = 2.94, p = .40. There was a significant difference in depression between ethnic groups (White, n = 118; Black, n = 12; Asian, n = 21; Mixed, n = 11) x^2 (3, n =162) = 12.54, p = .06 such that White and Asian participants had more depressive symptoms than any other ethnic group. There was also a significant difference in PANSS positive symptoms between ethnic groups (White, n = 118; Black, n = 12; Asian, n = 21; Mixed, n = 11) x^2 (3, n =162) = 8.43, p = .03 such that Mixed participants had more positive symptoms than any other ethnic group.

Most patients had a diagnosis of schizophrenia (n = 181, 87.4%). A Kruskal-Wallis Test was used to assess differences between diagnosis (schizophrenia, schizoaffective disorder, schizophreniform, delusional disorder, psychotic disorder not otherwise stated or not known) and PANSS positive scores; PANSS negative scores; expressive deficits; avolition-apathy and CDSS depression scores. This revealed that there were no significant differences between diagnoses and PANNS positive scores, x^2 (10, n =203) = 13.03, p = .22; PANSS negative scores, x^2 (10, n =203) =15.07, p =.12; expressive deficits, x^2 (10, n =203) = 14.22, p = .16; avolition-apathy, x^2 (10, n =203) = 6.15, p =.80; and CDSS depression, x^2 (10, n =203) = 11.94, p = .28.

5.3.1.1.1 Depression

The MINI revealed that over half of the participants did experience depression at baseline (n = 135, 65%). Using a total CDSS score of six as a cut off identified 80 (39%) participants in a current depressive episode at baseline.

5.3.1.2 **Description of longitudinal depression scores**

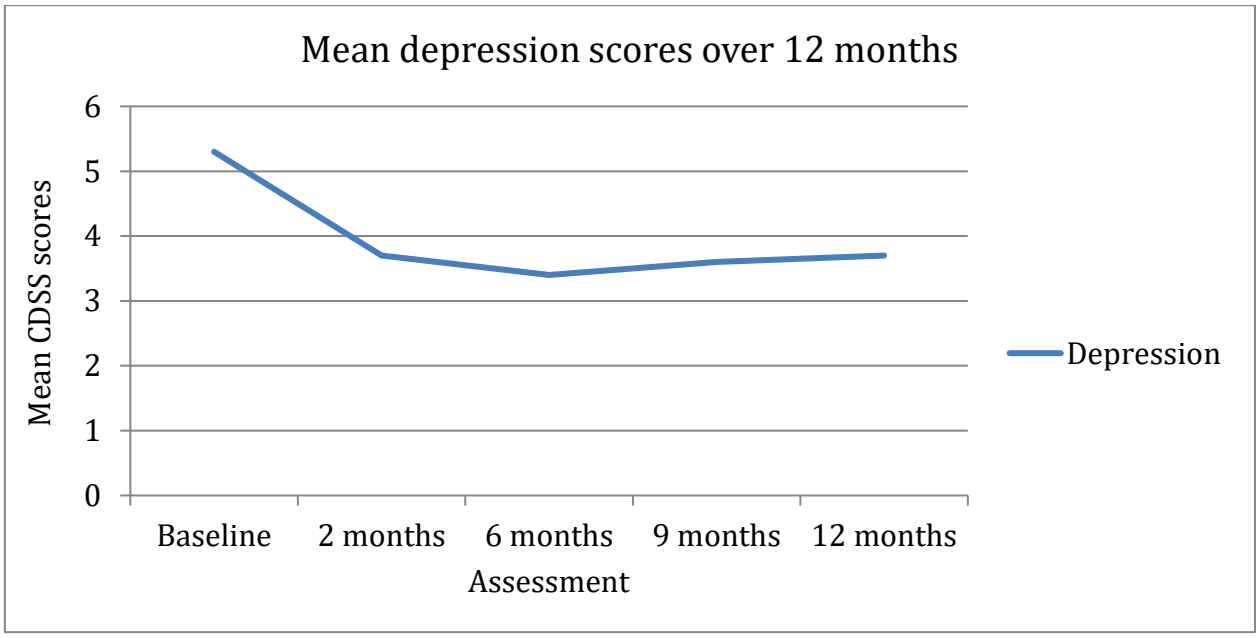
All participants were included in the analysis, from each of the participating BeneMin sites. Table thirteen shows the minimum, maximum, mean and standard deviation of the total score of the CDSS over the twelve months of participation.

Table 13: The minimum, maximum, mean, median and standard deviation of the total score of the CDSS total over the twelve months of study participation.

Visit	Number	Minimum	Maximum	Median	Mean (M)	Standard deviation (*SD*)
Baseline CDSS total	204	0	20	4.50	5.3	4.6
2-month CDSS total	169	0	17	2.00	3.7	3.9
6-month CDSS total	135	0	14	1.00	3.4	4
9-month CDSS total	131	0	16	1.00	3.6	3.8
12-month CDSS total	126	0	17	1.00	3.7	4.2

Figure fourteen shows the depression scores longitudinally.

Figure 14: The mean CDSS scores over twelve months of study participation.



5.3.1.3 **Description of longitudinal positive and negative symptom scores**

The mean, minimum, maximum, median and standard deviation of each of the PANSS domains (including the avolition-apathy sub-domain and expressive deficits sub-

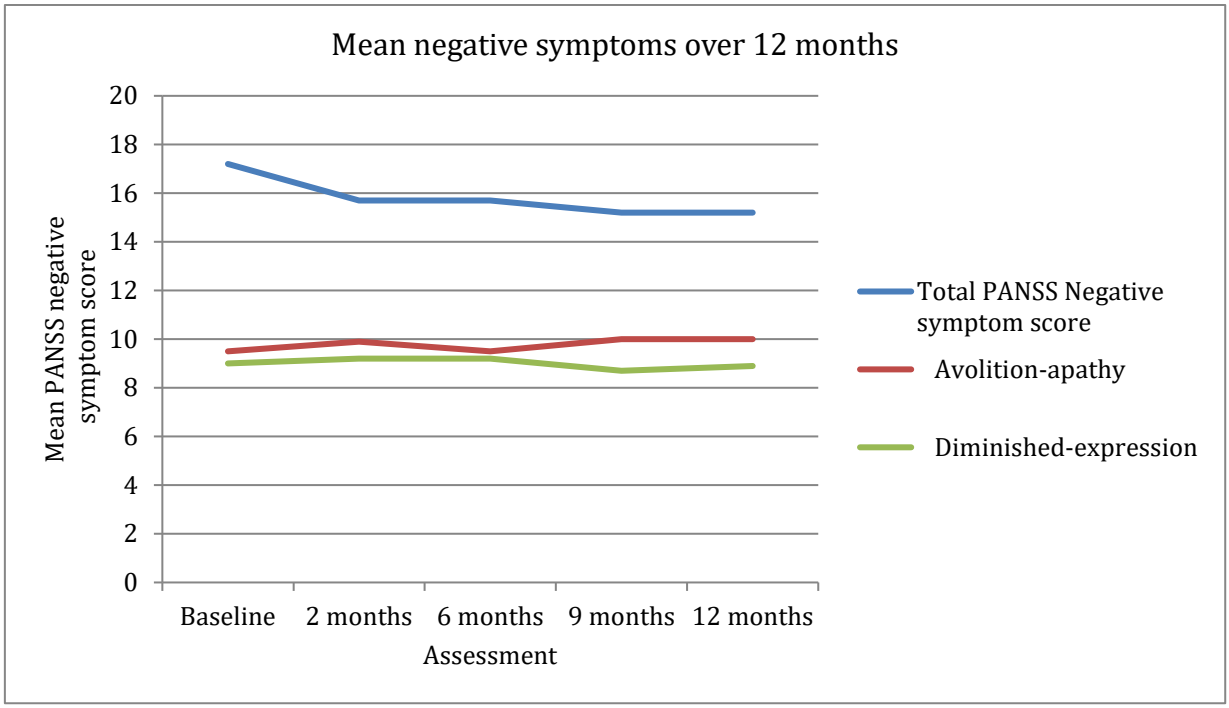
domain of negative symptoms) over the twelve months of participation is detailed in table fourteen.

Table 14: The minimum, maximum, mean, median and standard deviation for the PANSS, including the two separate sub-domains; avolition-apathy and expressive deficits.

PANSS domain	Visit	Minimum	Maximum	Mean (M)	Median	Standard deviation (*SD*)
Positive symptoms	Baseline	9	30	16.8	16	4.7
	2 months	7	27	14.1	13	4.6
	6 months	7	29	13.9	13.5	5.1
	9 months	7	26	13.1	12	4.8
	12 months	7	34	13.7	12	5.4
Negative symptoms	Baseline	7	37	17.2	16	5.6
	2 months	7	35	15.7	14	5.7
	6 months	7	34	15.7	14	6.1
	9 months	7	33	15.2	14	5.6
	12 months	7	32	15.2	14	5.7
Avolition-apathy	Baseline	7	19	9.5	7	2.7
	2 months	7	17	9.9	5	2.6
	6 months	7	17	9.5	4	2.7
	9 months	7	17	10	3.5	2.6
	12 months	7	16	10	3	2.6
Expressive deficits	Baseline	7	16	9	6	1.9
	2 months	7	15	9.2	4.5	1.9
	6 months	7	14	9.2	3	1.9
	9 months	7	14	8.7	3	1.8
	12 months	7	14	8.9	3	1.9
Total negative symptoms	Baseline	39	118	68.2	67	14.3
	2 months	32	104	59.8	59	15.2
	6 months	30	94	58.4	58	16.2
	9 months	30	93	56.9	55	14.6
	12 months	31	109	58.0	56	17.2

The mean scores of the avolition-apathy sub-domain and expressive deficits sub-domain are detailed in figure fifteen.

Figure 15: The mean and median total PANSS negative symptom scores, avolition-apathy and expressive deficits sub-domain scores over twelve months.



5.3.1.4 **Description of longitudinal Extrapyramidal Side Effects**

The mean, minimum, maximum, mean and standard deviations from the SAS over the twelve months of participation are detailed in table fifteen.

Table 15: The minimum, maximum, mean and standard deviation for the SAS for each assessment over the twelve months of participation.

	Minimum	Maximum	Mean	Std. Deviation
SAS baseline	0	15	1.5	2.4
SAS 6-month assessment	0	10	.9	1.7
SAS 12-month assessment	0	13	.8	2.0

5.3.2 Bivariate analyses

Preliminary analyses revealed that the data was not normally distributed; therefore, a Spearman's correlation was used to assess the correlation between key outcome variables at each time point (see table 16).

Table 16: The correlation between positive symptoms, negative symptoms avolition-apathy, expressive deficits, and depression across the 12 months of study participation.

	Baseline	2 months	6 months	9 months	12 months
	CDSS	CDSS	CDSS	CDSS	CDSS
Total PANSS positive	.28**	.29**	.43**	.38**	.45**
Total PANSS negative	.12	.31**	.20*	.28**	.30**
Expressive deficits	.00	-.16*	.33**	.34**	.35**
Avolition-apathy	.20**	-.11	.45**	.57**	.45**

**p < .005, *p < .05.

DUP did not correlate with any of the PANSS items at two months, six months, nine months or twelve months.

5.3.3 Multivariate analyses

5.3.3.1 Linear mixed effect model – general design

Separate linear mixed effect models were constructed for longitudinal total negative symptom scores and for the two sub-domains as dependant variables.

Longitudinal scores of the predictor variables were included in the models if they significantly correlated with negative symptoms and sub-domains or predicted this domain in the linear regression models (see appendix 9). All variables were treated as time-varying co-variates. The study design involved clustering effects (visit nested within participants), so a hierarchical model of one layer was developed to reflect the study design and investigate the relationship between depression and negative symptoms. A restricted maximum likelihood approach was adopted in the construction of the model to estimate covariance parameters and provides an estimate of the variance components taking into consideration an unbalanced or incomplete data set. An unstructured covariance matrix was specified in the analysis, to ensure that there were no constraints on the values and therefore each variance and covariance was estimated from the data thus providing the best model fit and an accurate reflection of the data.

5.3.3.2 **Linear mixed effect model – total negative symptoms**

Table 17: The linear mixed effects model predicting longitudinal negative symptom scores from and longitudinal depression, positive symptoms, assessment and EPSE.

Domain	B Coefficient	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
Depression	.15	.05	2.63	**.008**	.03	.26
EPSE (SAS)	.24	.10	2.33	**.020**	.03	.45
Positive symptoms	.24	.05	4.57	**.000**	.13	.34
Assessment	-.31	.11	-2.81	**.005**	-.53	-.09

Log likelihood = -1391.76. Wald $\chi^2 = 78.49$, $p < .001$.

Significant predictors of negative symptoms were depression, EPSE, positive symptoms, and assessment time point. The B coefficients indicate that as CDSS depression

scores increase by one, negative symptoms will increase by .15 units; as EPSE increase by one unit, negative symptoms will increase by .24 units; as positive symptoms increase by one unit, negative symptoms increase by .24 units; and negative symptoms reduce by .31 units at each assessment.

5.3.3.3 **Linear mixed effect model – avolition-apathy**

Table 18: The linear mixed effects model predicting longitudinal avolition-apathy from longitudinal depression and EPSE.

Domain	B Coefficient	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
Depression	.16	.02	5.40	**.000**	.10	.21
EPSE (SAS)	.07	.05	1.42	.156	-.03	.19
Assessment	-.20	.06	-4.33	**.000**	-.38	-.14

Log likelihood = -1102.21. Wald $\chi^2 = 64.79$, $p < .001$.

Significant predictors of avolition-apathy were depression and assessment time point. EPSE was not significantly associated with avolition-apathy longitudinally. B coefficients indicate that as depression increases by one unit, avolition-apathy scores increase by .16 units; and avolition-apathy scores reduce by .20 units with each assessment. This analysis was repeated, excluding the non-significant EPSE scores, which included all 204 participants with 765 observations. This indicated that depression was significantly associated with avolition-apathy.

5.3.3.4 **Linear mixed effect model – expressive deficits**

Table 19: The linear mixed effects model predicting longitudinal expressive deficits from longitudinal depression and EPSE.

Domain	B Coefficient	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
Depression	.01	.03	.52	.601	-.04	.07
EPSE (SAS)	.13	.06	2.20	**.028**	.01	.25
Assessment	-.12	.06	-2.08	**.038**	-.24	-.00

Log likelihood = -1139.54. Wald χ^2 = 10.85, p = .01.

Significant predictor of the expressive deficits sub-domain is EPSE. CDSS depression was not significantly associated with expressive deficits. B coefficients indicate that as EPSE scores increase by one unit, expressive deficits scores increase by .13 units; and expressive deficits scores reduce by .12 units at each assessment.

5.4 **Discussion**

The aim of this study was to explore the longitudinal relationship between depression and negative symptoms, adopting a sub-domain approach to negative symptoms. This study revealed that the negative symptom sub-domains relate differentially to depression; specifically, depression was associated with avolition-apathy longitudinally. However, depression was not associated with expressive deficits in the regression model. Depression was associated with total negative symptoms; however, this may be largely driven by the strong association between avolition-apathy and depression.

Viewing negative symptoms as being formed of two sub-domains may account (in part) for the inconsistent findings previously generated. Total negative symptoms largely reflect the sum of avolition-apathy and expressive-deficits. However, examining total negative symptoms ignores the variability in avolition-apathy and expressive-deficits scores within, which may appear the same in the PANSS total negative symptom score, but reflect differing sub-domain scores. Therefore, previous findings exploring the relationship between depression and negative symptoms that have examined negative symptoms using a total negative symptom score may not have detected a relationship between depression and negative symptoms. This may be because this relationship is driven by avolition-apathy, which may have been masked by expressive-deficits making up the total negative symptom score.

This study was the first study to explore the longitudinal relationship between depression and negative symptoms, viewing negative symptoms as being formed of two sub-domains. Some studies have identified correlations between negative and depressive symptoms (Pogue-Geile, 1989, Dollfus and Petit, 1995b). However, more recent studies (particularly studies using more sophisticated measures to assess depression such as the CDSS) have been able to discern depressive features from negative symptoms (Rocca et al., 2005), however few have separated expressive deficits from avolition-apathy. Stiekema et al. (2016) identified that depression is related to avolition-apathy and not expressive deficits in a chronic schizophrenia sample, however the stability of this relationship in early schizophrenia had not been explored. This relationship between depression and negative symptoms is in agreement with the systematic review presented in the introductory chapters by Krynicki et al. (2018). This review found that the area of symptom co-occurrence between depression and negative symptoms appears occupied by the avolition-apathy sub-domain. The present study also confirms these findings and establishes that this influence may occur longitudinally.

The difference in males and females experiencing negative symptoms is consistent with previous findings. Ring et al. (1991) identified that men were nearly twice as likely to experience negative symptoms, thus supporting the present findings that men had higher negative symptoms than women. Depression was found to be higher in White and Asian participants. This is in line with previous research findings that has reported that those of a minority ethnic background experience more depressive symptoms in schizophrenia (Delahanty et al., 2001). The present findings that White individuals experience high rates of depression may be due to the high number of white participants.

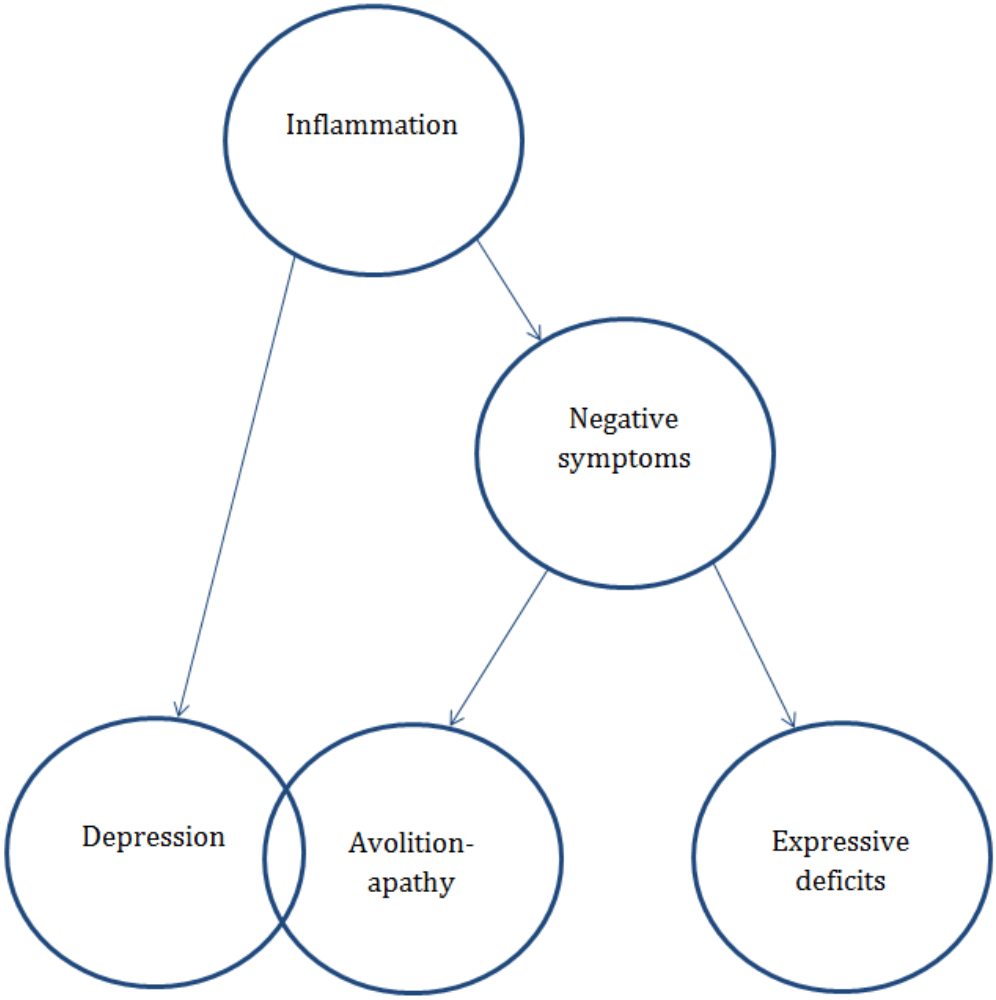
It is clear from the regression model presented in the baseline data analysis that the presence of depression accounts for just 5% of avolition-apathy, suggesting that the vast majority of variance in avolition-apathy cannot be accounted for by the presence of depression, thus confirming that depression and avolition-apathy are two separate constructs. However, given that there is no clear measure of anhedonia (unlike the Schedule for the Assessment of Negative Symptoms - SANS) (Andreasen, 1989), and that the negative symptom section of the PANSS interview does not naturally lend itself to being assessed using a sub-domain approach to negative symptoms, this figure may not represent a participants hedonic experience, and therefore may not accurately reflect the relationship between negative symptoms and depression. That said, these findings do provide partial support for the dimensional approach to psychosis (McGorry et al., 1998), suggesting overlapping domains or dimension of symptoms as depression and some negative symptoms are associated with one another. These findings suggest that these symptom domains overlap and should therefore be considered as belonging to both the depressive and negative syndromes.

It is important to acknowledge the significant correlations between expressive deficits and depression, and the lack of significant correlation between avolition-apathy and depression at the two month assessment. Both depression and negative symptoms have been associated with low dopaminergic activity in the prefrontal cortex. The relationship between dopaminergic dysfunction and negative symptoms has been highlighted by Okubo et al. (1997), who used PET imaging in patients with schizophrenia and found that irregular availability of cortical dopamine receptors in the prefrontal cortex is related to the severity of negative symptoms (such as emotional withdrawal) and

performance on the Wisconsin card sorting task. Depression may relate differentially to negative symptoms, such as a neurocognition relates with expressive deficits and not avolition-apathy. This may also account for the lack of correlation between avolition-apathy and depression at the two month assessment, as depression and avolition-apathy may be related through an emotion dysregulation of depression.

The schematic presented in the introductory chapters hypothesises that there is an association between depression and avolition-apathy (see figure 16). The present data suggests that depression and avolition-apathy are associated, and expressive deficits are independent of this relationship. However, it is currently unclear which mechanism underpins this relationship.

Figure 16: Putative relationship between depression and negative symptoms being associated with inflammation.



The association between depression and avolition-apathy may reflect a common biological aetiology underpinned by dopaminergic dysfunction, which has been implicated in both depression and negative symptoms (Voruganti et al., 2001, Dunlop and Nemeroff, 2007). Negative symptoms have been linked to diminished dopaminergic neurotransmission, particularly in the prefrontal cortex (Weinberger and Laruelle, 2002, Davis et al., 1991). This has been supported by both imaging studies (Weinberger and Laruelle, 2002) and studies that have observed treatment response following medication

administration known to enhance dopaminergic functioning, such as amisulpride (Boyer et al., 1995). There is also evidence of impaired dopaminergic functioning in depression (Swerdlow and Koob, 1987, Dunlop and Nemeroff, 2007), therefore these two symptom clusters may be the consequence of dopaminergic hypofunctioning, particularly in the prefrontal cortex, which may account for the association between these symptom clusters. This may be triggered by activation of pro-inflammatory cytokines (Maes et al., 1990, Maes, 1995, Noto et al., 2015a) leading to neuro-inflammation, white matter abnormalities, neuronal degeneration and decreased neurogenesis with a disturbance of dopaminergic functioning (Monji et al., 2009). However, this explanation does not account for the preferential relationship between depression and avolition-apathy (excluding expressive-deficits).

Psychological explanations to negative symptoms may hold explanatory power to understanding this relationship. Beck describes a cognitive triad of depression, whereby "life is pointless, the future is hopeless, and the self is worthless" (Beck, 1970). Depression may therefore lead to increasing social isolation which is exacerbated by a lack of positive reinforcement, which may lead to increased apathy and social isolation. Furthermore, anhedonia may emerge following social isolation, which may contribute to the development of the negative syndrome, secondary to depression. The stigma and shame that may be experienced by individuals with schizophrenia may be a source of depression, which may cause negative symptoms due to feelings of worthlessness contributing to apathy and anhedonia.

The stability of this relationship also indicates that early depression may be a useful marker of later avolition-apathy (as depression has been shown to be associated with avolition-apathy in the regression model). Therefore, the early identification of depression is crucial, not least because of its association with deliberate self-harm (Harvey et al., 2008, Siris, 2001). Moreover, depressive symptoms have been associated with increased psychotic relapse and hospitalisation (Tollefson et al., 1999, Conley et al., 2007) and a poorer quality of life (Reine et al., 2003). In a prospective study of 2228 people with schizophrenia over three years by Conley et al. (2007), those who were depressed at enrolment (n = 877, 39.4% of the total sample) compared to non-depressed were less likely to engage in the mental health services (such as emergency psychiatric services), pose a safety concern (such as violence, suicidal or victimised), experience greater social problems (such as substance misuse) and report poorer life satisfaction, quality of life and poorer family relationships. Interestingly in this sample, those who changed their depressed status (i.e. recovered from their depressive episode) reported improved changes in their functional outcomes, and those who became depressed reported poorer functional outcomes compared to the non-depressed group. These findings indicate that depression is a key predictor of outcome in schizophrenia.

Depression may also be intrinsic to the schizophrenia disease process itself. The Jacksonian hierarchy postulates that dysfunctional neurobiological pathways sufficient enough to cause schizophrenia will also cause disruption to lesser neural networks responsible, for example, those responsible for depression. Therefore, suggesting that lesser neurobiological abnormalities will be present if the mental illness is sufficiently severe (Upthegrove, 2009) rather than being a consequence of neurotoxicity following

antipsychotic treatment (as EPSE was controlled for in the analysis). Depression has also been explained as being 'revealed' following successful treatment of psychotic symptoms (Upthegrove et al., 2016); although results in this present study do not wholly support this, as positive symptoms were significant in only some of the models. Another explanation for this relationship is that depression may emerge because of a person's appraisal of their positive symptoms (such as voices), and adopt a subordinate relationship to these experiences, or new social status (Upthegrove et al., 2014b). These factors may lead to biases in low expectations of success and pleasure, driving avolition-apathy in the negative syndrome (Rector et al., 2005).

Conceptualising negative symptoms as being formed of two distinct sub-domains may enhance our understanding of how these disabling symptoms interact with other dimensions within psychosis. Previous findings have suggested that avolition-apathy and expressive deficits differ in terms of their course of illness (Strauss et al., 2012, Strauss et al., 2013). Avolition-apathy has also been shown to be associated with poorer functional outcomes (such as employment), have greater social cognitive deficits and have a gradual onset of psychosis (Messinger et al., 2011, Strauss et al., 2013), whereas expressive deficits are associated with deficits in emotional perception and are more likely to require a longer hospital admission (Strauss et al., 2013). Research investigating the relationship between functional recovery and negative symptoms has suggested a direct relationship between functional recovery and negative symptoms. A longitudinal study of 209 patients with schizophrenia by Álvarez-Jiménez et al. (2011) found that functional recovery predicted later negative symptoms, suggesting that functional and vocational recovery is crucial in preventing the development of negative symptoms. Moreover, Stiekema et al.

(2016) found expressive deficits to be associated with aspects of everyday functioning, behavioural problems and cognitive disability whereas avolition-apathy was related with social and emotional aspects of functioning. Being able to differentiate these two sub-domains early in the course of illness may not only allow early targeted intervention to improve outcomes but may also serve as a prognostic marker to predict future course of illness thus providing a therapeutic target to develop intervention techniques. Finally, early intervention in depression in schizophrenia may have important implications in functional recovery (Fenton Wayne, 2010), as depression is a key predictor of functional recovery in early schizophrenia (Conley et al., 2007), therefore implying that depression is a viable target for improving outcomes.

5.4.1 Strengths and limitations

One strength of this study is the relatively large number and longitudinal assessments. Another advantage is the statistical method in the analysis. Linear mixed effect modelling is used to assess the longitudinal relationship between variables, including all participants in the analysis. As all participants are entered into the analysis, statistical power is not compromised due to attrition.

However, findings from this study must be considered in light of its limitations. The method used to assess negative symptoms has its disadvantages. Whilst the PANSS interview offers adequate coverage of assessing global pathology, there is a lack of emphasis on the subtle nuances of negative symptoms. Indeed, there is no measure of anhedonia, which is one of the negative symptoms. Other negative symptom scales such

as the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984) or the Clinical Assessment Interview for Negative Symptoms (CAINS) (Horan et al., 2011) provides a more comprehensive coverage of the negative symptoms as well as reflecting current conceptions of sub-domains within negative symptoms (such as the CAINS) (Horan et al., 2011). That said, the PANSS interview has been shown to support this sub-domain approach in factor analytic studies (Stiekema et al., 2016). An important limitation to recognise in this study is that the CDSS was created specifically to separate depression from negative symptoms in schizophrenia. Therefore, the findings from this study are particularly significant given the positive association between depression and avolition-apathy.

Furthermore, DUP was calculated on just 121 participants, thus falling far below the total number of participants included in the analysis. However, given that DUP did not correlate with any of the predictor variables, it can be safe to conclude that DUP would be unlikely to significantly impact on the results of the regression analyses, if the data set was complete. Finally, the majority of white participants (n = 111) may be a potential source of bias and therefore effect the generalisability of results. Given that the sites were chosen to reflect both urban and rural populations and ethnically diverse groups, the high number of white participants may suggest selection bias.

These limitations are particularly important considerations and may limit the conclusions that are drawn from this data set, particularly the generalisability to other ethnic groups. Also, the high dropout rate may indicate that the assessments are not

suitable for this population, or because the sample was too unwell, therefore limiting conclusions drawn.

5.4.2 Implications

These findings have implications for our conceptualisation of negative symptoms. Distinguishing between avolition-apathy and expressive deficits is important given the differing clinical predictors and putative biological underpinnings associated with these two sub-domains (Strauss et al., 2013). Viewing negative symptoms as being composed of two sub-domains may enhance our understanding of its impact on prognosis, and may also provide a novel, more refined target for treatment.

Clinical implications can also be taken from these findings, albeit tentatively. Targeted therapy focusing on individual sub-domains may be a useful treatment option. Addressing erroneous cognitions thought to drive anhedonia (Staring et al., 2013) may lead to a reduction in avolition-apathy. Cognitive behavioural therapy may be used to treat anticipatory anhedonia (Staring et al., 2013) through targeting erroneous cognitions thought to underlie this symptom. Pharmacological treatment strategies have been found to preferentially treat either avolition-apathy (through the use of citalopram or selegiline) (Barnes et al., 2016) or expressive deficits (through the use of galantamine to treat alogia) (Conley et al., 2009, Azorin et al., 2014). This reduction in alogia through the use of galantamine may be due to cognitive enhancing properties of galantamine through its' action on the nicotinic receptor, which in turn improves processing speed and verbal memory (Conley et al., 2009). Furthermore, pharmacological treatment options such as

antidepressant medication, may lead to a reduction in avolition-apathy and depression, but had no effect on expressive deficits (Barnes et al., 2016). Therefore, a more tailored treatment plan could be used to help reduce either avolition-apathy or expressive-deficits rather than a single pharmacological agent to alleviate global negative symptoms. These findings bolster the potential relationship and shared putative biological markers underlying depression and avolition-apathy sub-domain of negative symptoms.

6. Immune dysfunction, depressive and negative symptoms in schizophrenia

6.1 Introduction

The negative symptoms of schizophrenia, comprising of affective blunting, alogia, anhedonia, asociality and avolition, apathy (Andreasen, 1982), have been associated with longitudinal grey matter tissue loss as reported in a structural brain imaging study. McKechanie et al. (2015) used MRI and whole brain tensor-based morphometry on forty-three adolescents at risk for psychosis at two time points over six years and found those with significant negative symptoms had marked grey matter loss particularly in the left temporal lobe, the left cerebellum, the posterior cingulate and the left inferior parietal sulcus compared with those without significant negative symptoms, over the six-year period.

One mechanism for grey matter loss in schizophrenia is neuro-inflammation. Inflammation is the body's response to harmful stimuli and is triggered by the immune system, consisting of the innate (older and less able to adapt to pathogens) and adaptive (acts on immunological memory and re-exposure) immune system. These two arms are co-ordinated by cytokines, which can either be pro- or anti- inflammatory. Consistently replicated findings have shown elevated pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8 (Lin et al., 1998, Zanardini et al., 2003) as well as reduced anti-inflammatory cytokines, such as IL-4, and IL-10 (Noto et al., 2015a) in medicated

schizophrenia patients. These findings have been supported by a meta-analysis of sixty two studies (n=2298 with schizophrenia, 1858 healthy controls) (Potvin et al., 2008) and a meta-analysis of positron emission tomography (PET) (n = 5) and a post mortem study (n = 12) by (Laskaris et al., 2016). Khandaker et al. (2014) examined a sample of over 4500 children and found that those with higher IL-6 levels at nine years of age were more likely to be depressed/diagnosed with a psychotic disorder at age eighteen compared with those with low IL-6 levels at age nine. Moreover, two meta-analyses involving medication naïve patients (Upthegrove et al., 2014a), and those in acute relapse (Miller et al., 2011) appear to have higher cytokine concentrations (such as IL-6 and TNF-α). Increased pro-inflammatory cytokine concentrations have been associated with positive symptomology (Khandaker and Dantzer, 2016), and appear to normalise upon administration of antipsychotic medication (Miller et al., 2011). Negative symptoms in those at clinical high risk for psychosis have also been associated with immune dysfunction. Goldsmith et al. (2019) found that baseline TNF and IL-6 were associated with the development of negative symptoms, and depressive symptom severity, in thirty-seven individuals considered at clinical high risk for psychosis, who were followed up over twelve months. These findings add to the evidence base of immune dysfunction in schizophrenia, but also suggests that inflammatory cytokines may be specific to negative and depressive symptoms in those at risk for psychosis.

One putative explanation to account for grey matter loss in schizophrenia is that microglia, in the activated state, produce pro-inflammatory cytokines (such as IL-6). Microglial cells represent the first line of immune defence in the central nervous system (Kettenmann et al., 2011) and, in the non-activated state, are involved in synaptic pruning,

maintenance and phagocytosis of debris from damaged cells. When activated, microglia adopt an amoeboid morphology to enable migration to the pathogen site (Kettenmann et al., 2011). Abnormal microglial functioning may be consequent of the abnormal neural pruning, prenatal complications resulting in an immune reaction following ischemic brain trauma, or schizophrenia-associated immune dysfunction (Munn, 2000). The microglial hypothesis of schizophrenia (Monji et al., 2009, Munn, 2000) postulates microglia becomes activated following stressful life events, which triggers the release of several pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6 and IL-8) and free radicals (such as nitric acid, and superoxide). This causes secondary neural degeneration, and both white and grey matter abnormalities, decreased neurogenesis, synaptic dysfunction, and enlarged ventricles (Owens et al., 1985) contributing to disease progression (Monji et al., 2009, Munn, 2000). Support for microglial activation has come from recent PET imaging findings that found individuals with schizophrenia and at risk of psychosis showed elevated microglial activity indicating neuro-inflammation is linked with risk for psychosis (Bloomfield et al., 2016). However, this study drew from a small sample and few participants at ultra-high risk for schizophrenia transitioned to schizophrenia. Moreover, these findings have not been replicated in other studies (Di Biase et al., 2017).

The glutamate hypothesis of schizophrenia implies there is glutamatergic neurotransmitter dysfunction at the N-methyl-D-aspartate (NMDA) receptor (Tamminga, 1998). NMDA receptors are excitatory cation channels which are important in synaptic transmission and neuronal plasticity (Coyle, 2006). NMDA hypofunctioning has been associated with schizophrenia, and healthy individuals medicated with ketamine (NMDA channel blocker) experienced symptoms similar to the positive and negative syndrome

(such as alterations in perception and impaired performance on cognitive assessments) (Krystal et al., 1994). Sustained increase in pro-inflammatory cytokines (such as IL-6 and TNF-α) raises kynurenic acid concentration (Erhardt et al., 2007, Schwieler et al., 2015). An increase in kynurenic acid leads to an increased production of nitric oxide, which may contribute to NMDA hypofunctioning. This acts as an NMDA receptor antagonist, which may account for the glutamatergic hypofunction seen in schizophrenia (Erhardt et al., 2007).

Depression has also been associated with activation of the immune system (Maes, 1995). Elevated pro-inflammatory markers, such as IL-6, TNF-α and CRP, have been reported in people with depression (Young et al., 2014, Valkanova et al., 2013, Haapakoski et al., 2015, Liu et al., 2012). Furthermore, administration of anti-depressant medication has been suggested to exert an immunoregulatory effect, which results in a decrease in production of pro-inflammatory markers and increase in anti-inflammatory cytokines (Maes et al., 1999, Xia et al., 1996, Sutcigil et al., 2007). As outlined above, evidence suggests that schizophrenia is associated with an elevation in inflammatory markers, characterised by increases in IL-6, TNF-α and CRP concentrations compared to healthy controls (Sasayama et al., 2013, Al-Hakeim et al., 2015). A cross-sectional study of thirty-nine individuals with schizophrenia or schizoaffective disorder by Noto et al. (2011), found positive correlations between brain derived neurotrophic factor (BDNF, which is a polypeptide involved in neuronal growth, differentiation, survival and repair) and depression, and IL-1β and severity of PANSS scores. These findings suggest there are distinct immunological pathways to depression and schizophrenia. However, Khandaker et al. (2014) has suggested there may be a common underlying pathway leading to

depression and schizophrenia. Khandaker et al. (2014) followed up 4500 individuals from childhood into adulthood obtaining clinical and blood samples from participants. They found that participants in the top third of IL-6 scores at age nine (compared to the bottom third) were more likely to be depressed or have psychotic experiences at eighteen years of age. These findings indicate that there may be a common immunological pathway underlying depression and schizophrenia.

A longitudinal study of thirty-nine hospitalised patients with schizophrenia over five years found that negative symptoms (such as psychomotor retardation, flattened affect and poverty of speech) did not significantly reduce over time (Pogue-Geile and Harrow, 1985). These findings were supported by Dollfus and Petit (1995b), who found symptoms such as affective flattening and attentional impairment did not significantly change over three years. These findings suggest that negative symptoms may remain stable throughout the course of schizophrenia (Pogue-Geile and Harrow, 1985). However, much of the previous research adopted the view that the negative symptoms represented a single symptom domain, rather than being composed of two sub-domains.

In a study by Upthegrove et al. (2010) of ninety-two patients with schizophrenia who were followed up over twelve months, 22% were depressed at each follow up assessment, 17% were depressed in the prodrome and acute phases but were not depressed at follow-up, 20% were not depressed in the prodrome but were depressed at the follow-up assessment and 20% did not experience any depressive episode throughout study participation. These findings suggest that depression may fluctuate throughout the course of schizophrenia (Upthegrove et al., 2010). Thus, the implication may be that

depression would be associated with state inflammatory markers (such as IL-6 and CRP), whereas trait cytokines (such as TNF-α) would be associated with negative symptoms.

Given that depression may have some specific association to the avolition-apathy sub-domain of negative symptoms, as shown in the previous studies of this thesis, it is predicted that increases in state cytokines and acute phase proteins (such as IL-6 and CRP) will be associated with depression and avolition-apathy sub-domain of negative symptoms in schizophrenia. It is also predicted that increased trait cytokines (such as TNF-α) will be associated with the expressive-deficits sub-domain of negative symptoms.

6.1.1 Aim

This research aimed to further elucidate the relationship between depression and negative symptoms. Previous research presented in this thesis has identified that avolition-apathy and depression were related, and expressive deficits were independent of this relationship. To further explore this relationship, this research focused on pro-inflammatory markers that are associated with depression and negative symptom domains in schizophrenia.

6.1.2 Predictions

1. Depression and the avolition-apathy sub-domain of negative symptoms will be associated with state pro-inflammatory markers (IL-6 and CRP).
2. The expressive deficits sub-domain of negative symptoms will be associated with stable, trait markers (TNF-α).

6.2 Methods

6.2.1 Design

This study employed an experimental design to define the pro-inflammatory markers associated with depression and negative symptoms in schizophrenia, and how these might change longitudinally over twelve months.

6.2.2 Sample

Two hundred and seven participants experiencing psychosis were recruited between April 2013 and March 2015 from all eight of the participating BeneMin sites. Participants were drawn from the EIP services. Participants adhered to the following inclusion criteria (see table 20).

Table 20: The inclusion and exclusion criteria for study participation.

Inclusion criteria	Exclusion criteria
Be male or female aged 16 to 40	Not currently abuse illicit substances
Meet DSMIV criteria for schizophrenia, schizophreniform or schizo-affective psychosis	Not pose a serious suicidal or violent risk
Be in a current episode as defined by the presence of positive symptoms (a score >2 on P1, 2, 3 or 6 on PANSS).	Not have taken an investigational medicinal product within 30 days of baseline assessments.
Be within 5 years of symptom onset	
Be fluent in English	
Have capacity to provide informed consent	

6.2.3 Materials

Demographic information such as age, gender and ethnicity were obtained at baseline, as well as height and weight at baseline and 12 months to calculate BMI (as BMI has been suggested to impact on cytokine levels, especially IL-6 (Lee et al., 2017).

Negative symptoms were assessed at all time points (baseline, two month, six month, nine month and twelve month assessments) using the PANSS (Kay et al., 1987) (see appendix 6). A measure of avolition-apathy was constructed using PANSS items G13 (disturbance of volition), N2 (emotional withdrawal) and N4 (passive/apathetic social withdrawal). Expressive deficits were assessed using PANSS items N1 (blunted affect), N3 (poor rapport) and N6 (Lack of spontaneity and flow of conversation). This method of differentiating between the avolition-apathy and expressive deficits sub-domains of negative symptoms appears to provide an adequate reflection of these sub-domains, and this was based on factor analysis used in previous research (Barnes et al., 2016, Liemburg et al., 2013).

Depression was assessed by the total score of the Calgary Depression Scale in Schizophrenia (see appendix 7) at all time points (baseline, 2 months, 6 months, 9 months and 12 months).

Extrapyramidal side effects were assessed using the Simpson Angus scale at baseline and twelve months (see appendix 8).

Inflammatory-marker analysis: blood samples were taken at baseline, six months, and twelve months of participation. Samples were taken in a 9ml ethylenediamine tetraacetic acid (EDTA), which were then placed in a centrifuge for fifteen minutes at 2000g. Plasma was then extracted, frozen (at -80^{o}C) and then processed by Meso Scale Discovery Piezoelectric Plate, with high sensitivity immunoassays at King's College London. Multiplex-assays included IL-6, TNF-α, IFN-gamma, IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-13, GM-CSF, IL-1alpha, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF, and CRP. For this analysis IL-6, TNF-α, and CRP were selected as primary inflammatory markers of interest because there is a strong evidence base to suggest that these inflammatory markers are consistently raised in medication naïve patients with schizophrenia (Upthegrove et al., 2014a). Also, given that CRP and IL-6 are state inflammatory markers and TNF-α is a trait pro-inflammatory cytokine (Miller et al., 2011), these markers were selected to assess whether state/trait cytokines are associated differentially with negative symptoms, and depression. This was supported by a factor analysis, which can be found in the exploratory analysis section in appendix ten.

6.2.4 Statistical analysis

All data were analysed using Statistical Package for the Social Sciences (SPSS v. 22) and STATA (v. 10). All data were treated as interval data and analyses were carried out were of one tailed significance unless otherwise stated and the alpha level was set at .05. Spearman's correlation was used to explore the correlation between symptom severity of each of the clinical variables of interest (depression, total negative symptoms, avolition-apathy and expressive deficits) and key markers of inflammation (IL-6, CRP and TNF-α).

Participants with a CRP of above ten were excluded from this analysis, as this would indicate an acute increase in the immune response (such as resulting from acute infection). Significant correlating variables were then entered into a linear mixed effects modelling, along with non-significant cytokines and BMI, to assess the longitudinal association between the clinical measures of symptom severity and cytokines.

6.3 **Results**

6.3.1 Descriptive statistics

A description of the sample and clinical measures has been provided in the previous study of this thesis. 188 participants were included in the analysis at baseline, 109 at six months and 97 at twelve months as blood samples were only available in these participants.

Most of the cytokines were not normally distributed (with the exception of TNF-α at baseline and six months) and data remained non-normally distributed after log transformation, therefore non-parametric tests were used. Table twenty-one shows the Kolmogorov-Smirnov test for normality for the variables entered into analysis. Variables with a *p* value of $p<.05$ were not normally distributed.

Table 21: The Kolmogorov-Smirnov test for normality of the cytokines/acute phase proteins included in the analysis.

Cytokine/Acute phase protein	Visit	Statistic	Significance
Hs-CRP	Baseline	.21	.00
	6 months	.32	.00
	12 months	.35	.00
IL-6	Baseline	.15	.00
	6 months	.20	.00
	12 months	.18	.00
TNF-α	Baseline	.06	.20
	6 months	.06	.20
	12 months	.09	.05

The mean, median, range and standard deviation of each cytokine/acute phase protein has been separated by visit (see table 22).

Table 22: The range, mean, median and standard deviation for each inflammatory marker broken down by each visit after excluding participants with a CRP greater than 10 at each time point.

Cytokine/Acute phase protein	Visit	Range	Mean	Median	Standard deviation (*SD*)
Hs-CRP (mg)	Baseline (n = 188)	.10 – 9.70	2.51	1.45	2.37
	6 months (n = 109)	.10 – 9.10	2.46	2	2.14
	12 months (n = 97)	.10 – 7.90	2.47	1.90	2.10
IL-6 (pg/mL)	Baseline (n = 188)	.01 – 3.83	.70	.57	.48
	6 months (n = 109)	.01 – 2.04	.73	.64	.38
	12 months (n = 97)	.01 – 3.97	.74	.67	.52
TNF-α (pg/mL)	Baseline (n = 188)	1.07 – 5.77	2.50	2.43	.64
	6 months (n = 109)	1.36 – 4.75	2.52	2.49	.52
	12 months (n = 97)	1.55 – 5.25	2.56	2.40	.64

Figures seventeen to nineteen show the mean scores of each inflammatory marker of interest (CRP, IL-6 and TNF-α) over twelve months of study participation.

Figure 17: Longitudinal mean CRP levels (mg).

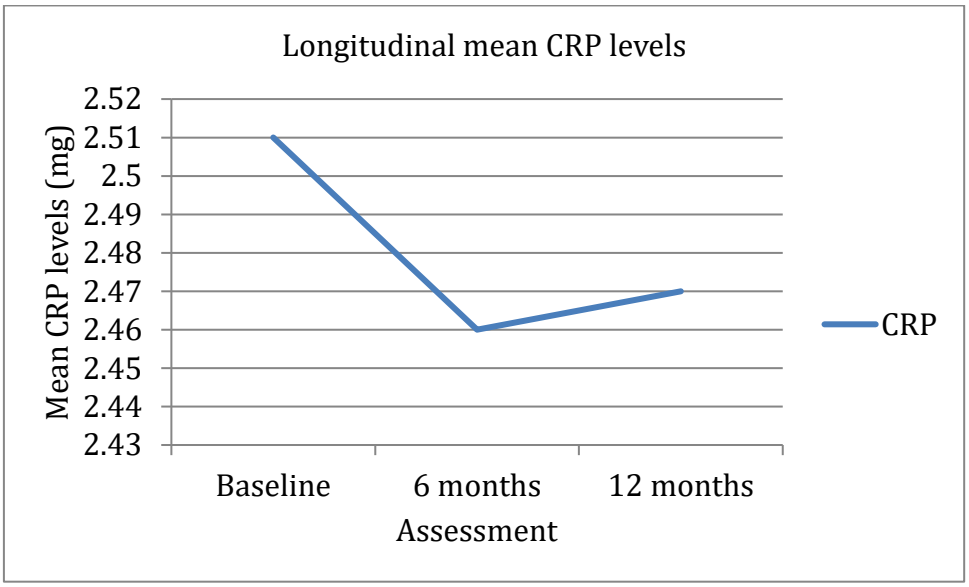


Figure 18: Longitudinal mean IL-6 levels (pg/mL).

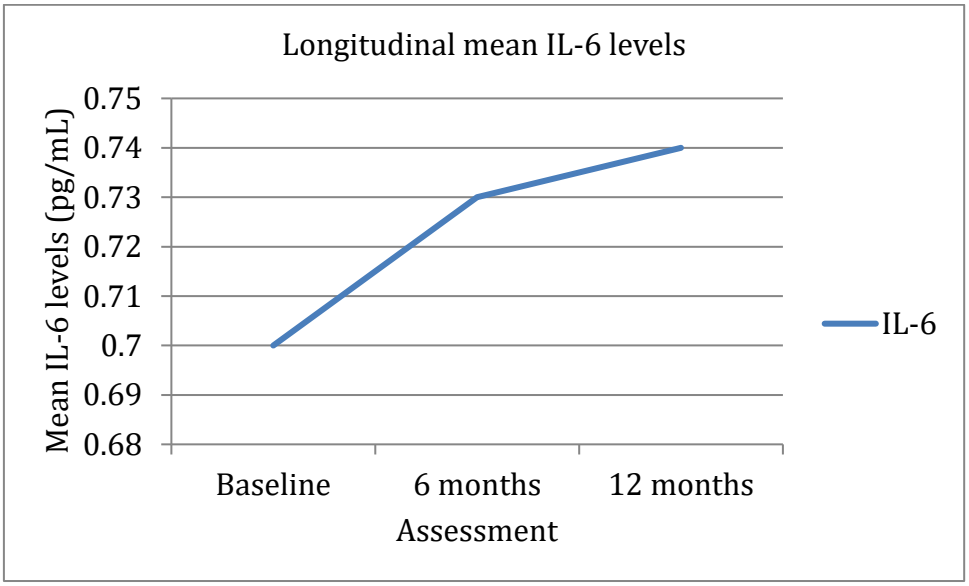
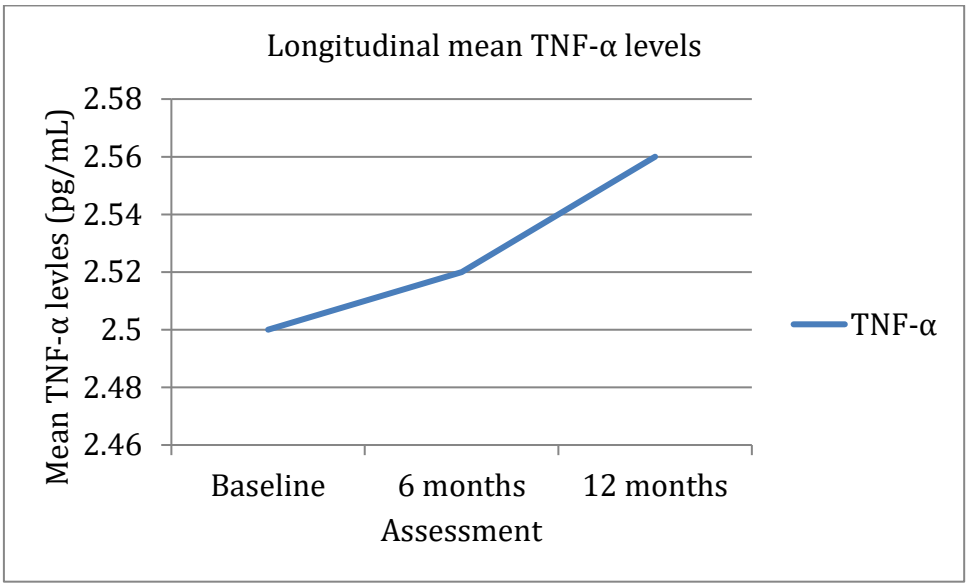


Figure 19: Longitudinal mean TNF-α levels (pg/mL).



Wilcoxon Signed Rank Tests did not reveal statistically significant changes in any marker of inflammation over time (CRP, z = -1.00, p = .31, effect size = .10, baseline Md = 1.45, 12-month median = 1.90; IL-6, z = -.10, p = .91, effect size = .01, baseline Md = .57, 12-month median = .67; TNF-α, z = -1.37, p = .16, effect size = .14, baseline Md = 2.43, 12-month median = 2.40).

Body mass index (BMI) was assessed using routine measures and calculated at baseline and twelve months as BMI is considered to exert some influence on inflammatory cytokines. BMI ranged from 16.3-60.2 at baseline, (n = 197, M = 27.9, *SD* = 7.0), and 17.9 – 54.9 at twelve months (n = 111, M = 29.4, *SD* = 7.1). Wilcoxon Signed Rank Test revealed a statistically significant reduction in BMI over 12 months z = -3.20, p < .001, effect size = .22.

6.3.2 Bivariate analyses

6.3.2.1 Correlations between key outcome variables, cytokines and BMI

Table twenty-three shows the correlations between positive symptoms, the two sub-domains of negative symptoms (expressive deficits and avolition-apathy), total negative symptoms, depression, and BMI.

Table 23: Spearman's correlation between total positive symptoms, total negative symptoms, expressive deficits avolition-apathy, depression, BMI, and EPSE (SAS) at baseline, 6 months and 12 months.

	Baseline			6 months	12 months		
	BMI	CDSS	SAS	CDSS	BMI	CDSS	SAS
Total PANSS positive	-.12	.28**	-.04	.43**	.11	.45**	.03
Total PANSS negative	.02	.12	-.00	.20*	.07	.30**	.26**
Expressive deficits	.03	.00	-.03	.33*	.03	.35**	.50**
Avolition-apathy	.04	.20**	.01	.46**	.06	.45**	.44**

***p <.001, *p < .05*

Table twenty-four shows the correlations between cytokines/acute phase proteins (CRP, IL-6 and TNF-α) at baseline, six months and twelve months.

Table 24 Spearman's correlation between inflammatory markers CRP, IL-6 and TNF-α at baseline, 6 months and 12 months.

	Baseline		6 months		12 months	
	IL-6	TNF-α	IL-6	TNF-α	IL-6	TNF-α
CRP	.44**	.28**	.42**	.34**	.48**	.29*
IL-6	-	.34**	-	.28**	-	.34**

***p <.001, *p < .05*

At baseline, there were significant but small positive correlations between Calgary depression scores and IL-6, r = .14, n = 187, *p* .04 and TNF-α, r = .14, n = 187, *p* = .04. There was also a significant positive correlation between expressive-deficits and TNF-α at the twelve-month assessment r = .25, n = 101, *p* .01. There were no other significant positive correlations between cytokines and depression, total negative symptoms, avolition-apathy or expressive-deficits (See appendix 10 for correlations between inflammatory markers, CDSS and PANSS).

6.3.3 Multivariate analyses

6.3.3.1 Regression models for cytokines associated with total negative symptoms

A linear mixed effect model was constructed, for each of four dependent variable scores: PANSS negative; avolition-apathy, expressive deficits and Calgary Depression. The three inflammatory markers CRP, IL-6 and TNF-α at baseline, six and twelve months, and BMI at baseline and twelve months were entered as the predictor variables. Both

dependant and independent variables were considered to have random effects and are time-varying covariates. Each of the time points (baseline, two months, six months, nine months and twelve months) were included as co-variates in the model to assess whether the dependent variable changes over the twelve months of study participation. The study design involved clustering effects (each assessment - PANSS score was nested within participant – so there were five observations or assessments for each participant who has a completed data set), so a hierarchical model of one layer was developed to reflect the study design and investigate which cytokines are associated with total negative symptoms longitudinally. A restricted maximum likelihood approach was adopted in the construction of this model to estimate covariance parameters and provide an estimate of the variance components taking into consideration an unbalanced or incomplete data set. An unstructured covariance matrix was specified in the analysis, to ensure that there were no constraints on the values and therefore each variance and covariance was estimated from the data, thus providing the best model fit and an accurate reflection of the data.

Table 25: Linear mixed effects model predicting longitudinal total negative symptoms from assessment and cytokine/acute phase protein concentrations.

Domain	B coefficients	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
CRP	-.02	.13	-.18	.85	-.28	.23
IL-6	.03	.61	.05	.96	-1.16	1.23
TNF-α	.90	.47	1.49	.13	-.22	1.62
Assessment	-.56	.11	-4.17	**.00**	-.79	-.32

Log likelihood = -1196.32. Wald $\chi^2 = 24.02$, $p < .001$.

Table twenty-five shows that no cytokine was significantly associated with total negative symptoms as indicated by the low B values and *p* values of >.10. Assessment was significant, indicating that negative symptoms reduced by .56 points over the study

participation time frame. BMI was included in the original regression model; however, BMI was not significantly associated with total negative symptoms and was therefore removed in the final regression model.

6.3.3.2 **Regression models for cytokines associated with avolition-apathy**

Table 26: Linear mixed effects model predicting longitudinal avolition-apathy from longitudinal cytokine/acute phase proteins scores.

Domain	B coefficients	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
CRP	.04	.06	.59	.558	-.09	.17
IL-6	.29	.32	.91	.362	-.34	.93
TNF-α	.00	.24	.01	.994	-.48	.48
Assessment	-.33	.07	-4.79	**.000**	-.47	-.19

Log likelihood = 941.45. Wald $\chi^2 = 24.14$, $p < .001$.

No marker of inflammation was significantly associated with avolition-apathy. Assessment was significant suggesting that avolition-apathy scores reduced by .33 units over the course of participation. BMI was included in the original regression model; however, BMI was not significantly associated with total negative symptoms and was therefore removed in the final regression model.

6.3.3.3 **Regression models for cytokines associated with expressive deficits**

Table 27: Linear mixed effects model predicting longitudinal expressive deficits from longitudinal cytokines/acute phase protein scores.

Domain	B coefficients	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
CRP	-.08	.07	-1.10	.269	-.23	.06
IL-6	-.15	.34	-.44	.661	-.82	.52
TNF-α	.75	.26	2.83	**.005**	.23	1.26
Assessment	-.10	.06	-1.61	.107	-.23	.02

Log likelihood = -970.82. Wald χ^2 = 10.88, p = .02.

TNF-α was significantly associated with expressive deficits. These results indicate that as TNF-α scores increase by one unit, expressive deficits will increase by .75 units. Assessment was not significant, suggesting that expressive deficits scores remained stable throughout study participation. BMI was included in the original regression model; however, BMI was not significantly associated with expressive deficits and therefore removed in the final regression model.

6.3.3.4 **Regression models for cytokines associated with depression**

Table 28: Linear mixed effects model predicting longitudinal depression from longitudinal cytokines/acute phase protein scores.

Domain	B coefficients	SE B	Z	*p*>|z|	95% C.I. of the coefficient	
					Lower	Upper
CRP	.07	.10	.70	.483	-.13	.28
IL-6	.79	.49	1.60	.109	-.17	1.77
TNF-α	-.35	.37	-.94	.345	-1.10	.38
Assessment	-.53	.10	-5.07	**.000**	-.74	-.32

Log likelihood = -1109.61. Wald χ^2 = 29.33, p < .001.

Cytokine concentrations were not significantly associated with longitudinal depression. Assessment was significant indicating that depression scores decrease by .53 over the course of the study.

6.4 Discussion

The findings from this study suggest that inflammatory markers are not associated with avolition-apathy longitudinally. TNF-α correlates with expressive deficits at twelve months and was significantly associated with longitudinal expressive deficits (table twenty-seven). IL-6 and TNF-α correlate with depression at the baseline assessment, however, was not associated with depression scores longitudinally.

The aim of this study was to identify inflammatory markers that may predict negative and depressive symptoms in schizophrenia. Consistently replicated findings have shown changes in pro- and anti-inflammatory cytokines are related to schizophrenia symptomology (Potvin et al., 2008, Lin et al., 1998, Zanardini et al., 2003, Laskaris et al., 2016, Wium-Andersen et al., 2014, Miller et al., 2014, Noto et al., 2015a). Moreover, elevated pro-inflammatory markers have been found in medication naïve (Upthegrove et al., 2014a), treatment-resistant first episode psychosis (Mondelli et al., 2015a) and acute relapse patients (Miller et al., 2011). However, much of the previous research findings have considered the global symptoms of schizophrenia when assessing the impact of immune dysfunction and have not explored whether inflammatory markers predict certain symptom domains. The presented findings have shown that although total negative symptoms are not associated with any marker of inflammation, expressive

deficits are strongly associated with increases in TNF-α. These findings suggest that expressive-deficits may emerge following activation of pro-inflammatory cytokine-induced macrophage activation of the innate immune system (M1) as well as a pro-inflammatory adaptive immune (Th1) cell response.

Negative symptoms (particularly primary negative symptoms) have been previously conceptualised as enduring (Barnes and Paton, 2011). Given that expressive deficits are associated with TNF-α (trait cytokines) (Miller et al., 2014, Baumeister et al., 2014, Miller et al., 2011), the enduring nature of negative symptoms may be a consequence of raised TNF-α. Avolition-apathy improved over twelve months of follow-up, suggesting that avolition-apathy may fluctuate throughout the course of illness. The finding that expressive deficits and avolition-apathy differ in terms of their biological underpinnings and course of illness, this adds to the growing evidence base of two distinct sub-domains within negative symptoms. The enduring nature of negative symptoms has been disputed by Savill et al. (2015), who found that negative symptoms reduced over time; our results suggest the potential improvement for negative symptoms may be limited to avolition-apathy rather than expressive deficits.

Previous findings have suggested that depression is associated with immune dysfunction, both within schizophrenia (Noto et al., 2011) and as a major depressive disorder (Haapakoski et al., 2015), specifically, depression has been associated with increased CRP and IL-6. Furthermore, Khandaker et al. (2014) found that increased childhood concentrations of IL-6 was associated with a risk for depression in adulthood, suggesting that there may be a causal link between increased concentrations of IL-6 and

depression. The present findings support this at baseline, as IL-6 and CRP were significantly correlated with depression. However, the longitudinal analysis failed to support this as depression was not associated with increased concentrations of IL-6 or CRP. This may, in part, be due to the high dropout rate of the most severely depressed patients.

There are several explanations for these differences in results from previous literature regarding immune activation associated with depression. The median CDSS scores for most of the assessments was one, suggesting that the majority of the sample was not depressed. Also, the mean and median levels of CRP, IL-6 and TNF-α were low compared to previous research (Upthegrove et al., 2014a), which may explain the differences in the present findings compared to previous research. Another explanation for the differences in the present findings to that in the previous literature may have controlled for medication in the analysis.

6.4.1 Strengths and limitations

The statistical technique used to assess the relationship between cytokines, negative symptoms and depression represents a particular strength with this study. Linear mixed effect modelling allows for the analysis of incomplete data sets over time, therefore statistical power was not compromised from participant drop out. Just fewer than 200 participants were included in the analysis, furthermore, linear mixed effect modelling allows for a longitudinal assessment of this relationship, which is not achieved using standard linear regression models.

However, there are disadvantages associated with this study. Firstly, medication was not controlled for, as this data was not available at the time of analysis. This represents a problem with this research as both antipsychotic and antidepressant medication has been suggested to have at least some effect on cytokines (Potvin et al., 2008), which may not be considered when medication usage is not entered in the analysis, thus masking any relationship between immune dysfunction, depression and negative symptoms. Therefore, including current medication usage in the analysis would allow for this relationship to be assessed, and to see how medication can impact on putative markers of inflammation underpinning the negative and depressive syndromes. However, Upthegrove et al.'s (2014a) systematic review found that IL-6, IL-1β, sIL-2r and TNF-α were raised in schizophrenia in those who were medication naïve, which differed from those who were medicated, as medicated patients show a reduction in inflammatory cytokines compared to unmedicated patients (Upthegrove et al., 2014a). These findings exemplify the importance of controlling for these variables in the analysis. This is particularly important when assessing which inflammatory markers may be associated with depression or negative symptoms as cytokine concentrations may be associated with medication usage and not depression or negative symptoms.

Secondly, other key variables (such as smoking status, illicit drug use) were also not included in the analysis as this data was not captured, which represents another disadvantage with the current study, particularly given that smoking can impact on concentrations of inflammatory markers (O'Connor et al., 2009). However, BMI was controlled for in this study, which represents an advantage as BMI has been shown to significantly impact on cytokine levels.

6.4.2 Conclusion

The relationship between inflammatory markers, negative symptoms and depression remains complex. The current findings suggest that expressive deficits can be distinguished from avolition-apathy both clinically and with biological markers. Expressive deficits are associated longitudinally with TNF-α indicating M1 and Th1 cell activation. Global negative symptoms, avolition-apathy and depression are not associated with any marker of inflammation longitudinally. These findings have important implications for our conceptualisation of the relationship between these symptom domains. Given that expressive deficits are associated with increases in TNF-α, it is possible that immune dysfunction/inflammation may account for the enduring nature of negative symptoms. Therefore, therapeutic options that specifically target TNF-α may alleviate these symptoms.

7. Targeting the immune system to treat depression and negative symptoms in schizophrenia

7.1 Introduction

Negative symptoms of schizophrenia include impaired quality of life, social isolation, reduced emotional responsiveness, self-neglect and anhedonia (Barnes, 1993). Negative symptoms have been associated with poor quality of life, and impaired social and occupational functioning, and for around 15-20%, are enduring (Buchanan, 2007, Bobes et al., 2010). A review of 168 randomised placebo-controlled trials using 6503 patients in the treatment arm and 5815 placebo, found current treatment options (antipsychotic or antidepressant medication, psychological therapy etc.) statistically reduce negative symptoms, but few treatments were clinically meaningful (as measured by the clinical global impression severity scale) (Fusar-Poli et al., 2015).

Depression is a mood disorder characterised by sadness, pessimism, feelings of guilt, shame and worthlessness, and anhedonia (Beck, 1967). Depression is commonly seen in schizophrenia (Upthegrove et al., 2010, Koreen et al., 1993, Hafner et al., 2005a) and may be an integral syndrome in the illness (Murray et al., 2005, Lindenmayer et al., 1995, Yung et al., 2006).

There has been much interest in the possibility that abnormal inflammatory processes may underlie depression and schizophrenia. The evidence for an inflammatory process underlying schizophrenia has strengthened over recent years. Chronic low grade

inflammation has been observed in schizophrenia (Kirkpatrick and Miller, 2013). A systematic review found significant elevation in pro-inflammatory cytokines (such as IL-6 and TNF-α), particularly in medication naïve schizophrenia patients (Upthegrove et al., 2014a) suggesting that there is immune-dysregulation in schizophrenia, specifically with an increase in pro-inflammatory cytokines. Moreover, immune dysfunction has been associated with the development of negative and depressive symptoms, specifically elevated IL-6 and TNF, in those at clinical high risk for psychosis (Goldsmith et al., 2019).

Inflammation is the body's response to help fight infection, remove damaged chemicals and repair damaged tissue (O'Neill, 2008), and has been observed as being chronic and low grade in schizophrenia (Upthegrove et al., 2014a). One explanation to account for inflammation in schizophrenia is the microglial hypothesis. Microglial cells form part of the body's first line of immune defence (Kettenmann et al., 2011) and adopt a ramified position. These cells are responsible for synaptic pruning, maintenance and phagocytosis of debris from damaged cells. Once activated, these cells change function and assume an amoeboid morphology and produce pro-inflammatory cytokines (such as IL-6) (Laskaris et al., 2016) contributing to neuro-inflammation. The microglial hypothesis of schizophrenia (Monji et al., 2009) postulates microglia becomes activated following a stressful life event, thus triggering the release of several pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6 and IL-8) and free radicals (such as nitric acid, and superoxide). Evidence for microglial activation has been found in patients with schizophrenia (van Berckel et al., 2008) and in those in the ultra-high-risk for psychosis group (Bloomfield et al., 2016) using Positron Emission Tomography (PET) scanning.

Sustained increase in pro-inflammatory cytokines (such as IL-6 and TNF-α) raises kynurenic acid concentration (Erhardt et al., 2007, Schwieler et al., 2015). This acts as an NMDA receptor antagonist, which along with glutamatergic hypofunction (Erhardt et al., 2007), may be causal in the symptomology of schizophrenia. Despite the lack of evidence for overt neurodegeneration in schizophrenia, structural MRI studies have shown subtle loss of grey matter in those in the ultra-high risk for schizophrenia who transitioned to psychosis compared to those who didn't transition to psychosis (Dazzan et al., 2012), the early course of schizophrenia (Lieberman et al., 2005b), and after five years of the illness (Cahn et al., 2006).

Minocycline is a second-generation broad spectrum, tetracycline antibiotic that possesses anti-inflammatory and anti-apoptotic properties, that is commonly used to treat infection (Levkovitz et al., 2010). There is preliminary evidence to suggest minocycline may be effective in schizophrenia. When administered as an adjunctive to antipsychotic medication, minocycline has demonstrated some benefit in reducing symptoms of psychosis compared with antipsychotic monotherapy (Kelly et al., 2011, Miyaoka et al., 2007, Miyaoka et al., 2008), and has shown particular benefit in improving negative symptoms (Chaudhry et al., 2012, Levkovitz et al., 2010). However, Levkovitz et al. (2010) only treated participants for six months with minocycline at a single site, and there was no blood test taken in both studies to assess cytokine levels in order to understand the mechanistic action of minocycline in schizophrenia.

There are several explanations of the beneficial role minocycline may have in reducing symptoms of schizophrenia. Firstly, minocycline may exert its action on the

glutamate system. The glutamate hypothesis of schizophrenia implies there is glutamatergic neurotransmitter dysfunction at the NMDA receptor (Tamminga, 1998), which leads to an increased production of nitric oxide, which is blocked by minocycline (Levkovitz et al., 2010). Secondly, a putative neuroprotective role of minocycline may be through reducing microglial activation, and thus decrease the production of cytokines (Frick et al., 2013, Domercq and Matute, 2004, Kloppenburg et al., 1996).

Some clinical trials suggest minocycline may treat depression (Soczynska et al., 2012, Pae et al., 2008), which maybe the result of minocycline's anti-inflammatory properties. Immune dysfunction has been reported in depression (Maes et al., 1990, Maes et al., 1991, Maes et al., 1993, Young et al., 2014). Replicated findings have consistently shown an increase in pro-inflammatory markers (such as IL-6, TNF-α, and CRP) (Young et al., 2014, Valkanova et al., 2013, Haapakoski et al., 2015, Liu et al., 2012), indicating an increase in pro-inflammatory cytokines may contribute to the pathology of depression (Maes, 1995, Smith, 1991). Moreover, anti-depressant medication may contain some immune-regulatory properties and decrease the production of pro-inflammatory cytokines (such as IL-6) and increase the production anti-inflammatory cytokines (such as IL-10) (Maes et al., 1999, Xia et al., 1996, Sutcigil et al., 2007). Therefore, minocycline may help reduce depression in schizophrenia by suppressing the release of toxic pro-inflammatory cytokines.

The evidence presented suggests that the administration of minocycline will lead to a reduction of depression symptoms and avolition-apathy through its effect on inflammatory mechanisms associated with schizophrenia. These symptoms may be

associated with state inflammatory markers. Trait cytokines remain constantly high throughout the course of illness and will therefore remain unaffected by treatment with minocycline as these markers are consistently raised in schizophrenia (Kirkpatrick and Miller, 2013). Trait cytokines have been associated with symptoms that correspond with the expressive deficits sub-domain (such as blunted affect and alogia) in those with chronic schizophrenia (Goldsmith et al., 2018). Given that trait cytokines have been associated with expressive deficits, these symptoms will not improve following treatment of minocycline.

7.1.1 Aim

This thesis aimed to assess the relationship between depression and negative symptoms. To do this, the effect of minocycline was observed to explore whether a single anti-inflammatory agent would reduce both depressive and negative symptoms, thus suggesting a common mechanism underpinning these two symptom domains.

7.1.2 Predictions

1. In schizophrenia, minocycline will lead to an improvement in depressive symptoms and avolition-apathy.
2. Minocycline will not lead to a reduction in expressive deficits.

7.1.3 Mechanistic hypothesis

1. Minocycline will lead to a reduction of state pro-inflammatory markers (such as IL-6 and CRP).
2. There will be no impact of minocycline on trait pro-inflammatory markers (such as TNF-α). This is because trait inflammatory cytokines will not respond to anti-inflammatory agents.

These hypotheses predict that a reduction in state pro-inflammatory markers will lead to an improvement of depression and the avolition-apathy sub-domain of negative symptoms but will not lead to a reduction in expressive-deficits.

7.2 Methods

7.2.1 Design

An experimental design was employed to assess the effect of minocycline on depressive and negative symptoms. Ethical approval was sought and received by the North West Greater Manchester Central UK NHS Research Ethics Committee reference 11/NW/0218, (trial Registration: Current Controlled Trials ISRCTN49141214, NIHR EME grant number 2010-022463-35) which was then followed by local R&D approval at the participating sites.

7.2.2 Planned intervention

Minocycline, or a matching placebo, was taken as 100mg capsules twice daily for two weeks and then increased to 300mg daily for the remainder of the twelve-month study period. Participants were then withdrawn from the investigational medicinal product and assessed again at fifteen months from baseline (however this data is not presented). Participants were randomly allocated to either the treatment (minocycline) arm or placebo arm of the study, which was operated by the online software, OpenCDMS.

7.2.3 Sample

Two hundred and seven participants experiencing psychosis were recruited between April 2013 and March 2015 across all eight of the participating BeneMin sites (universities of Manchester; Birmingham; Cambridge; Edinburgh; London – both King's College, London; and University College London; Lancashire care trust; and Cheshire and Wirral NHS trust). Participants were drawn from the EIP services at the discretion of the RMO who determined eligibility, and consent to contact the patient was obtained from a member of the patient's care team. Eligibility was then confirmed by a screening assessment which yielded a research diagnosis obtained from assessments. Participants adhered to the following inclusion criteria (see table 29).

Table 29: The inclusion and exclusion criteria for study participation.

Inclusion criteria	Exclusion criteria
Be male or female aged 16 to 40	Not currently abuse illicit substances
Meet DSMIV criteria for schizophrenia, schizophreniform or schizo-affective psychosis	Not pose a serious suicidal or violent risk
Be in a current episode as defined by the presence of positive symptoms (a score >2 on P1, 2, 3 or 6 on PANSS).	Not have taken an investigational medicinal product within 30 days of baseline assessments.
Be within 5 years of symptom onset	
Be fluent in English	
Have capacity to provide informed consent	

7.2.4 Materials

Demographic information was obtained including age, gender, ethnicity and DUP. BMI as recorded at baseline and twelve months was also included in this analysis.

Negative symptoms were assessed using the PANSS (Kay et al., 1987) (see section 4, subsection 4.7.1.1). Avolition-apathy was constructed using PANSS items G13 (disturbance of volition), N2 (emotional withdrawal) and N4 (passive/apathetic social withdrawal), and the expressive deficits was made up of PANSS items N1 (blunted affect), N3 (poor rapport) and N6 (Lack of spontaneity and flow of conversation), at all-time points (baseline, two months, six months, nine months and twelve months). This method of differentiating between the avolition-apathy and expressive deficits sub-domains of negative symptoms appears to provide an adequate reflection of these sub-domains, and this was based on factor analysis used in previous research (Barnes et al., 2016, Liemburg et al., 2013).

Depression was assessed by the total score of the Calgary Depression Scale in Schizophrenia, which was administered at each time point (baseline, two months, six months, nine months and twelve months).

Extrapyramidal side effects were assessed using the Simpson-Angus scale (SAS) (Simpson and Angus, 1970), and was administered at baseline, six months and twelve months.

Cytokine analysis was carried out using blood samples that were taken at baseline, six months, and twelve months of participation. Samples were taken in a 9ml ethylenediamine tetraacetic acid (EDTA) tube which was then placed in a centrifuge for fifteen minutes at 2000g. Following centrifugation, the plasma supernatant was extracted and pipetted into 6 identical aliquots and equally divided into two boxes. The samples were stored locally in a -80^{o}C freezer before being transported to King's College, London for analysis. The samples were then processed by Meso Scale Discovery Piezoelectric Plate, with high sensitivity immunoassays at King's College London. Multiplex-assays included IL-6, TNF-α, IFN-gamma, IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-13, GM-CSF, IL-1alpha, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF, and CRP. For this analysis IL-6, TNF-α, and CRP were used as previous findings have consistently shown these cytokines are raised in schizophrenia (Upthegrove et al., 2014a). Also, given that CRP and IL-6 are state inflammatory markers and TNF-α is a trait cytokine (Miller et al., 2011), these markers were selected to assess whether state/trait inflammatory markers are associated differentially with negative symptoms, and depression. Participants with a CRP of above 10 were excluded from this analysis, as this would indicate an acute increase

in the immune response (characterised by an increase in inflammatory marker concentrations).

7.2.5 Statistical analysis

All data were analysed using Statistical Package for the Social Sciences (SPSS v. 22) and STATA (v. 10). All data were treated as interval data and analyses that were carried out were of one tailed significance unless otherwise stated and the alpha level was set at .05.

Following descriptive statistics, univariate analyses were used to explore the differences between placebo and treatment groups in twelve-month total depression and negative symptoms (including avolition-apathy and expressive deficits) scores. Given that both depression and negative symptoms were not normally distributed (even after log transformation), Mann Whitney U was used to assess whether depression and negative symptoms significantly differed. Univariate analyses were also used to assess differences between placebo and treatment groups in twelve-month cytokines (CRP, IL-6 and TNF-α) to test the mechanistic hypothesis that minocycline will lead to a reduction in state inflammatory markers (such as CRP and IL-6). Participants with a CRP greater than ten were excluded from the analysis, as this would indicate acute inflammation, and not the chronic low-grade inflammation that maybe identified in individuals with schizophrenia.

To test whether minocycline leads to a reduction of depression and negative symptoms, four linear mixed effects models were created (one for longitudinal total

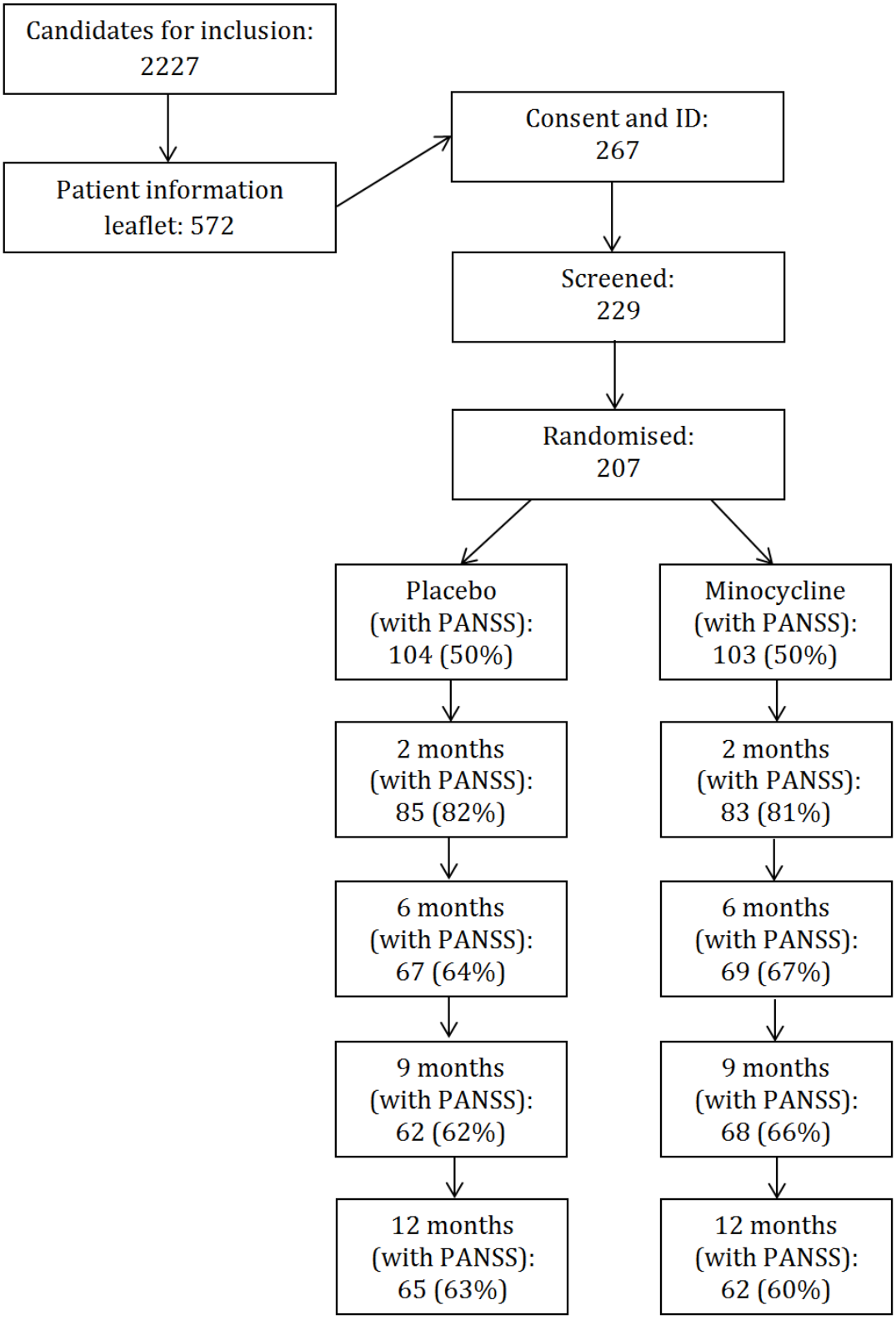
negative symptom scores, one for longitudinal avolition-apathy scores, one for longitudinal expressive deficit scores and the final one for longitudinal depression scores).

Further exploratory analysis of minocycline effect was conducted using depression scores, stratified by CRP.

7.3 **Results**

Two hundred and seven participants were recruited between April 2013 and May 2015 and were randomised to the two arms of the trial, minocycline or placebo. Numbers of participants assessed for eligibility, screened and randomised to treatment arm, as well as retention rates, can be found in figure twenty.

Figure 20: The number of participants who took part in the trial at each assessment, separated by allocation group.



38% of participants who had been randomised dropped out of the study, chiefly due to participants being lost to follow-up or participants request to withdraw (81% of withdrawals). The numbers of participants who dropped out was evenly split by treatment arm (39 on placebo and 40 on minocycline).

7.3.1 Descriptive statistics

At baseline, participants were matched on age, and had a similar BMI and EPSE. The mean age of participants was twenty-five in both arms of the study. Also, there was predominance for male participants in both groups. Clinical characteristics and demographic information captured, separated by allocation group can be found in table thirty (excluding participants with a CRP greater than ten).

Table 30: The mean and standard deviation of the baseline demographic and clinical characteristics of the patient's randomised treatment arm (see abbreviations table on page XIV for the abbreviations).

Variable	Placebo	Minocycline
Sex N (%)	77male (75%), 27 females (25%)	73 males (71%), 30 females (29%)
Age (SD)	25.71 (5.22)	25.36 (5.29)
DUP (SD)	14.44 (35.69)	25.36 (51.30)
BMI (SD)	27.82 (6.93)	27.11 (6.32)
SAS (SD)	1.42 (1.96)	1.55 (2.30)

7.3.2 Descriptive statistics: clinical variables

Depression and negative symptom scores were fairly evenly matched across treatment arm at baseline. At baseline the mean CDSS score was over five in both groups

(median = 4.5, SD = 4.63). Data was not normally distributed and remained not normally distributed following log transformation. In the placebo arm, 41% of participants were depressed and in the treatment arm, 38% were depressed. Moreover, the mean total PANSS score is comparable among treatment arms and was low (mean PANSS total in the placebo arm was 69.3 and 67.1 in the minocycline arm) – indeed a score of sixty or below is indicative of being in remission (Opler et al., 2007).

Table thirty-one shows the clinical scores at each assessment in the placebo and minocycline treated groups. Drop out patterns at the follow-up assessments were similar among treatment arms.

Table 31: The clinical characteristics of the sample separated by treatment arm at the baseline and follow up assessments.

		Placebo				Minocycline			
		N	M	SD	Range	N	M	SD	Range
Positive symptoms	Baseline	104	17.35	5.30	9-29	103	16.31	4.08	9-30
	2 months	86	14.50	4.82	7-27	83	13.78	4.53	7-27
	6 months	67	14.42	5.24	7-29	69	13.43	5.00	7-26
	9 months	63	13.60	5.01	7-26	68	12.76	4.59	7-26
	12months	65	13.98	4.82	7-28	63	13.43	6.06	7-34
Negative symptoms	Baseline	104	16.78	5.47	7-33	103	17.70	5.87	8-37
	2 months	85	15.14	5.75	7-30	83	16.40	5.62	7-35
	6 months	67	15.67	5.78	7-33	69	15.75	6.53	7-34
	9 months	62	14.53	4.86	7-28	68	15.85	6.27	7-33
	12months	65	14.17	5.15	7-28	62	16.37	6.24	7-32
PANSS total	Baseline	103	69.30	15.35	39-118	103	67.14	13.17	43-108
	2 months	85	60.08	15.74	32-104	83	59.55	14.91	34-103

	6 months	66	59.41	16.77	30-94	69	57.51	15.72	30-93
	9 months	62	56.81	16.68	30-93	68	56.99	14.65	31-91
	12 months	65	57.09	17.26	31-97	62	59.03	17.32	31-109
Expressive deficits	Baseline	102	6.39	3.19	3-19	103	6.82	3.43	3-18
	2 months	85	6	3.39	3-17	83	6.45	3.16	3-16
	6 months	67	5.93	2.92	3-17	69	6.51	3.49	3-16
	9 months	63	5.98	2.80	3-15	68	6.46	3.63	3-17
	12 months	65	5.63	2.92	3-15	63	6.94	3.65	3-16
Avolition-apathy	Baseline	102	7.09	2.79	2-15	103	7.78	2.57	3-16
	2 months	85	6.86	2.93	3-15	83	7.14	2.70	3-14
	6 months	67	6.82	3.09	3-13	69	6.51	3.03	3-14
	9 months	63	5.90	2.57	3-14	68	6.47	2.74	3-13
	12 months	65	5.86	2.66	3-14	63	6.59	2.93	1-14
CDSS total	Baseline	101	5.52	4.99	0-20	103	5.17	4.27	0-18
	2 months	86	3.67	4.03	0-17	88	3.53	3.87	0-16
	6 months	73	3.47	4.28	0-14	77	2.81	3.65	0-13
	9 months	66	3.30	3.91	0-16	71	3.62	3.82	0-16
	12 months	64	3.80	4.44	0-17	65	3.62	4.07	0-17

Figure twenty-one shows the mean scores of avolition-apathy and expressive deficits in the placebo and minocycline treated groups. This graph shows little variation in PANSS scores corresponding to avolition-apathy and expressive deficits over twelve months.

Figure 21: The association between the avolition-apathy sub-domain and expressive deficits sub-domain longitudinally over twelve months of study participation in those treated with placebo and minocycline

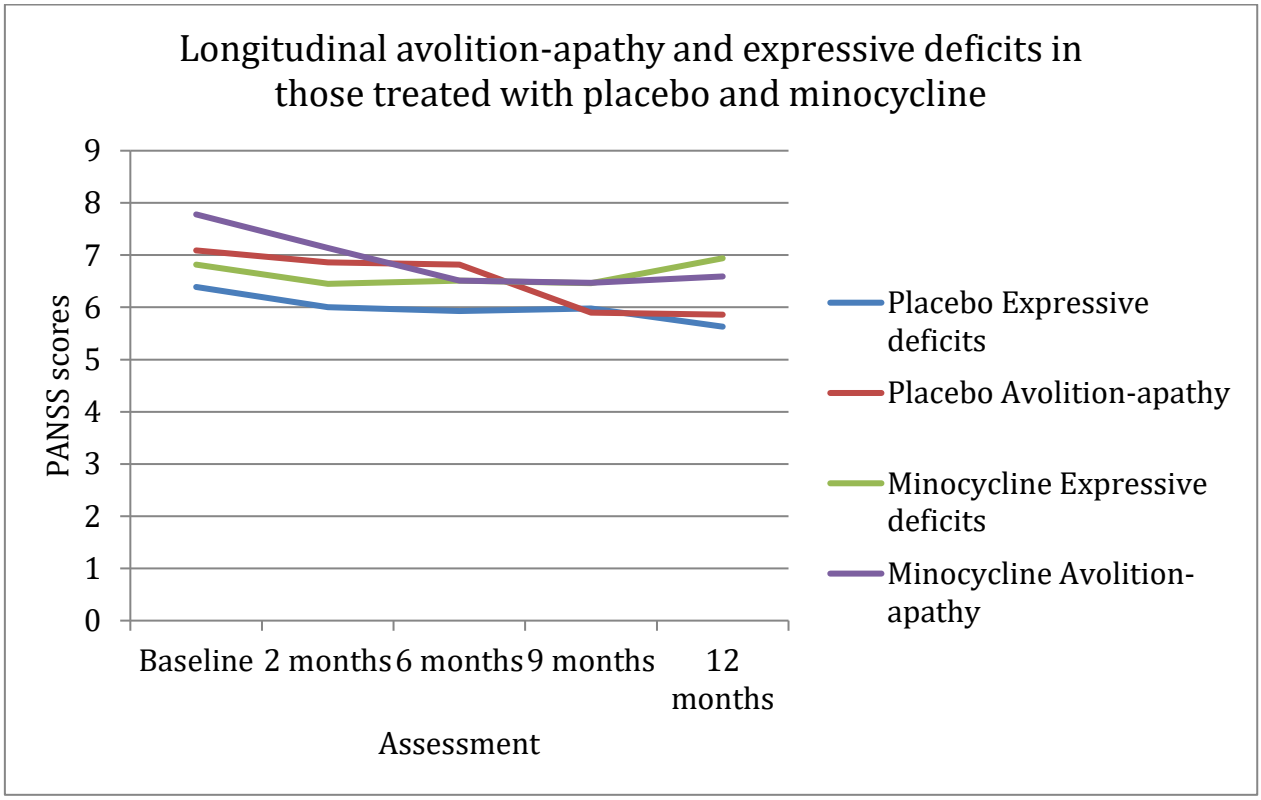
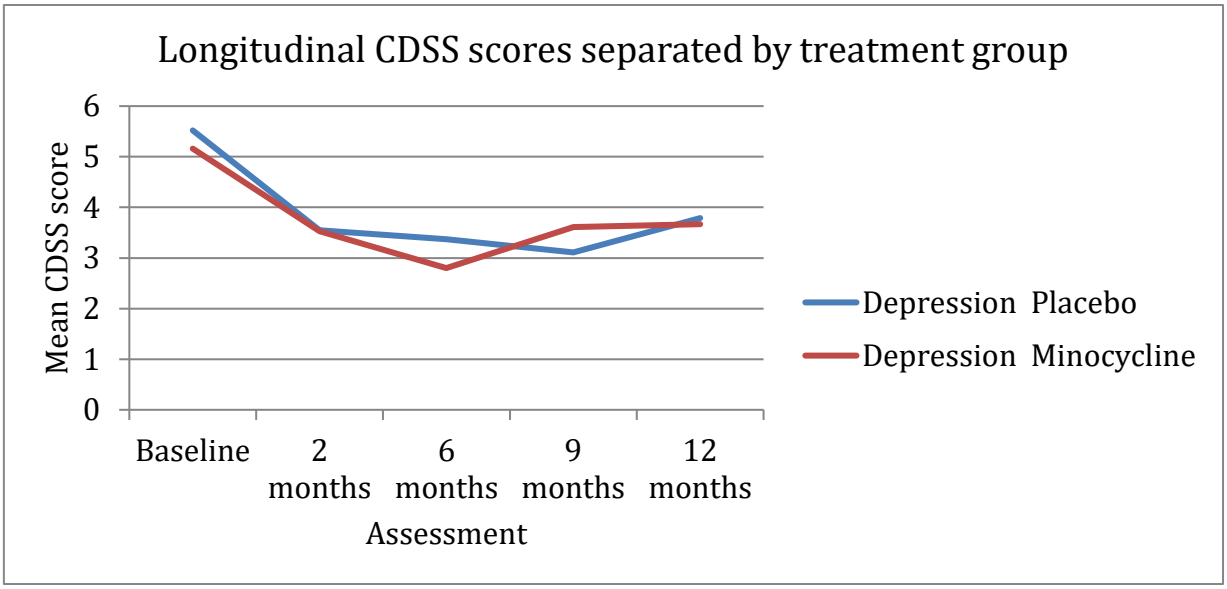


Figure twenty-two shows the mean depression scores over twelve months of study participation separated by treatment group.

Figure 22: The change in depression scores over twelve months of study participation in those treated with placebo and minocycline



7.3.3 Cytokines

Participants with a CRP of above ten were excluded from this analysis, as this would indicate a possible acute infection or inflammatory reaction.

Table 32: The number and median of CRP, IL-6 and TNF-α concentration in those treated with placebo and minocycline of patients with a CRP below ten.

Cytokine/Acute phase protein	Visit	Placebo		Minocycline	
		n	Md	n	Md
Hs-CRP (mg)	Baseline	91	1.40	97	1.50
	6 months	57	2.40	52	1.85
	12 months	50	2.40	47	1.65
IL-6 (pg/mL)	Baseline	91	.62	97	.53
	6 months	57	.64	52	.63
	12 months	50	.68	49	.59
TNF-α (pg/mL)	Baseline	91	2.40	97	2.46
	6 months	57	2.34	52	2.62
	12 months	50	2.47	47	2.36

7.3.4 Univariate analyses

7.3.4.1 Clinical variables

Depression and negative symptom scores were not normally distributed, so Mann Whitney U was used to assess the difference between depression and negative symptoms.

Table 33: The differences between minocycline and placebo groups for each clinical variable (depression, total negative symptoms, avolition-apathy and expressive deficits) at twelve months.

Clinical domain	Visit	Treatment	n	Md	U	z	p
1) Total negative symptoms	12 months	Placebo	65	14	1600.00	-2.00	.04
		Minocycline	62	16			
2) Avolition-apathy	12 months	Placebo	65	5	1733.00	-1.51	.13
		Minocycline	63	6			
3) Expressive deficits	12 months	Placebo	65	5	1606.00	-2.13	.03
		Minocycline	63	6			
4) Depression	12 months	Placebo	64	2.5	2066.50	-.06	.94
		Minocycline	65	3			

Md = Median

The Mann Whitney U test revealed there was a significant difference between the placebo and minocycline group at baseline and twelve months in total negative symptoms between placebo (Md = 14, n = 65) and minocycline (Md = 16, n = 62) groups, with the placebo group conversely showing lower levels of total negative symptoms U = 1600.00, z = -2.00, p = .04. This difference may be driven by the significant difference in expressive deficits between placebo (Md = 5, n = 65) and minocycline (Md = 6, n = 63) groups U = 1606.00, z = -2.13, p = .03. There were no significant differences in depression between

treatment arms at twelve months, or differences in avolition-apathy between treatment arms (see table thirty-three).

7.3.4.2 **Cytokines**

Cytokine concentrations were not normally distributed, so the non-parametric Mann Whitney U test was used to test for significance of group differences.

Table 34: Median cytokine concentrations by treatment arm at twelve months

Cytokine/Acute phase protein	Visit	Treatment	n	Md	U	z	p
Hs-CRP (mg)	12 months	Placebo	51	2.60	974	-1.75	.08
		Minocycline	48	1.60			
IL-6 (pg/mL)	12 months	Placebo	51	.70	1238	-.25	.80
		Minocycline	50	.62			
TNF-α (pg/mL)	12 months	Placebo	51	2.42	1224	-.34	.72
		Minocycline	50	2.35			

Md =median

There were no significant differences in cytokines between treatment arms at twelve months (see table thirty-four).

7.3.5 Multivariate analyses: Treatment effect of minocycline

To assess the effect of minocycline on longitudinal clinical variables, linear mixed effect models were created for the four domain scores; 1) total negative symptoms, 2) avolition-apathy, 3) expressive deficits and 4) depression. The fixed variable was treatment allocation. Clinical variables were considered random effects and are time-

varying co-variates. The study design involved clustering effects (visit was nested within participants), so a hierarchical model of one layer was developed to reflect the study design. A restricted maximum likelihood approach was adopted in the construction of this model to estimate covariance parameters and provides an estimate of the variance components taking into consideration an unbalanced or incomplete data set. An unstructured covariance matrix was specified in the analysis, to ensure that there were no constraints on the values and therefore each variance and covariance was estimated from the data thus providing the best model fit and an accurate reflection of the data. Table thirty-five shows the four linear mixed effect models assessing the effect of minocycline on clinical variables.

Table 35: Four linear mixed effect models showing the effect of minocycline vs placebo on the four symptom domain scores

Clinical domain	n	B	SE B	Z	p>|z|	95% C.I. of the coefficient		Log likelihood	Wald χ2	p
						Lower	Upper			
1) Negative symptoms	207	.62	.69	.90	**.37**	-.73	1.98	-2239.97	40.94	<.001
2) Avolition-apathy	206	.44	.31	1.42	**.15**	-.16	1.04	-1785.01	48.47	<.001
3) Expressive deficits	205	.34	.40	.84	**.39**	-.45	1.13	-1779.68	7.97	.01
4) Depression	204	-.26	.48	-.54	**.58**	-1.20	.68	-2159.62	34.43	<.001

The non-significant *p* values (in bold) show that treatment allocation was a non-significant predictor in these models. Therefore, being allocated to either placebo or minocycline arms of the study did not predict any of the clinical variables (total negative symptoms, avolition-apathy expressive deficits or depression). Minocycline did not lead

to a reduction of total negative symptoms, avolition-apathy, expressive deficits or depression in this sample.

7.3.6 Exploratory analysis

It would be predicted that only those who have an inflammatory response would experience a reduction of depression or negative symptoms after being administered minocycline. Therefore, individuals with a CRP of between 1 and 10 at *all-time points* were included in the following linear mixed effects models.

Based on Addington et al. (2014), participants who had a total CDSS score of six and above have been viewed as depressed, with sensitivity of 75% and specificity of 79%. Participants with a score of 0-5 have been treated as non-depressed. Immune dysfunction has been shown in individuals with depression, therefore, it would be predicted that those who are depressed and show an inflammatory response would respond to minocycline therapy compared to the placebo group. Table thirty-six shows number and percentage of participants who were depressed and not depressed at each time point. The number of depressed and non-depressed individuals was equal between minocycline and placebo groups.

Table 36: The number and percentage of participants who were depressed and not depressed using a cut of score of 6 on the CDSS to indicate depression.

Visit	Depressed (%)	Not depressed (%)
Baseline CDSS total	80 (39)	124 (61)
2-month CDSS total	51 (30)	118 (70)
6-month CDSS total	39 (29)	96 (71)
9-month CDSS total	33 (25)	98 (75)
12-month CDSS total	35 (28)	91 (72)

7.3.6.1 **Immune activation**

Four linear mixed effect models were created, using the same method as described above (total negative symptoms, avolition-apathy, expressive deficits and depression) with allocation (placebo or minocycline) but selecting individuals who had an immune activation as indicated by a CRP of between one and ten. Being allocated to treatment or placebo arm was not a significant predictor in any of the regression models (depression: B = -.69, p = .28, n = 152; total negative symptoms: B = .48, p = .60, n = 151; avolition-apathy: B = .08, p = .84, n = 151; expressive deficits: B = .34, p = .51, n = 151), indicating that minocycline does not lead to a reduction in depression or total negative symptoms in those with an active immune response, including those separated into avolition-apathy and expressive deficits.

However, in the linear mixed effects regression model, IL-6 was significantly associated with depression (B = 1.09, p = .03, n = 152), indicating that as IL-6 increases; depression will also increase by 1.09 units. No other cytokines were significantly associated with depression.

Expressive deficits were significantly associated with TNF-α (B = 1.19, $p < .000$, n = 151), as were total negative symptoms (B = 1.34, $p = .02$, n = 151). No cytokine was significantly associated with avolition-apathy.

7.3.6.2 **Immune activation and clinically depressed**

To assess whether there was a treatment response in individuals who had a CDSS score of 6 or above *and* who had immune activation, four mixed linear mixed effects models were created, using the same method described above (total negative symptoms, avolition-apathy, expressive-deficits and depression) but only including participants who were depressed (therefore scoring 6 or above on the CDSS) and with a CRP of between 1 and 10 (indicating immune activation).

Being allocated to treatment or placebo arm was not a significant predictor in any of the regression models (depression: B = -.93, $p = .15$, n = 67; total negative symptoms: B = -1.24, $p = .33$, n = 67; avolition-apathy: B = -.79, $p = .19$, n = 67; expressive deficits: B = -.53, $p = .47$, n = 67), indicating that minocycline does not lead to a reduction in depression or total negative symptoms (including separated into avolition-apathy and expressive deficits). No inflammatory marker was significantly associated with avolition-apathy or total negative symptoms. TNF-α was the only cytokine that was significantly associated with expressive deficits (B = 1.55, $p = .01$, n = 67) and conversely, was negatively associated with depression (B = -2.05, $p < .001$, n = 67).

7.4 Discussion

The results of this double-blind randomised placebo-controlled study indicate that the addition of minocycline adjunctive to standard antipsychotic and antidepressant therapy in early schizophrenia does not lead to an improvement of negative or depressive symptoms. It was hypothesised that the administration of minocycline would lead to a reduction in cytokines associated with avolition-apathy and depression, therefore reducing these markers using minocycline would lead to an improvement of these symptoms. However, cytokines were not associated with avolition-apathy, and state cytokines did not statistically significantly differ between treatment groups. IL-6 was, however, significantly associated with depression in those with depression and with an inflammatory response (as measured by an increase in CRP). Interestingly, expressive deficits were associated with TNF-α, and at the 6-month assessment TNF-α scores statistically significantly differed, with the minocycline group showing higher levels of TNF-α, however at baseline and twelve months, this difference was not observed. There was no treatment effect of minocycline on expressive deficits.

There is no evidence from this study that minocycline reduces negative symptoms or depression in early schizophrenia. Previous findings have suggested minocycline may improve negative symptoms (Chaudhry et al., 2012, Miyaoka et al., 2007, Miyaoka et al., 2008, Kelly et al., 2011, Levkovitz et al., 2010). Two of these were case reports (Miyaoka et al., 2007, Kelly et al., 2011), and one was an open label trial (Miyaoka et al., 2012). Chaudhry et al. (2012) reported on a multicentre study; a smaller centre in Brazil (n = 24) and a larger one in Pakistan (n = 70) (total n = 94). The total sample size was half the

sample used in the present study (94 participants vs. 127). Both sites reported an improvement in negative symptoms in the minocycline group compared to the placebo. The differences in the present study and the Pakistan study may reflect a chance variation in study findings. Also both Chaudhry et al. (2012) and Levkovitz et al. (2010) report higher negative symptoms (in both studies, PANSS negative symptoms = 22) compared to the present study (PANSS negative symptoms = 17) which may account for the discrepancy in findings, suggesting that minocycline may only be effective in alleviating more severe negative symptoms.

The findings from a meta-analysis by Xiang et al. (2017) suggest that negative symptoms are reduced by the administration of minocycline. However, this review included relatively few studies (n = 8) and participants (n = 548 – 286 on minocycline, 262 on placebo), and the treatment effect was mainly observed in studies employing the SANS (Andreasen, 1989), which differed from the present study which assessed negative symptoms using the PANSS. This may reflect a potential limitation of the present study. Nevertheless, the present study failed to detect any improvement in negative symptoms in those being administered minocycline. Moreover, this review presented studies using a sample of chronic schizophrenia patients and studies from various countries. Therefore, phase of illness and cultural differences between studies presented in this meta-analysis may explain the discrepancy between the present findings and the findings from this review.

Minocycline was hypothesised to reduce negative symptoms and depression through its putative neuroprotective role in reducing microglia activation, which has been

found in those with schizophrenia by van Berckel et al. (2008) and Bloomfield et al. (2016) through the use of PET imaging. However, in a narrative review of the literature, others have failed to find evidence of microglial activation in schizophrenia (Kahn and Sommer, 2015). Indeed, Di Biase et al. (2017) who looked at UHR for psychosis, recently diagnosed and chronic schizophrenia patients found no evidence of microglial activation. Also, of the UHR patients who showed microglial activation (Bloomfield et al., 2016), only one transitioned to psychosis, suggesting that microglial activation may not underpin all cases of schizophrenia. These findings highlight an important phenomena; that not all UHR transition to first episode psychosis or schizophrenia. A meta-analysis of 2502 high risk for psychosis patients across twenty-seven studies found that 29% transitioned to psychosis in the thirty-one months following their initial clinical presentation (Fusar-Poli et al., 2012), and most who transition to psychosis receive a diagnosis of schizophrenia (Yung, 2017). Indeed, there are some who do not transition to schizophrenia who may present with attenuated psychotic symptoms and display many of the hallmarks of schizophrenia (such as high negative symptoms and cognitive dysfunction), and others who transition but have few negative symptoms and intact neurocognitive functioning (Yung et al., 2010). Moreover, psychotic like experiences occur in the non-clinical population. A systematic review of general population surveys found that attenuated delusional thinking and hallucinations were present in between 5 and 8% of the healthy population (van Os et al., 2009). Those who meet criteria for UHR but do not transition to psychosis may recover from these symptoms, but these symptoms may reoccur later in life. This accounts for around 15% of UHR patients (Huber and Gross, 1989). Finally, those who do not transition to psychosis have been found to experience some functional recovery (in terms of social and role functioning), but continue to show more functional

impairment compared to non-clinical controls (Addington et al., 2011). "Transition to psychosis" may represent an arbitrary cut off point of positive symptom severity that bares little in common with negative symptom severity or cognitive dysfunction, and may not be useful in predicting course of illness, response to treatment or prognosis. Moreover, this may not have any bearing on the presence of underlying biomarkers. Indeed, the lack of reliable clinical and biological markers and heterogeneity of UHR patients suggest that some individuals with different presentations may have differing underlying psychopathology.

Minocycline has been shown to exert an antidepressant effect in clinical (Miyaoka et al., 2012) and preclinical studies (Zhang et al., 2006), and has been shown to offer neuroprotection against the various brain insults associated with depression such as neurotoxicity associated with 5-HT and dopamine. The beneficial effect has also been supported by numerous meta-analyses (Husain et al., 2017b, Pae et al., 2008, Rosenblat and McIntyre, 2017). The antidepressant effect of minocycline may be a product of the anti-inflammatory properties of minocycline, which helps reduce neurotoxic pro-inflammatory cytokines found to underlie depression (Miller et al., 2009). To date, there have been three published randomised controlled clinical trials that have used minocycline to treat depression (Dean et al., 2017, Husain et al., 2017a, Emadi-Kouchak et al., 2016), two of which suggest that minocycline may improve depressive symptoms (Husain et al., 2017a, Emadi-Kouchak et al., 2016). However, in all of these studies, the sample size is considerably smaller than the present study, the largest study to find an effect of minocycline being Emadi-Kouchak et al. (2016) (n = 50). Interestingly, the largest study by Dean et al. (2017) (n = 71) found no significant effect of minocycline on

depression. Moreover, these trials have only followed participants up for either six weeks (Emadi-Kouchak et al., 2016) or twelve weeks (Dean et al., 2017, Husain et al., 2017a), which is much shorter than the present study (although this is not uncommon in drugs trials in depression).

The present study is much larger than previous studies in both schizophrenia and depression, with a longer phase of treatment, which may account for the negative results in comparison to previous work. An alternative explanation for these differences in results may be that depression in schizophrenia and depression involve different neurobiological processes. This is supported by Chuang et al. (2014), who identified differences in brain structures associated with depression in schizophrenia compared to those with major depressive disorder using MR imaging. In depressed patients, symptoms such as blunted affect, alogia and withdrawal, and cognitive impairment were inversely associated with grey matter volume in the bilateral cerebellum. In patients with schizophrenia, however, anhedonia and avolition was inversely related to white matter volume in the left anterior limb of the internal capsule/anterior thalamic radiation and positively in the left superior longitudinal fasciculus, indicating depression and schizophrenia result from differing underlying biological processes. However, this seems less likely as depression in schizophrenia responds to antidepressant medication (Gregory et al., 2017).

Previous evidence has suggested raised IL-6 and CRP prior to treatment in those with schizophrenia, which then normalises in those who respond to treatment whilst they remain high in treatment resistant (Mondelli et al., 2015a, Miller et al., 2011). Given the

relatively low PANSS total scores in this study, it would suggest that few participants would be treatment resistant. This may explain the surprisingly low mean scores of markers used to assess inflammation, compared to previous findings, and the lack of treatment response. Whilst this does not exclude the possible role of the immune system in schizophrenia, it may suggest that a sub-group of individuals with an 'immune activated psychosis' may benefit from minocycline adjunctive to antipsychotic medication, or that minocycline may be most beneficial to those with treatment resistant schizophrenia, as this sub-group are most likely to show increased neuro-inflammation. However, both this study (including the exploratory analyses) and a previous study of a similar design and methodology (Chaudhry et al., 2012) failed to identify an 'inflammatory' sub-group of patients. A possible explanation for the lack of inflammatory response in this sample may be that the intervention was administered too late, and that immune dysfunction occurs earlier on in the At Risk Mental State (ARMS) group leading to psychosis, which then normalises upon recovery.

Despite not identifying an inflammatory sub-group of patients who did respond to minocycline, the exploratory analyses did reveal some interesting findings. In those with an inflammatory response, depression was associated with greater concentrations of IL-6. This supports Khandaker et al. (2014), who reported raised childhood IL-6 levels predicted future depression. One explanation for these findings is that the number of participants diminished, compromising statistical power. Also, the exploratory analyses strengthened the already robust finding of expressive-deficits' association with TNF-α, from previous studies in this thesis. The anomalous negative relationship between TNF-α and depression cannot be explained by previous literature, nor is there a sufficient

biological explanation for this finding. Upthegrove et al. (2010) identified a sub-group of depressed participants who remained depressed longitudinally, indicating a trait depression. Raised TNF-α would suggest that individuals who were consistently depressed would be associated with this trait inflammatory mechanism. However, exploratory analysis using the present data suggests that there was a negative relationship between depression and TNF-α suggesting that the majority of patients in this sample did not experience a trait depression, associated with trait cytokines, but instead experienced depressive episodes that improved over time.

7.4.1 Limitations

There were several potential limitations associated with this study. Firstly, this study suffered a high dropout rate, which can explain the reduction in CDSS scores from baseline as those experiencing the highest level of depression may have dropped out after the baseline assessment. To overcome this limitation, a linear mixed effect model was used to analyse data, which can account for missing data and retain statistical power in analysis. Another limitation with this study is that antipsychotic and antidepressant medication has not been included as a co-variate in the analysis as this data was not ready at the time of analysis. This raises a potential limitation as antipsychotic and antidepressant medication is known to impact on cytokines (Mondelli et al., 2015a). Therefore, it is essential to include this variable in the analysis to assess the impact of medication on cytokines. Finally, this study was not designed to assess the benefit of minocycline to treat depression in schizophrenia.

7.4.2 Future implications

Evidence from the present study suggests that minocycline, when given in combination with an antipsychotic medication, does not lead to a reduction of negative or depressive symptoms in early schizophrenia. However, this does not rule out the potential beneficial effect of other anti-inflammatory medication. One such anti-inflammatory drug, celecoxib, has been shown to reduce negative symptoms, in a meta-analysis of eight randomised controlled trials (Zheng et al., 2017). Future studies focusing on either medication naïve patients or patients in the ultra-high-risk category for psychosis may take advantage of anti-inflammatory medication and show a beneficial effect, particularly on expressive deficits. Furthermore, studies exploring the effect of anti-inflammatory agents on negative symptoms should employ a more sensitive measure of negative symptoms such as the CAINS or the SANS, which may identify subtle changes in negative symptoms, not captured by more global measures of psychopathology, such as the PANSS.

8. Discussion

The five year forward plan put forward by the NHS (2017) reports that despite considerable time and financial investment into early intervention in psychosis (EIP) services, outcomes for people with schizophrenia remain pessimistic. A systematic review of 19072 patients with first episode psychosis across seventy-nine studies by Lally et al. (2018) demonstrated that only 58% of people achieve remission following their first episode of psychosis and a mere 30% of people with schizophrenia recover previous role and occupational functioning (Lally et al., 2018). Thus, there remains considerable scope for understanding the complex presentation of psychosis and development of new more targeted treatments. Negative symptoms and depression are both common in schizophrenia, share some symptoms (such as avolition, anhedonia and apathy), potentially share aetiological pathways, and can be difficult to treat. This thesis has investigated the relationship between depression and negative symptoms, their interaction over twelve months and the potential common pro-inflammatory biological aetiology, together with an exploration of the effects of a novel therapeutic agent, minocycline.

8.1.1 Aims

This thesis set out to test four key hypotheses. Firstly, that the sub-domain of negative symptoms avolition-apathy is mechanistically related to depression and so these symptoms will change together over the twelve month study, correlating with each other

at each time. Secondly, it was predicted that the sub-domain of negative symptoms expressive deficits will be independent of depression longitudinally. Thirdly; avolition-apathy and depression will share an inflammatory pathogenesis and so fluctuations over time will be associated with changes in state-dependant cytokine inflammatory markers. In contrast, expressive deficits will be more stable and associated with trait-dependent cytokine. Finally, administration of minocycline will reduce state-dependent cytokine concentrations (IL-6) and CRP, mediating a reduction in avolition-apathy and depression, without affecting trait cytokine levels and expression deficits.

8.2 Study summaries

8.2.1 Phenomenological investigation: longitudinal relationship between negative symptoms and depression

The first study investigated the longitudinal relationship between depression and negative symptoms in early schizophrenia in a sample of 204 patients, capturing data at baseline, two, six, nine and twelve months. At baseline, depression was associated with avolition-apathy and not with expressive deficits. Longitudinally, depression was associated with avolition-apathy. Depression did positively correlate with expressive deficits at the six, nine and twelve month assessment and negatively correlate at the two month assessment, but depression was not associated with expressive deficits longitudinally in the regression model. The results highlighted three important points. Firstly, that depression was associated with avolition-apathy longitudinally. Secondly, that two sub-domains; avolition-apathy and expressive deficits could be distinguished,

and that this distinction holds longitudinally. Finally, these findings may explain some of the conflicting findings of previous research examining the relationship between depression and negative symptoms that used total negative symptom scores rather conceptualising negative symptoms as being composed of two sub-domains.

8.2.2 Immune dysfunction, depression and negative symptoms

The immune dysfunction study focused on examining whether potential pro-inflammatory markers IL-6, TNF-α and CRP were associated with depression, total negative symptoms, avolition-apathy and expressive deficits. It was predicted that depression would be associated with cytokines that increase during acute exacerbations and normalise upon recovery (state cytokines) and expressive deficits would be associated with cytokines that remain elevated during the acute exacerbations and upon recovery (trait cytokines). Depression correlated with IL-6 in cross-section but was not associated in the longitudinal analysis. Linear mixed effects modelling found that no state inflammatory (e.g. CRP. IL-6) was associated with avolition-apathy or depression over twelve months. However, TNF-α, considered an indication of trait inflammation, was associated with expressive deficits longitudinally. These findings may suggest that expressive deficits may be associated with distinct psychopathology involving the prolonged activation of the immune system, specifically by a TNF-α pathway.

8.2.3 Targeting the immune system to treat depression and negative symptoms

The final study utilised data from the placebo-controlled, randomised controlled trial to assess the benefits of targeting the immune system to treat depression and negative symptoms using the antibiotic minocycline. This was a novel approach as trial data was re-analysed assessing the effect of minocycline on depression and the two sub-domains of negative symptoms; avolition-apathy and expressive deficits. This study found that minocycline did not treat depression, total negative symptoms, avolition-apathy or expressive deficits. Moreover, minocycline did not lead to a reduction in pro-inflammatory markers. Further exploratory analysis did not identify a treatment effect of minocycline on negative and depressive symptoms in a stratified subgroup of patients with raised CRP (indicating an inflammatory response), although did identify an association between depression and IL-6 in those who were depressed. These findings suggest that minocycline is not effective in the treatment of negative symptoms or depression, however the results must be considered in light of its limitations (e.g. medication was not controlled for).

8.3 Main outcomes

8.3.1 Co-occurrence of negative symptoms and depression

Findings from the longitudinal analysis of this thesis suggest that depression may be related with avolition-apathy. Psychological approaches to negative symptoms may explain their emergence and how they relate with depression. Negative symptoms may

emerge as a negative belief about performance encompassing negative views of the self and fear of failure (Rector et al., 2005), from low expectancies of pleasure (deVries and Delespaul, 1989), or due to stigma associated with the diagnosis of schizophrenia (Estroff, 1989). Depression is characterised by low mood, apathy and social withdrawal. Beck describes a "cognitive triad" in depression whereby "life is pointless, the future hopeless and the self is worthless" (Beck, 1970), of which anhedonia is central. Therefore, certain negative symptoms that correspond with the avolition-apathy sub-domains, which are phenomenologically similar to anhedonia, may be secondary to depression. However, it is also possible that anhedonia may present differently in depression and in schizophrenia, with consummatory being hypothesised as being present in depression and anticipatory anhedonia being hypothesised as being present in schizophrenia (Gard et al., 2007). Furthermore, Upthegrove et al. (2014b) has described the emergence of depression in schizophrenia as being the result of stigma and shame, which has been suggested to drive negative symptoms, which may be through low mood and aberrant cognitions leading to increased social withdrawal, apathy and anhedonia or low expectancy of pleasure or success (Rector et al., 2005). One interpretation of these findings may be that there may be a common underlying psychopathological mechanism that underpins avolition-apathy and depression.

Depression was not associated with expressive deficits in psychosis. Previous studies examining the relationship between depression and negative symptoms have generated contradictory findings. Barnes et al. (1989b) confirmed a depression syndrome which was distinct from the negative syndrome symptoms and was not a result of pharmacotherapy. Pogue-Geile and Harrow (1984), however, failed to find a difference

between depression and negative symptoms. The finding that depression is related to symptoms such as poor motivation, emotional withdrawal and social withdrawal (avolition-apathy) confirms previous research by Sax et al. (1996). These findings also support Whiteford et al. (1987) who found that certain symptoms (such as poor attention and alogia) were associated with the negative syndrome, whereas others (such as anergia and anhedonia) were present in both depressive and negative domains. However, one explanation for this finding may be that the high association between these negative and depressive symptoms may be artefactual and the product of similarly worded items of different rating scales, that may appear to show an association between these two syndromes. Also, the discrepancy in findings of Sax et al. (1996) and Pogue-Geile and Harrow (1984), and the current findings presented in this thesis may be due to Sax et al. (1996) and Pogue-Geile and Harrow (1984) drawing from a relatively small sample size and that failure to identify a relationship between depression and negative symptoms based on cross-sectional data, thus ignoring the complexities of this relationship captured in longitudinal studies.

8.3.1.1 **Dimensional model of depression and negative symptoms**

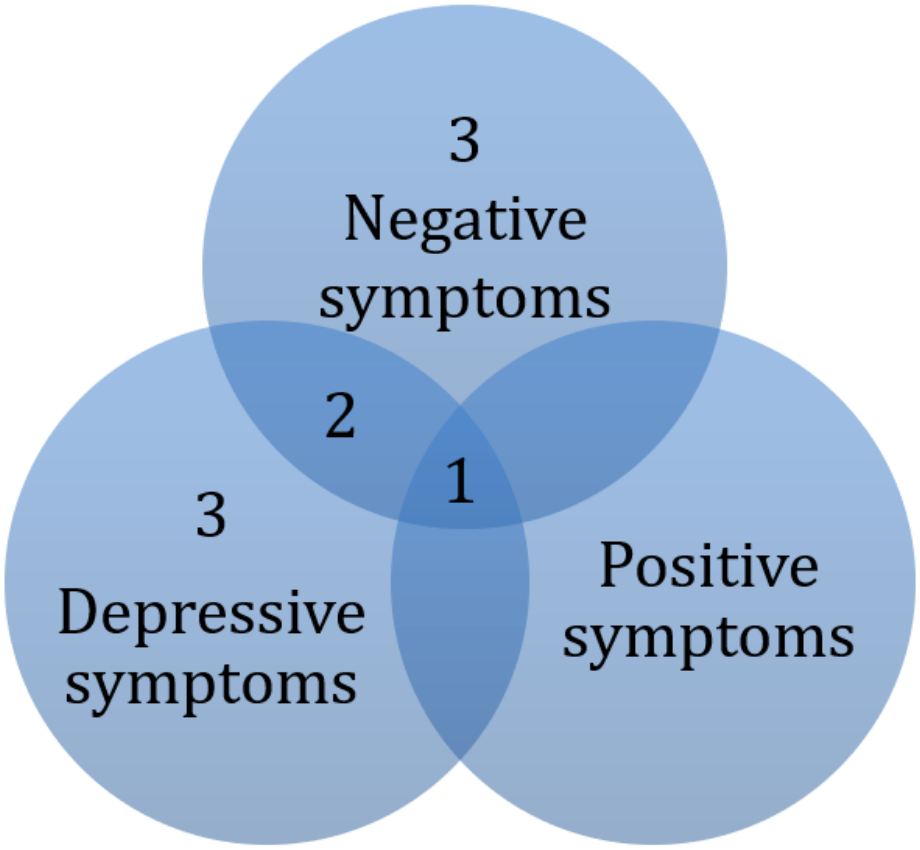
The systematic review presented in this thesis exemplified the complexity of the relationship between negative symptoms and depression, and suggests that differences in the previous literature may, in part, be due to considering negative symptoms being composed of a single syndrome. Differentiating expressive deficits from social amotivation may clarify the relationship between depression and negative symptoms. Specifically, depression appears associated with social amotivation (similar to avolition-

apathy) and not expressive deficits. The systematic review presented in this thesis by Krynicki et al. (2018), included twenty-eight studies and supports the experimental findings of this thesis, as well as by previous literature (Stiekema et al., 2016), which identified a relationship between depression and avolition-apathy and not expressive deficits in a chronic schizophrenia sample. This is an important distinction to make, as a global score of negative symptoms ignores the distinction between its two sub-domains and may have implications for future research examining both the phenotype of psychosis but also predictors of poor functional outcome.

This study presented a dimensional model within domains representation of the relationship between depression and negative symptoms (see figure twenty-three). This model suggests that certain symptoms span both depressive and negative domains (such as avolition, apathy and anhedonia) (2), others represent a core symptom specific to depression (such as depressed mood, suicidal ideation) (3) and others specific to negative symptoms (such as blunted affect and alogia) (3) and others represent general, attenuated non-specific symptoms that are not associated with positive, depressive or negative symptomology (1). The co-occurrence of depression and avolition-apathy (as predicted by this model) has been supported by the empirical studies presented in this thesis. Furthermore, the lack of relationship between expressive deficits and depression in the longitudinal analysis can also be explained by this relationship as representing a core feature of the negative syndrome. The finding that TNF-α is associated with expressive deficits but not depression and avolition-apathy longitudinally further supports the suggestion that expressive deficits may have a different psychopathological underpinning to depression and avolition-apathy. Moreover, this model may account for the

improvement of depression and avolition-apathy following administration of citalopram by Barnes et al. (2016) as being underpinned by a shared biological mechanism that responds to the same pharmacological agent. However, given that the study by Barnes et al. (2016) was underpowered, any conclusions of the relationship between depression and negative symptoms, and the treatment effect of citalopram on negative symptoms must be made with caution. This model however ignores the finding that depression and expressive deficits correlated in cross-section and that depression may be composed of differing 'sub-types', that may relate with negative symptoms independently (as discussed in section 8.3.2).

Figure 23: Dimensional model of negative, depressive and positive symptoms in schizophrenia. 1= General, attenuated non-specific symptoms; 2. Symptoms that span both depressive and negative symptoms; and 3. Symptoms specific to the depressive or negative symptoms domains



Expressive deficits represent a core body of negative symptoms that are not just the consequence of extrapyramidal side effects (EPSE). Early work exploring the nature of negative symptoms typically included patients who had been medicated using typical antipsychotic medication (usually at a higher dose than currently recommended), which resulted in EPSE potentially being primarily attributed as a negative symptom. The findings presented in the longitudinal study of this thesis suggest that not all cases of expressive deficits can be attributed as being caused by antipsychotic medication, and that these can be identified early in the course of psychosis. In this sample, EPSE scores

were strikingly low and EPSE was included as a proxy measure for antipsychotic medication, which was shown to not be significant in predicting expressive deficits in the regression models. These findings suggest that expressive deficits represent a core feature of the negative syndrome and are not the just result of EPSE.

The use of the CDSS in studies presented in this thesis may account for some discrepancies with previous research such as Pogue-Geile (1989) and Koreen et al. (1993). Early research examining depressive symptoms in schizophrenia relied heavily on the Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1967), the Beck Depression Inventory (BDI) (Beck et al., 1961) and the depression item on the PANSS interview (PANSS-D) (Kay et al., 1987). However, these scales have been criticised for their use in schizophrenia. Whilst adequately identifying depression in schizophrenia, it has been argued that these scales poorly separate depression from negative and extrapyramidal symptoms of schizophrenia (Collins et al., 1996). The Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al., 1993) has been shown not only to identify depressive symptoms in schizophrenia, but also able to distinguish them from negative and extrapyramidal symptoms (Collins et al., 1996, Addington et al., 2014, Addington et al., 1996, Liu et al., 2009).

8.3.2 Immune dysfunction

Findings from the immune dysfunction study in this thesis indicate that expressive deficits were associated with the increased inflammatory cytokine TNF-α, suggesting that there may be a distinct psychopathological process. TNF-α is a Th1 pro-inflammatory

cytokine that is involved in the regulation of immune cells. TNF-α is elevated in people with schizophrenia compared to healthy controls (Mansur et al., 2012), and is consistently raised throughout the course of illness, independent of clinical stability (trait cytokine) (Miller et al., 2011). These findings and others indicate that schizophrenia may be associated with immune dysfunction (Khandaker and Dantzer, 2016). Early brain changes and grey matter volume (GMV) loss may be initiated by immune cell activation which triggers the release of cytokines. Increased circulating pro-inflammatory cytokines are known to generate microglia activation, which in its activated state, act as mononuclear phagocytes causing synaptic pruning and grey matter volume loss. This sequence of events contributes to the neuronal degeneration aberrant neuronal architecture and white matter abnormalities (Monji et al., 2009) observed in schizophrenia.

Previous studies have found elevated inflammatory markers (such as CRP, IL-1β, IL-6 and TNF-α) related to schizophrenia symptom severity. These markers have also been raised in those who were medication naïve, as reviewed by Upthegrove et al. (2014a) and those who were treatment resistant (Mondelli et al., 2015a) and acute relapse patients (Miller et al., 2011). Interestingly, those who responded to treatment showed a reduction in cytokine concentrations (Mondelli et al., 2015a). Others have focused on immune dysfunction associated with specific symptoms within schizophrenia. Noto et al. (2015b) found depression was associated with TNF-α, but no cytokine or acute phase protein was associated with negative symptoms. This finding is surprising given the symptom co-occurrence of depression and avolition-apathy, and the hypothesis that TNF-α is considered a trait cytokine, whereas depression is found to fluctuate throughout the course of illness. However, the sample size used by Noto et al. (2015b) was much smaller

than the present sample size. Moreover, Noto et al. (2015b) did not control for BMI in their analysis, known to impact on cytokine levels which was and controlled for in the present study. Therefore, discrepancies in the results may be due to these factors. Goldsmith et al. (2018) found an association between TNF-α predicted blunted affect and alogia (i.e. expressive deficits) and total negative symptoms in a sample of fifty-six chronic schizophrenia patients versus twenty-eight healthy controls. They concluded that negative symptoms are associated with increased inflammation and may therefore represent a target for future therapies. This suggests that diminished emotional expression may be brought about by increases in TNF-α.

Functional MRI data has found inflammatory cytokines target specific brain regions and circuits including the basal ganglia (Capuron et al., 2012) and corticostriatal reward circuitry (Felger et al., 2015) which are involved in motivation and motor activity. Recent evidence provides support for a specific brain circuitry that underlies expressive deficits; in an fMRI study by Dlabac-de Lange et al. (2018) which presented twenty participants with schizophrenia the wall of faces task found that those with high expressive deficits showed hypoactivation of the fronto-thalamic circuitry (consisting of thalamic, prefrontal, precentral, parietal and temporal brain areas) during ambiguous social appraisal. One interpretation from these findings may be that increased levels of the pro-inflammatory cytokine TNF-α may lead to microglial activation causing synaptic pruning and GMV loss by phagocytosis, resulting in decreased connectivity within the fronto-thalamic circuitry, which contributes to the development of expressive deficits. However, this remains yet to be established. The association between expressive deficits

and TNF-α may suggest that expressive deficits represent a distinct psychopathology that is driven by immune dysfunction.

The studies presented in this thesis identified an association between avolition-apathy and depression. One explanation for this is that avolition-apathy is a manifestation of a neurobiological process that is common with depression. Preliminary evidence to support this assertion comes from Barnes et al. (2016) who found that both depression and avolition-apathy improved following treatment using citalopram whereas expressive deficits showed no improvement.

The evidence for immune dysfunction in depression is well established. Pro-inflammatory markers (such as IL-6 and CRP) have been reported in depressed individuals (Young et al., 2014, Valkanova et al., 2013, Haapakoski et al., 2015, Liu et al., 2012). The immune dysfunction study of this thesis showed that IL-6 correlated with depression at baseline but not longitudinally, where the sample was at its greatest and the depression scores were highest. This may suggest that the state cytokine IL-6 was associated with depression where depression was at its highest and reduced upon recovery of depression. Furthermore, depression was associated with IL-6 longitudinally in those with an inflammatory response. A longitudinal study of 4500 individuals by Khandaker et al. (2014) found that those with increased IL-6 in childhood were more likely to be depressed at eighteen years of age. A meta-analysis of forty studies by Miller et al. (2011) has found increased levels of IL-6, CRP and TNF-α in first episode and acutely relapsed patients. They also found that IL-6 then normalised upon successful treatment with antipsychotic medication (Miller et al., 2011). The sample included in this thesis

however, had surprisingly low levels of IL-6, CRP and TNF-α. Given that these levels were low; this may explain the lack of a strong association between state cytokines and CRP, and avolition-apathy and depression, longitudinally. Moreover, the PANSS scores and CDSS of the present sample suggest that the sample were not very symptomatic and were improving over time. The low PANSS scores and low levels of inflammatory markers therefore mean that the role of inflammation in depression and avolition-apathy cannot be ruled out.

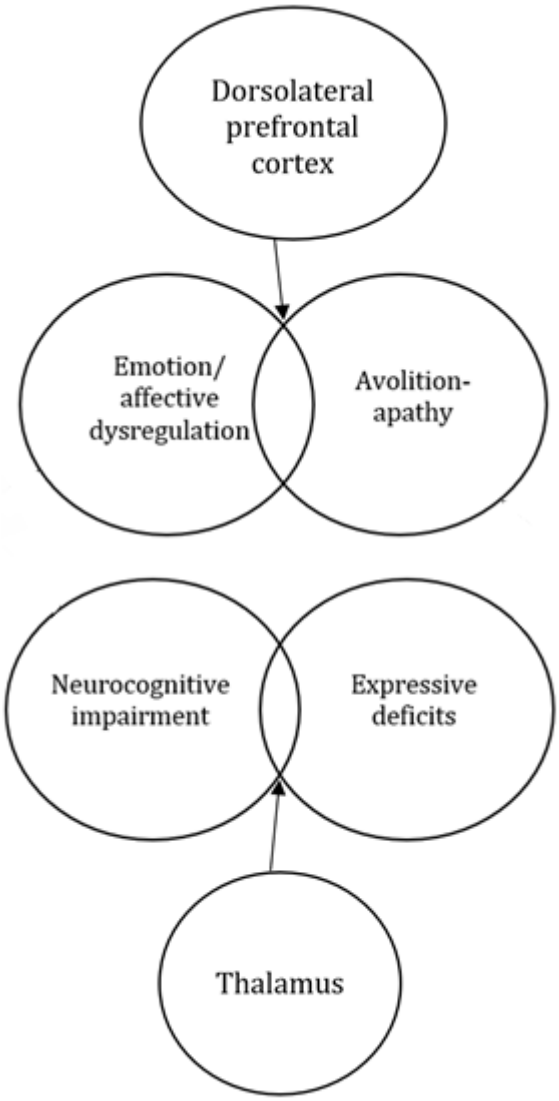
The findings of this thesis suggest that there is a relationship between depression and negative symptoms via the avolition-apathy sub-domain. However, depression and expressive deficits also correlated at all time points except baseline. Despite not being significant in the linear mixed effects model, this finding suggests that depression may be related to expressive deficits. Depression is composed of differing symptoms that may independently relate with avolition-apathy and expressive deficits. Depression has been associated with circadian dysfunction (Wirz-Justice, 2006, Germain and Kupfer, 2008), emotional/affective dysregulation (Gratz et al., 2004) and neurocognitive impairment (Mohn and Rund, 2016). Individuals with major depressive disorder (MDD) have been shown to have impaired neurocognition, and this symptom domain has been included in the diagnostic criteria for depression (Diagnostic and Statistical Manual) (American Psychiatric Association, 2013). Mohn and Rund (2016) compared thirty-three patients with matched healthy controls and found that those with MDD experienced impaired neurocognition especially working memory and processing speed, which was related to severity of depression. Desynchronised circadian rhythms have also been implicated in mood disorders (as reviewed in Goodwin and Jamison (2007), perhaps through phase

disturbance that regulates temperature, cortisol, melatonin and REM sleep (Germain and Kupfer, 2008). Indeed, treatments that specifically target the sleep-wake cycle and circadian rhythms have been found to be clinically effective in managing major depression (reviewed by Hickie et al. (2013). Circadian dysregulation has also been suggested to increase the release of pro-inflammatory cytokines in both human and animal models (reviewed in McClung (2013). Finally, emotion/affective dysregulation has been defined as an awareness, understanding and acceptance of emotions (Marganska et al., 2013) and mood. Gratz et al. (2004) have conceptualised emotion dysregulation as deficits in one or more of the following abilities associated with the regulation of emotion: awareness/understanding of emotions, acceptance of emotions, control of impulsive behaviour and use of appropriate emotion regulation strategies. Therefore, depression may emerge following poor emotion regulation in response to negative affect (Hofmann et al., 2012). The three symptom clusters of depression may relate to negative symptoms differently, with emotional dysregulation being related to avolition-apathy. Expressive deficits may relate to neurocognition, given the co-occurrence of neurocognitive dysfunction seen in expressive deficits (Sevy et al., 2018) and depression (Mohn and Rund, 2016).

The evidence presented above also suggests that immune dysfunction may contribute to the emergence of depression and negative symptoms in schizophrenia. This may be associated with one pathological process that targets different brain regions that are responsible for negative and depressive symptomology. One hypothesis is that increased inflammation contributes to pathology in the dorsolateral prefrontal cortex (dlPFC) and the thalamus. Hypoactivation of the thalamus has been implicated in both

expressive deficits and neurocognitive deficits in depression (Elliott et al., 1997), suggesting that targeted inflammation in the thalamus may contribute to both neurocognitive deficits in depression and expressive deficits in schizophrenia. Abnormalities in the dlPFC have been found in depressed patients (Drevets, 2000) suggesting that this region may also be important in understanding the relationship between depression and negative symptoms. The dlPFC has primarily been associated with cognitive functions, however more recent appraisals suggest that this region may pertain to emotions and affect (Golkar et al., 2012). Given the association between depression and avolition-apathy, and the suspected involvement of the dlPFC in depression, inflammation associated pathology in the dlPFC may contribute both to emotion/affective dysregulation in depression and avolition-apathy. A diagram of this relationship can be seen in figure twenty-four, detailing the symptoms of depression and how they relate with negative symptoms, and potential brain regions that may be responsible for these sub-domains.

Figure 24: Diagrammatic representation of the putative relationship between symptom profiles associated with depression and negative symptoms, and putative pathological changes.



The longitudinal association between depression and negative symptoms, and the cross-sectional correlation between depression and expressive deficits suggest that the relationship between depression and negative symptoms is dynamic, with a strong

association between depressive symptoms and avolition-apathy, but also cross-sectional correlations between expressive deficits and depression suggest that depression and expressive deficits may be related. This diagram shows the putative relationship between the different symptoms of depression and negative symptoms accounting for the significant correlations found in this thesis potentially being related via a neurocognitive impairment pathway. This distinction between depression symptom clusters was not possible for the present research, as the CDSS does not lend itself to separating out depression but this distinction may be important for future research to further elucidate the relationship between depression and negative symptoms. Importantly, viewing depression as being made up of 'sub-types' may have important clinical implications, that target specific aspects of depression (such as circadian rhythm being treated using light therapy), in addition to implications to how depression is viewed and investigated. Moreover, focusing on depressive 'sub-types' and how these may relate with negative symptoms may indicate a psychopathological mechanism (as suggested above) that may be held to account for these symptoms, which can provide a target for therapy, as well as understanding how depression relates with other symptom domains. Taken together, the findings from this thesis suggests that the relationship between depression and negative symptoms is complex and requires further investigation and will be the focus of on-going research.

8.3.3 Targeting the immune system to treat depression and negative symptoms

8.3.3.1 Minocycline and schizophrenia

There was no evidence from the final study presented within this thesis that targeting the immune system with minocycline will reduce inflammation and cause a correlated reduction in negative and depressive symptoms. This is through the putative neuroprotective role of minocycline which was hypothesised to reduce microglial activation. The microglial hypothesis for schizophrenia (Monji et al., 2009) postulates that pro-inflammatory cytokines are released or elevated following stressful life events which activate microglia and lead to a cascade of events causing neuronal degeneration, decreased neurogenesis and white matter abnormalities. However, the present findings differ from the previous literature. Chaudhry et al. (2012) found negative symptoms were reduced in those treated with minocycline compared to those in the placebo arm of this double blind, placebo-controlled trial. Furthermore, a meta-analysis of eight randomised controlled trials with 548 participants (286 patients on minocycline and 262 on placebo) by Xiang et al. (2017) identified a treatment effect of minocycline, thus supporting these findings. However, six of the eight studies reporting a treatment effect of minocycline drew from a more symptomatic sample, (mean illness severity scores were much higher than the current sample). Moreover, seven of the eight studies included in this meta-analysis were much shorter in duration of treatment. As the present study is much larger, and the treatment and follow up times are greater, and less symptomatic than previous studies, this may account for the discrepancies in previous studies exploring minocycline in schizophrenia.

8.3.3.1.1 Microglial activation in ultra-high risk for psychosis and schizophrenia

Those considered ultra-high risk for psychosis are so defined based on various risk factors, such as age, functional decline, prodromal symptoms and genetic risk (Yung and Nelson, 2013) however, a major challenge has been predicting which individuals go onto develop psychosis and which recover/remain UHR. Indeed, the concept of psychosis is an arbitrary one, and divides those with psychosis from those without psychosis, and those who transition to psychosis can be clinically indistinguishable from those who do not transition to psychosis. However, psychosis has been suggested to be on a continuum of frequency, duration of psychotic symptoms and intensity (Yung et al., 2010). Indeed, the arbitrary cut off may result in an individual with low negative symptoms, intact neurocognition and unaffected social functioning transitioning to psychosis, whereas another individual with a presentation closer to the schizophrenia construct (such as high levels of neurocognitive and social dysfunction) not transitioning to psychosis but who may have a poorer outcome. The lack of predictability between those who transition and those who do not remains a challenge, given the clinical implications, such as early intervention resulting in better outcomes (Malla et al., 1999).

Structural changes in the brain may serve as a biomarker to predict transition to psychosis. Dazzan et al. (2012) found that those who transition to psychosis experienced greater GMV loss compared to those who did not transition to psychosis. Moreover, (Ziermans et al., 2012) found those who transition to psychosis showed volume loss in the left anterior cingulate, precuneus and temporo-parieto-occipital area. A large multicentre study (total n = 182, transitioned n = 48) assessing the brain structure of

those in the UHR for psychosis found that those who transitioned displayed greater GMV loss in the inferior frontal, parahippocampal and superior temporal corticies compared to those who did not transition (Mechelli et al., 2011). These findings suggest that GMV loss may serve as an appropriate biomarker to predict who transitions to psychosis and which individuals do not.

Microglial activation has been found in patients with schizophrenia (van Berckel et al., 2008) and in those in the ultra-high-risk for psychosis group (Bloomfield et al., 2016) using Positron Emission Tomography (PET) scanning, adding to the weight of evidence implicating microglial activation in the pathology of schizophrenia. However, some PET studies have failed to provide support for microglial activation. Di Biase et al. (2017) looked at the binding potential in six key brain areas (dorsal frontal, orbito frontal, anterior cingulate, medial temporal, thalamus and insula) to assess whether there was microglial activation in UHR individuals, recently diagnosed and chronic schizophrenia. They found that there were no correlations in binding potential with regional grey matter, peripheral cytokine levels or clinical symptoms, suggesting that there was no microglial activation in those in the UHR for psychosis, recently diagnosed and chronically ill with schizophrenia. Moreover, of those who were UHR and identified as having microglial activation (Bloomfield et al., 2016), only one participant transitioned to psychosis, suggesting that activated microglial may only account for immune dysfunction in some individuals with schizophrenia. The inconsistent findings of microglial activation suggest that immune dysfunction does not underpin all cases of schizophrenia, highlighting the diverse psychopathology of schizophrenia and the many factors associated with the

emergence of the illness. This supports findings from the current study that some patients did not show immune cell activation in first episode psychosis.

8.3.3.2 **Minocycline and depression**

To date, there have been three published randomised controlled clinical trials have used minocycline to treat depression (Dean et al., 2017, Husain et al., 2017a, Emadi-Kouchak et al., 2016). Of these, two identified a treatment effect of minocycline to treat depression (Emadi-Kouchak et al., 2016, Husain et al., 2017a). Discrepancies between the present findings and these studies may be due to a much smaller sample size being used in these studies (the largest study to find an effect of minocycline being Emadi-Kouchak et al. (2016) has a sample of fifty participants). Indeed, Dean et al. (2017) conducted the largest study used a sample of seventy participants and found no effect of minocycline on depression. Another explanation for the differences in these findings may be that these studies followed up participants for either six weeks (Emadi-Kouchak et al., 2016) or twelve weeks (Husain et al., 2017a, Dean et al., 2017), which is much shorter than the present study. A final explanation of these findings is that depression is different to that in schizophrenia (regardless of the presence of depression), where there is not activated microglia for minocycline to act on.

8.4 Limitations and methodological considerations

8.4.1 Sample limitations

There were limitations associated with the sample of participants. Firstly, many of the patients included in the studies had been patients under the early intervention in psychosis services for up to five years and had been treated with antipsychotic and antidepressant medication since their first admission. Given the prevalence of depression and negative symptoms in both UHR samples and first episode psychosis, studying the relationship between depression and negative symptoms in patients earlier in the course of illness using unmedicated patients, and even those prior to frank onset of illness may have revealed a common inflammatory mechanism underpinning depression and avolition-apathy.

A second, related limitation to the studies presented is that all patients were taking antipsychotic medication, and some were taking antidepressant medication. Both antipsychotic medication and antidepressant medication have a known effect on markers of inflammation, many of which result in a reduction in pro-inflammatory markers. This may account for the surprisingly low levels of pro-inflammatory markers in the sample. Furthermore, this presents a key methodological weakness; as antidepressant and antipsychotic medication are known to reduce markers of inflammation, then minocycline would not be effective in treating depression or negative symptoms as the mechanism for this would be to reduce pro-inflammatory markers. There are other ways in which minocycline has been hypothesised to reduce negative symptoms, such as

through minocycline's putative action on the glutamate system implicated in schizophrenia, by reversing a disease-related impairment of NMDA function thought to underpin negative symptoms. However, this was not directly assessed in the studies presented in this thesis.

Finally, the majority of patients included in this sample were male (n = 149, 73%), thus suggesting that the results may not be generalisable to female patients with schizophrenia. Whilst this is a limitation of this study, the sample reflects the increased prevalence of schizophrenia in the male population compared to women (McGrath et al., 2008, Kirkbride et al., 2006).

8.4.2 Evaluation of analysis

One advantage of the studies presented in this thesis is the method of analysis. Linear mixed effects regression modelling proves superior compared to the standard ANOVA in several ways. Mixed regression models can be used on a sample that is not normally distributed, whereas this cannot be performed by ANOVA. Also, mixed regression models can also handle missing data so that all participants are included in the analysis, unlike missing data analysed using ANOVA, which discards incomplete data sets due to ANOVA's use of list wise deletion, which can cause bias and reduce power. This was important when considering which statistical technique to use to analyse the data, as some of the data was missing.

A disadvantage with the analyses presented in all the studies in this thesis is the exclusion of antipsychotic and antidepressant medication from the multivariate analyses as current medication usage and medication history was not available at the time of analysis. To overcome this disadvantage, measures assessing extrapyramidal side effects (EPSE) have been included in the analysis if they correlate in the exploratory analyses. EPSE was used as a proxy measure in the cross-sectional and longitudinal analyses presented in study one, with the assumption that drug induced EPSE may mimic negative symptoms, which may therefore be misattributed to negative symptoms. This would be detected using the Simpson-Angus Scale (SAS) (Simpson and Angus, 1970), which is an assessment specifically designed to assess EPSE. However, both antipsychotic and antidepressant medication have a known effect on inflammatory cytokines, therefore controlling for medication in the multivariate analyses presented in studies two and three may have benefited the analyses and enriched the results. Finally, the lack of other key variables from the analysis such as length of illness, duration of untreated psychosis and neurocognitive functioning represents another limitation with this research. The addition of duration of untreated psychosis and length of illness is important not least given the hypothesis that microglial activation occurs early in the schizophrenia disease process, therefore administering an antibiotic may prevent microglial activation, but also as the nature of the relationship between depression and negative symptoms may change given the phase of illness. Considering cognitive symptoms in the analysis may have elucidated the relationship between expressive deficits and depression and provides a target for future work.

8.4.3 Evaluation of methodology

The methods used to assess the clinical variables are not without fault. Many of the earlier scales such as the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982) do not reflect the findings from the systematic review presented in the introductory chapters of this thesis by Krynicki et al. (2018) which suggests that the negative symptoms are composed of two symptom domains; the avolition-amotivation and the diminished emotional expression domains. This has provided a challenge when attempting to assess these two sub-domains. Furthermore, some of the global score of schizophrenia do not consider all aspects of the negative symptoms, such as the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) which does not assess anhedonia. Moreover, several scales assessing negative symptoms fail to distinguish between primary and secondary negative symptoms (Lincoln et al., 2016). Finally, several of the global scores of psychopathology ignore the subjective experience of these symptoms, thus representing a major disadvantage of these assessments, such as the PANSS (Kay et al., 1987) and the SANS (Andreasen, 1989). To overcome this methodological limitation, the Clinical Assessment Interview for Negative Symptoms (CAINS) (Kring et al., 2013) has been developed, which provides two composite scores on 'motivation and pleasure' and 'expression'.

The method used to assess depression in the studies presented in this thesis has been shown to provide an accurate reflection of depressive symptoms in schizophrenia. The use of the CDSS in these studies represents a particular strength of the studies presented in this thesis. When assessing the relationship between depression and

negative symptoms, it is essential for a scale to reliably measure depression and misattribute EPSE or negative symptoms as depressive symptoms. The CDSS has been specifically developed to comprehensively assess depression in schizophrenia, minimising the risk of erroneously considering EPSE or negative symptoms as depression. This highlights the CDSS as a superior method of assessing depressive features in schizophrenia compared with the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), Beck Depression Inventory (BDI) (Beck et al., 1961) and PANSS-D (Kay et al., 1987). The use of the CDSS however, represents a methodological limitation to the present research. This thesis aimed to understand the relationship between depression and negative symptoms. Therefore, using a scale that was specifically developed, through factor analysis of other symptom domains including global negative symptoms, to separate depression from negative symptoms may cloud the true nature of the relationship between these two syndromes. However, to overcome this limitation, adopting a sub-domain approach to examining negative symptoms (rather than global negative symptoms included in the original factor analysis by Addington et al. (1990) has enabled a critical examination of this paradigm.

Finally, this study drew data from the BeneMin trial, which was an RCT assessing the benefits of minocycline, therefore this sample may be biased to participants willing to take an investigational medicinal product, rather than represent the general schizophrenia population. Furthermore, the high dropout rate of participants may suggest that those who were most unwell left the study, therefore, limiting the conclusions of the research and restricting the results to those who remained in the study and those who were least unwell.

8.5 Future research and clinical implications

8.5.1 Clinical implications

The findings from this thesis have significant research and clinical implications. The absence of any therapeutic benefit of minocycline even when stratified by patients with immune activation suggests that minocycline does not have any benefit on reducing depression or negative symptoms in schizophrenia through its putative action on the immune system. However, other anti-inflammatory agents may still show promise: a meta-analysis of eight randomised controlled trials with 626 patients with schizophrenia (316 treated with 400mg of celecoxib and 310 placebo) (Zheng et al., 2017) identified a treatment effect on positive and negative symptoms and general psychopathology. Celecoxib is an nonsteroidal anti-inflammatory drug (Sommer et al., 2012) that selectively inhibits COX-2. It is not reported in these studies whether this beneficial effect preferentially reduces avolition-apathy over expressive deficits. However, there is no evidence from the current findings that minocycline has any effect on depression or negative symptoms in schizophrenia, which is supported the absence of any changes in peripheral pro-inflammatory markers, as measured by a blood sample.

Negative symptoms have long been a key predictor in functional outcome. Álvarez-Jiménez et al. (2011) followed up 209 first episode psychosis patients over 7.5 years. They found that full functional recovery at the fourteen month follow-up predicted functional recovery and remission of negative symptoms at the 7.5 year follow-up, suggesting that functional recovery and negative symptoms are closely related, and targeting functional

and vocational recovery may be an important therapeutic target to prevent the development of future (chronic) negative symptoms. The present findings suggest that negative symptoms may have two sub-domains with different neurobiological substrates and relationship with depression. Avolition-apathy and expressive deficits may have differing prognostic factors as well as biological predictors. For example, expressive deficits has been associated with an abrupt onset of psychosis and longer hospitalisation and avolition-apathy has been associated with poorer social functioning, disorganisation and unemployment (Strauss et al., 2013). Therefore, separating avolition-apathy and expressive deficits may have future clinical benefit in terms of treatment targets or prognostic indicators. Utilising the dimensional model of depression and negative symptoms proposed in the systematic review presented in the introductory chapters by Krynicki et al. (2018) to conceptualise the relationship between these symptom domains may have clinical benefit. Distinguishing between avolition-apathy and expressive deficits early may allow for early intervention and specific treatments thus potentially improving outcomes. Indeed, Barnes et al. (2016) found that avolition-apathy and depression were treated with citalopram, but there was no treatment effect on expressive deficits, therefore providing a target for treatment of avolition-apathy/depression and expressive deficits. Moreover, a review of the literature by Sarkar et al. (2015) has shown that psychological therapies such as Motivation and Engagement Training (MOVE) which utilises CBT may be used to treat diminished emotional expression. This suggests that distinct targeted therapy at either avolition-apathy or expressive deficits may offer therapeutic benefit.

Given the association between immune dysfunction (supported by raised TNF-α) and expressive deficits, and the relationship between negative symptoms and functional recovery (Álvarez-Jiménez et al., 2011), immune dysfunction may be related to poorer functional outcome. Raised pro-inflammatory markers have been shown to correlate with clinical status (i.e. raised during relapse and normalise upon recovery) (Mondelli et al., 2015b). This suggests that inflammation may be a key marker in predicting functional outcomes. The association between expressive deficits and immune dysfunction may suggest that inflammation may be a marker of poor functioning, and therefore a therapeutic target given the detrimental emotional effect of negative symptoms on the individuals experiencing these symptoms as well as the financial and societal burden of negative symptoms.

8.5.2 Future research

The present findings have several implications for future research, including how the negative sub-domains are assessed which may have important implications for targeted therapy or as potential prognostic indicators. Clearly, avolition-apathy and expressive deficits are related (the high correlation between avolition-apathy and expressive deficits in the cross-sectional analysis supports this assertion). However, the exact nature of how the two sub-domains relate is unknown. One explanation for this relationship may be that they are both the product of immune dysfunction, with expressive deficits emerging following an increase in TNF-α and avolition-apathy being driven by the increase of another cytokine. Therefore, future research should focus on

how these two sub-domains are related, how they may differ in terms of biological underpinnings, and how this may impact on the development of future therapies.

Future analyses examining immune dysfunction in schizophrenia should include a UHR sample and employ a stratified approach to analysis. A medication naïve UHR sample or stratified analytical approach based on cytokine markers (i.e. those with raised inflammatory markers indicating immune dysfunction) was not achieved in the studies presented in this thesis or in much of the previous research. However, including a medication naïve sample may yield different results to much of the previous literature as antipsychotic medication is known to effect markers of inflammation. Furthermore, a stratified approach to analysis may show that certain groups of patients respond to a therapy depending on their inflammatory marker profile. Specifically, those who have increased levels of inflammation may respond to certain therapies over those without elevated levels of inflammation. However, sub-group analysis of the studies presented in this thesis found 'an inflammatory group' of patients showing increased levels of inflammation but did not show a reduction in negative or depressive symptoms after being treated with minocycline.

8.6 **Summary and Conclusion**

In summary, this thesis has presented a systematic literature review and novel analysis of data from a large, multicentre clinical trial in first episode psychosis, utilising gold standard methodologies, to help understand the complex relationship between

depression and negative symptoms, their relation to inflammatory cytokines and response to minocycline.

Depression was associated with avolition-apathy suggesting a common psychopathological mechanism underpinning these syndromes. Expressive deficits were not associated with depression longitudinally in the regression model but did correlate with depression in cross-section and were associated with a state-inflammatory marker indicating immune dysfunction. Immune dysfunction was also associated with depression. IL-6 was associated with depression in those with raised CRP. These findings suggest that expressive deficits may emerge following immune dysfunction indicating a distinct psychopathological pathway. However, one interpretation of these findings may be that as cytokines were surprisingly low in this sample, the patient group was relatively clinically stable in comparison to previous studies and state markers of inflammation were also low. Finally, minocycline does not lead to a reduction in depression or negative symptoms and there was a lack of effect of minocycline on inflammatory cytokines. A putative model has been proposed to explain the relationship between depression and negative symptoms, with avolition-apathy being associated with emotion/affective dysregulation, and expressive deficits being associated with neurocognitive dysfunction.

These findings suggest that the relationship between depression and negative symptoms is complex, however separating expressive deficits from avolition-apathy may in part, enhance our understanding. Also, findings suggest that inflammation may be more prominent in patients with certain negative symptoms yet may not explain the emergence of all negative symptoms. Whilst these findings suggest that minocycline has no effect on

depression or negative symptoms, these results do not mean that the immune system cannot be targeted in future studies aiming to relieve these symptoms. More research is needed here to accurately identify targets for future treatments focused on immune dysfunction in schizophrenia.

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10. Appendix 1 – Systematic review search strategy

	Database(s)	Search Term
1	Medline	exp SCHIZOPHRENIA/ OR SCHIZOPHRENIA, CATATONIC/ OR SCHIZOPHRENIA, CHILDHOOD/ OR SCHIZOPHRENIA, DISORGANIZED/ OR SCHIZOPHRENIA, PARANOID/
2	Medline	PSYCHOTIC DISORDERS/
3	Medline	(schizophren* OR psychosis OR psychoses OR FEP).ti
4	Medline	*(1 OR 2 OR 3)*
5	Medline	exp DEPRESSION/
6	Medline	(depres*).ti
7	Medline	DEPRESSIVE DISORDER/
8	Medline	DYSTHYMIC DISORDER/ OR DEPRESSIVE DISORDER, MAJOR/
9	Medline	*(5 OR 6 OR 7 OR 8)*
10	Medline	ANHEDONIA/
11	Medline	APATHY/
12	Medline	(alogia OR "affective flattening" OR "blunted affect" OR Anhedonia OR asociality OR avolition OR apathy).ti,ab
13	Medline	("social withdrawal" OR (loss ADJ1 motivat*) OR (loss ADJ1 interest) OR (lack ADJ1 motivat*) OR (lack ADJ1 interest)).ti,ab
14	Medline	("NEGATIVE SYMPTOM*").ti,ab
15	Medline	*(10 OR 11 OR 12 OR 13 OR 14)*
16	Medline	*(4 AND 9 AND 15)*
17	Medline	*16 [DT 2014-2017] [Languages English]*
18	PsycINFO	exp SCHIZOPHRENIA/
19	PsycINFO	PSYCHOSIS/
20	PsycINFO	(schizophren* OR psychosis OR psychoses OR FEP).ti
21	PsycINFO	*(18 OR 19 OR 20)*

☐	22	PsycINFO	MAJOR DEPRESSION/ OR RECURRENT DEPRESSION/ OR TREATMENT RESISTANT DEPRESSION/
☐	23	PsycINFO	DYSTHYMIC DISORDER/
☐	24	PsycINFO	(depres*).ti
☐	25	PsycINFO	*(22 OR 23 OR 24)*
☐	26	PsycINFO	POSITIVE AND NEGATIVE SYMPTOMS/
☐	27	PsycINFO	ANHEDONIA/
☐	28	PsycINFO	APATHY/
☐	29	PsycINFO	(alogia OR "affective flattening" OR "blunted affect" OR Anhedonia OR asociality OR avolition OR apathy).ti,ab
☐	30	PsycINFO	("social withdrawal" OR (loss ADJ1 motivat*) OR (loss ADJ1 interest) OR (lack ADJ1 motivat*) OR (lack ADJ1 interest)).ti,ab
☐	31	PsycINFO	("NEGATIVE SYMPTOM*").ti,ab
☐	32	PsycINFO	*(26 OR 27 OR 28 OR 29 OR 30 OR 31)*
☐	33	PsycINFO	*(21 AND 25 AND 32)*
☐	34	PsycINFO	*33 [DT 2014-2017] [Languages English]*
☐	35	EMBASE	*SCHIZOPHRENIA/ OR *PARANOID SCHIZOPHRENIA/ OR *PSYCHOSIS/
☐	36	EMBASE	(schizophren* OR psychosis OR psychoses OR FEP).ti
☐	37	EMBASE	*(35 OR 36)*
☐	38	EMBASE	*DEPRESSION/ OR *MAJOR DEPRESSION/
☐	39	EMBASE	*DYSTHYMIA/
☐	40	EMBASE	(depres*).ti
☐	41	EMBASE	*(38 OR 39 OR 40)*
☐	42	EMBASE	*BLUNTED AFFECT/ OR *ANHEDONIA/ OR *APATHY/ OR *NEGATIVE SYNDROME/
☐	43	EMBASE	("negative symptom*").ti,ab
☐	44	EMBASE	(alogia OR "affective flattening" OR "blunted affect" OR Anhedonia OR asociality OR avolition OR apathy).ti,ab
☐	45	EMBASE	("social withdrawal" OR (loss ADJ1 motivat*) OR (loss ADJ1 interest) OR (lack ADJ1 motivat*) OR (lack ADJ1 interest)).ti,ab
☐	46	EMBASE	*(42 OR 43 OR 44 OR 45)*

☐	47	EMBASE	*(37 AND 41 AND 46)*
☐	48	EMBASE	*47 [DT 2014-2017] [Languages English]*
☐	49	Medline	"MOOD DISORDERS"/
☐	50	Medline	("mood disorder*").ti
☐	51	Medline	*(49 OR 50)*
☐	52	Medline	*(4 AND 15 AND 51)*
☐	53	Medline	*(9 OR 51)*
☐	54	Medline	*(4 AND 15 AND 53)*
☐	55	Medline	*54 [DT 2017-2018]*
☐	56	PsycINFO	"AFFECTIVE DISORDERS"/
☐	57	PsycINFO	("mood disorder*").ti
☐	58	PsycINFO	*(56 OR 57)*
☐	59	PsycINFO	*(21 AND 32 AND 58)*
☐	60	PsycINFO	*(25 OR 59)*
☐	61	PsycINFO	*(21 AND 32 AND 60)*
☐	62	PsycINFO	*61 [DT 2017-2018]*
☐	63	EMBASE	*"MOOD DISORDER"/
☐	64	EMBASE	("mood disorder*").ti
☐	65	EMBASE	*(63 OR 64)*
☐	66	EMBASE	*(37 AND 46 AND 65)*
☐	67	EMBASE	*(41 OR 65)*
☐	68	EMBASE	*(37 AND 46 AND 67)*
☐	69	EMBASE	*68 [DT 2017-2018]*

☐	86	EMBASE	*SCHIZOAFFECTIVE PSYCHOSIS/
☐	87	EMBASE	81 AND 86 [Limit to: English Language]
☐	88	EMBASE	*AFFECTIVE PSYCHOSIS/
☐	89	EMBASE	86 OR 88
☐	90	EMBASE	81 AND 89 [Limit to: English Language]
☐	91	PsycINFO	SCHIZOAFFECTIVE DISORDER/

☐	92	PsycINFO	55 AND 91 [Limit to: English Language]
☐	93	MEDLINE	SCHIZOAFFECTIVE DISORDER/
☐	95	MEDLINE	AFFECTIVE DISORDERS, PSYCHOTIC/
☐	96	MEDLINE	93 OR 95
☐	97	MEDLINE	23 AND 96 [Limit to: English Language]

11. Appendix 2 - Studies included in the systematic review: characteristics and conclusions (Dx = depression/depressive symptoms; Nx = negative symptoms; Px = positive symptoms

Author	Year	Number of participants (Inc. gender)	Population	Depression scale	Negative symptoms scale	Antidepressant usage	Conclusions	Other information
Whiteford et al. (1987)	1987	40 male patients with chronic schizophrenia	Outpatients	HRSD,	SANS	Unknown	Nx and Dx overlap on loss of energy, drive and interest and anhedonia/asociality, but can be differentiated on features such as poverty of thought and speech, and poor attention/concentration, which were related to Nx but not Dx.	Cross-sectional
Barnes et al. (1989b)	1989	194 chronic schizophrenia	Inpatients	BDI	SANS (specific rating for Anhedonia).	Unknown	Confirmed a Dx syndrome which was distinct from Nx symptoms and was not a result of pharmacotherapy.	Cross-sectional
Pogue-Geile (1989)	1989	44 (40 with schizophrenia and 4 schizoaffective)	Outpatient	SADS-L; BDI,	SANS	Unknown	There was no significant difference between Nx and Dx in schizophrenia.	Cross-sectional
Kulhara et al. (1989)	1989	95 patients with schizophrenia	Patients were referred.	BPRS	BPRS (total score); SANS; SAPS	Unknown	Retardation, lack of energy and slowness were associated with Nx. Depressed mood was associated with Dx.	Cross-sectional

Ragin et al. (1989)	1989	25 (12 schizophrenia, 13 depressed patients)	Inpatient, and then followed up after discharge	SADS	TLC	Unknown	During inpatient admission, Dx patients experienced more poverty of speech than SCZ patients. At follow up, poverty of speech increased in SCZ patients but stayed the same in Dx patients.	Longitudinal
Newcomer et al. (1990)	1990	69 schizophrenia (all male)	Unmedicated inpatients	BPRS:DEP (depressed mood, guilt, anxiety and somatic concern); HRSD	BPRS: NEG (blunted affect, emotional withdrawal, motor retardation).	All unmedicated	High association between Nx measures and Dx measures. Other items reflecting other aspects of Dx did correlate with the Nx measures.	Retrospectively drawn sample
Koreen et al. (1993)	1993	70 patients with schizophrenia/ schizoaffective (39 male, 31 female).	Inpatients	Extracted scores on the HDRS	SADS; SANS; CGI-S	Following baseline assessment, patients prescribed antidepressants and antipsychotics	Dx are present and prevalent in the first episode and occur concurrently with psychotic symptoms and resolves as the psychotic episode remits. Dx is a core feature of schizophrenia and correlates with Nx and Px.	Prospective
Kibel et al. (1993)	1993	73 schizophrenia	Inpatients	Montgomery - Asberg	SANS; NSRS;	Unknown	Subjective sadness, pessimism and suicidal	Cross-sectional

		(51 male, 22 female)		Depression rating scale	PANSS (total), Manchester scale, Montgomery Schizophrenia Scale.		intent belong to the Dx. Observed sadness, lassitude and inability to feel were more strongly associated with Nx.	
Tharyan and Kuruvilla (1994)	1994	46 patients with schizophrenia	Unknown	HDRS	SANS	Initially unmedicated and then medicated	Depressed patients showed fewer negative symptoms than non-depressed.	Longitudinal
Sax et al. (1996)	1996	17 with schizophrenia (including schizophreniform and psychosis not specified), 25 with depression	Inpatient	HRSD	SANS; SAPS	Unknown	Dx and Nx correlated on poor motivation, social withdrawal and anhedonia, which were not attributable to any drug effects.	Cross sectional
Nakaya et al. (1997)	1997	89 with schizophrenia/ schizophreniform (39 male, 47 female	Inpatients	HRSD (17 item); PANSS depression item	PANSS (total negative symptom subscale)	Unknown	There was a lack of association between improvement of Dx and Nx.	Prospective and longitudinal
Nakaya et al. (1998)	1998	86 patients with schizophrenia	Inpatients	HRSD (17 item version)	PANSS (total negative	Unknown	Differences in the composition of the Dx syndrome were observed	Prospective and longitudinal

		or schizophreniform. (39 male, 47 female).			symptom subscale)		in the acute and chronic phase of schizophrenia. Dx unrelated to Px and Nx.	
Zisook et al. (1999)	1999	60 patients with schizophrenia, 60 healthy controls	Outpatients	HDRS and BPRS	SANS and BPRS	Medicated	Severity of Dx did not correlated with severity of Nx.	Cross-sectional
Baynes et al. (2000)	2000	120 with chronic schizophrenia (78 male, 42 female)	out-patients	BDI; HDRS; CGISS; BPRS (anxiety/depression items)	SANS	No antidepressants	No association between Dx and Nx in schizophrenia. Dx are more common in the active illness.	Cross-sectional
Romney and Candido (2001)	2001	54 schizophrenia 27 MDD (55 male 26 female)	36 in-patients; 45 out-patients	BDI; HRSD; MDI;	PANSS (total negative subscale score); PAS; SAS; Maine scale	MDD patients took antidepressants	Anhedonia is more closely aligned with Dx.	Cross-sectional - Factor analysis
Tapp et al. (2001)	2001	104 with schizophrenia and depression	Inpatients	HRSD	SANS; BPRS (total negative subscale)	Unknown	Dx are prevalent during acute psychosis and are reduced following treatment with antipsychotic medication.	Experimental – double blind trial.
Muller. et al. (2002)	2002	57 (53 schizophrenia,	Inpatients	CDSS	PANSS (total	14% patients	Dx is heterogeneous and occurs in schizophrenia.	Cross-sectional

		4 schizoaffective) 36 Male 21 female			negative subscale)	received antidepressants	These symptoms are separate from emotional blunting and emotional blunting is independent from Dx. Affective blunting was observed in patients with residual and disorganised schizophrenia.	
Oosthuizen et al. (2002)	2002	80 (41 male, 39 female) schizophrenia/ schizophreniform	Unknown	PANSS - D	PANSS (total negative subscale)	Antidepressants were used	Dx were common in the first episode and were also independent from Nx.	Experimental – open labelled trial.
Bottlender et al. (2003)	2003	76 (33 schizophrenia, 43 depression)	Inpatient	Montgomery-Asberg Depression Rating scale	SANS	Unknown	For Dx patients, Nx emerged for within 3 months prior to hospitalisation. In patients with SCZ, Nx were present for 12 months prior to admission and were more enduring than in those with Dx. Also, Nx in Dx patients was associated with Dx symptoms but not in SCZ patients.	Cross sectional and longitudinal

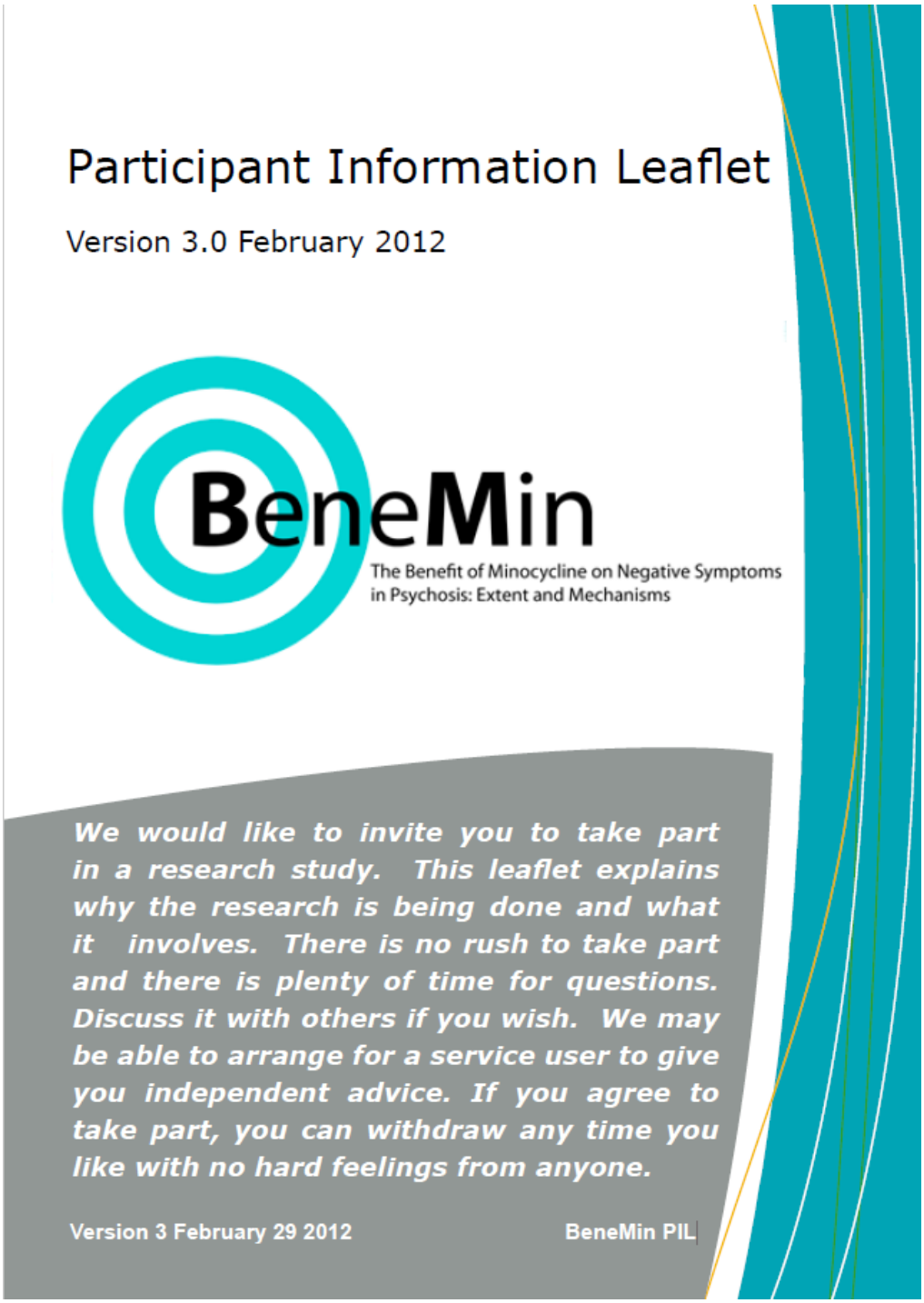
Maggini et al. (2004)	2004	128 chronic with schizophrenia (87 male, 41 female)	Unknown	CDSS	SANS; SAPS; SENS	Unknown	Dx patients with schizophrenia showed higher scores for Px, anhedonia, asociality, cognitive overload, basic emotional impairment and heightened awareness of functional deficits. Dx did not correlate with Nx.	Cross-sectional
Hafner et al. (2005a)	2005	130 first admission patients with schizophrenia. 130 with depression. 130 healthy controls	first admission patients	Retrospective Assessment of the Onset of Schizophrenia	IRAOS	81% of schizophrenia patients and 79% of depressed were drug naïve.	Dx and Nx are prominent in the prodromal stages of Dx and schizophrenia and transition to psychosis or depression later in the illness.	Cross-sectional
Hafner et al. (2005b)	2005	130 with schizophrenia (51%male, 49% female), 130 depressed (51%male, 49% female) and 130 controls (51%male, 49% female)	Patients from Mental health institution	IRAOS	IRAOS	Most were medication naïve (both antipsychotic and antidepressants)	Dx and schizophrenia begin with combined symptomology consisting of Dx and increasing impairment.	Cross-sectional

Rocca et al. (2005)	2005	78 (46 males and 32 females) with schizophrenia	Outpatient	CDSS	PANSS (total negative subscale)	Unknown	Nx were separate from those of Dx. Also, outcome for patients with Dx and Nx was less favourable. Dx and Nx did not share the same pattern of clinical and cognitive predictors.	Cross-sectional
Upthegrove et al. (2010)	2010	92 with FEP (87% - non-affective psychosis, 13% - affective psychosis.	EIP	Prodromal depression: SCAN; Baseline and follow up depression: CDSS	PANSS (total negative subscale)	Unknown	Dx occurs, waxes and wanes throughout early psychosis and predicts illness with prodromal Dx predicting Dx in the early course and future Dx episodes throughout the course of illness.	Prospective study
Majadas et al. (2012)	2012	90 (59% male, 41% female - 73 with schizophrenia, 2 schizophreniform and 15 with schizoaffective disorder)	Outpatient clinic	CDSS	PANSS (total negative subscale); SANS; SUMD	Patients with a diagnosis of depression or treated with antidepressants were excluded.	Dx is frequent in the stable phase of schizophrenia and is associated with greater severity of illness. Dx is indicative of greater severity of the illness. Dx and Nx are poorly associated.	Cross-sectional
Chiappelli et al. (2014)	2014	98 with schizophrenia (67% male,	Local community	Maryland Trait and State	BNSS	36 were taking antidepres	State and trait aspects of Dx and Nx can be separated in	Cross-sectional - Factor analysis

	33% female) and 117 control (43% male, 57% female)	mental health clinics.	Depression - scale		sants, 15 were taking mood stabilisers	schizophrenia. In the schizophrenia sample, Nx and Dx did not correlate.
Rossi et al. (2017)	2017 921 schizophrenia	outpatients	CDSS	BNSS	Unknown	High negative symptoms predict depression Cross-sectional

Beck Depression Inventory (BDI); Hamilton Depression Rating Scale (HDRS); Clinical Global Impression Severity Scale (CGI-S); Scale for the Assessment of Negative Symptoms (SANS); Extrapyramidal Rating Scale (EPRS); Brief Psychiatric Rating Scale (BPRS); Barnes Akathisia Rating Scale (BARS); Significant Others Scale (SOS); Brief Negative Symptoms Scale (BNSS); Interview for the Retrospective Assessment of the Onset of Schizophrenia (IRAOS); Schedule for Affective Disorders and Schizophrenia (SADS); Negative Symptom Rating Scale (NSRS); The Positive and Negative Syndrome Scale (PANSS); Structured Interview for Prodromal Symptoms (SIPS); Scale of Prodromal Symptoms (SOPS); Global Assessment of Functioning (GAF); Scale for the Assessment of Positive Symptoms (SAPS); Subjective Experience of Negative Symptoms (SENS); Toronto Alexithymia Scale (TAS); SUMD; Extrapyramidal Symptom Rating Scale (ESRS); Bech-Rafaelsen Melancholia Scale (BREMS); Calgary Depression Scale in Schizophrenia (CDSS); Abnormal Involuntary Movement Scale (AIMS); Operational Criteria Checklist [OPCRIT]; Multiscore Depression Inventory (MDI); Schedule for Clinical Assessment in Neuropsychiatry (SCAN); Physical Anhedonia Scale (PAS) and Social Anhedonia Scale (SAS)

12. Appendix 3 – Patient information leaflet



Why is the study being done?

Recent research at the University of Manchester has found that recovery from early psychosis is improved by taking a well-known antibiotic called minocycline. The aim of this research is to confirm the benefit of minocycline and to understand how it works.

Modern treatments are very effective at alleviating psychosis. However, once this has passed, some people lose motivation and full enjoyment of life and companionship. This tends to improve if there is no relapse of psychosis but it can become a long-standing problem. The ability to think and remember efficiently can also suffer. Standard treatments do not directly help these 'negative' symptoms but minocycline has shown benefit in the recent studies.

Minocycline is a common antibiotic that has been used for many years in the long-term treatment of acne (teenage spots). However, it has other actions that may be useful in the treatment of various brain disorders.

Minocycline may work by:

- stimulating mental activity through an action on a brain chemical called glutamate. *We will test this using brain scans and computer tasks.*
- reducing inflammation in the body. *We will measure signs of inflammation (cytokines) in blood samples.*
- preserving connections between brain cells and this may explain improved motivation and thinking. *The brain scan would tell us whether this occurs.*

Once we understand the action of minocycline, better medications can be developed to focus on the relevant action.

The study would involve taking minocycline for a year in addition to your usual treatment. You would have a brain scan before starting the treatment and in the last (12th) month.

Why have I been invited?

We are inviting you to participate because you are receiving NHS care for early treatment of psychosis. Your clinical team think you may be eligible and interested to take part. We are inviting you to take part in an interview with one of the research workers to find out if you are eligible for the study. A total of 225 people will participate in the study.

Do I have to take part?

It is up to you to decide whether or not to take part in the study. We will describe the study and go through this information leaflet, which you should keep. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. If you decide not to take part, this will not affect the standard of care you receive.

What will happen to me if I take part?

Beginning the study

You will first take part in an interview with a research worker to find out if you are eligible for the study. If you are eligible and agree to participate, the researcher will carry out further checks including reviewing your medical and drug treatment casenotes. They will arrange a medical check–up, routine blood and urine tests and, blood pressure to make sure there is no medical reason why you should not take part. If you agree, we will also ask your GP to let us know of any relevant medical problems.

At your next appointment, the researcher will check through your results and confirm that you understand the research and wish to continue. You will complete some standard questionnaires about your symptoms and carry out some tests of your thinking and reasoning skills. Most tests will be presented on a computer screen. These assessments are widely used in research and are done at your own pace. We will take a research blood sample for inflammation measures (cytokines). You will have a magnetic resonance imaging (MRI) brain scan at this visit and your last visit. This is described in a later section.

At this appointment or soon after you will receive a supply of your study medication to take in addition to your usual treatment. Your doctors will continue to offer the full range of treatments for you in the normal way.

Your treatment

You will be allocated at random to receive one of the following study treatments in addition to the treatment you receive from your doctors:

- Minocycline, 3 pills a day.
- dummy pills, 3 pills a day

The treatment continues for a year.

The pills can be taken once, twice or three times a day to a maximum of three pills in 24 hours, as you prefer.

We don't know how big the benefit of minocycline is or how quickly it happens. To find out we need to compare it with dummy (placebo) pills that look the same as minocycline pills. This ensures that no one knows who is taking which treatment. To try to make sure from the start that the people who receive minocycline are similar to those who take placebo, your treatment is chosen at random (by chance) by a computer program that has no information about you. You cannot choose which of the treatments you will receive.

Neither you nor your clinical team will know which treatment you had because your assessments will only be identified by a code number. The code can be broken during the study at any time just in case you experience any adverse effect.

The researcher or your community psychiatric nurse will ask about side-effects approximately every two weeks to begin with and every 1-2 months thereafter. Your medical team and the researcher will be available at all times should you have any concerns. The team will assist you in collecting your research medication.

Follow-up appointments

We will try and make appointments at times which suit you, at a location of your preference (your home or a different location if this is more convenient). We will see you after the 2nd, 6th and 9th month to repeat some of the questionnaires and find out how you are doing. We routinely do urine checks for cannabis and other drugs. Urine pregnancy tests will be carried out every 2-3 months. At some appointments we will repeat the blood sample for cytokines.

End of research treatment (12 months) and final follow-up (15 months)

The trial treatment will end at 12 months when we will repeat all the measures you carried out at the beginning including a MRI scan. We would repeat some of your assessments 3 months later at 15 months to see if any benefits outlast the treatment.

MRI brain scanning

MRI scanning is a routine procedure in all major hospitals. There are no dangers or side-effects of any kind. No chemicals, injections, blood samples are involved. There is no radiation. You lie with your head inside a short tunnel in a magnetic field. The field changes and this makes quite a lot of noise so you wear padded earphones. Very occasionally people find this claustrophobic so you can press a button to come out if you don't want to continue. The scanner creates images of your brain and we can use this to measure the shape and size of its regions. We will also ask you to perform some computer tasks in the scanner. You will be shown pictures of shapes, numbers, letters and faces. You will be asked to respond to the images by pressing buttons with your fingers. The scanning will last less than one hour.

What do I have to do?

To enter the study all you need do is to agree to attend all appointments, perform the assessments and take the study treatment. You agree not to take illegal substances throughout the study. You and anyone you have sex with must each take contraceptive precautions because of possible effects during pregnancy. Fertile men must use condoms and women must use the contraceptive pill, an IUD or a diaphragm with a spermicidal cream or equivalent. Further details are given at the end of this document.

What are the disadvantages and risks of taking part?

We have extensive experience of all our research tests and procedures and all have been used on very large numbers of patients world-wide and in the UK. It is essential that people find them acceptable otherwise research would fail.

Nevertheless, it is possible you might find some questions too personal, upsetting or tiresome. If you do we will treat this with respect and sympathy. Given the extensive use of minocycline for acne and other infections, you are unlikely to be harmed by it. As with any drug, minocycline might not agree with you, for example by causing nausea, stomach upset or headache. Similarly, you could develop an allergic skin rash. Very rarely (less than 1 in 10,000 people treated) a more serious skin condition may occur with minocycline, which may also affect the liver. We will do blood checks specifically for this before and during the study so it will be caught early. Patches of skin pigmentation are reported in about 2% in long-term treatment and we will be careful to check for this at each visit so you can stop the medication before it becomes a problem.

What are the possible benefits of taking part?

We hope that the treatment with minocycline and assessments will help you. It probably does not act quickly so it may be hard to tell whether you are benefitting. There is no reason to think it will make your psychosis worse. It is reported to lessen weight gain caused by antipsychotic drugs. However, we cannot promise this. At the end of the study when we compare the two groups, we will have a strong indication of how worthwhile it is to take minocycline and whether it points the way for new treatments.

What if new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens the research team will discuss this with you.

Will my taking part be kept confidential?

All information which is collected about you during the research will be kept strictly confidential and will conform to the Data Protection Act of 1998 with respect to data collection, storage and destruction. This covers any information from your NHS records that we collect to complete a research checklist and to note any relevant medical or other factors relevant to the research. We will not make photocopies of any part of your notes. Your name and address is not recorded with the research information, instead we use a code number to identify your research data. Research data will be stored on computers in an encrypted (i.e. scrambled) form that can only be read with a 'key' held by one researcher.

With your permission, your GP will be notified of your participation in the study as will your care coordinator and psychiatrist. However, unless there is information which puts you or others at serious risk of harm, information collected in the trial will not be fed back or exchanged without your consent.

What will happen if I don't want to carry on with the study?

You can withdraw from the study completely at any time and this will not affect your usual treatment. Alternatively, you may stop study treatment but agree to continue with the assessments with the research worker. Information collected may still be used, and a follow up review of your medical records may continue if you agree.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the researchers who will do their best to answer your questions (see contact number below). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Manchester Mental Health & Social Care Trust, who are the sponsors of this research, but you may have to pay your legal costs.

What will happen to the results of the research study?

We aim to publish the results of the study in scientific journals but will also make them available to all participants in a non scientific format. We do not expect the results to be available until after the end of the trial (2015).

What about expenses?

You will be reimbursed for travel expenses for all research clinic visits. You will be reimbursed £30 for the general inconvenience of each MRI scanning session.

Further details on contraception during the study

Acceptable methods of contraception for women are:

- Tubal ligation
- Physician documented placement of an intra-uterine device (IUD)
- Diaphragm with spermicidal form/gel/film/cream pessary
- Condom with spermicidal foam/gel/film/cream/pessary
- Male partner who has had a vasectomy
- Hormonal contraceptives

Acceptable methods of contraception for men are:

- Use of condom
- In addition to a condom, male subjects without a vasectomy must assure that their female partner uses another form of contraception such as an IUD, spermicidal foam/gel/film/cream/suppository, diaphragm with spermicide, oral contraceptive, injectable progesterone, subdermal implant or a tubal ligation if the female partner could become pregnant from the time of the first dose of trial medication until completion of follow-up procedures.
- Female partner would not be required to use another form of contraception if they are of non-childbearing age.

Who is organising and funding the research?

This study is funded by the National Institute of Health Research and the Medical Research Council. It is organised by the University of Manchester in partnership with Lancashire Care Foundation NHS Trust and Manchester Mental Health and Social Care Foundation NHS Trust. The other centres involved are in London, Cambridge and Edinburgh.

Who has reviewed the study?

The study was funded after approval of its scientific and ethical quality by independent international experts and the NIHR scientific committee. All research in the NHS is checked by an independent group of people, called Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Greater Manchester Central NRES Committee.

Contact for further information

If you require further information about the study you may contact one of the following people:

Trial manager: Kelly Byrne
Phone number:

Please affix sticker with local contact details

Thank you very much for taking the time to read this information booklet.

You will be given a copy of this information booklet and a copy of the signed consent

This research is part of a UK programme funded by the National Institute of Health Research (NIHR) and the Medical Research Council (MRC) to find better treatments for psychotic disorders. Six universities are involved in the minocycline study. Drug companies are not involved in this research.

Please affix sticker with local Trust & centre logo and contact details

MANCHESTER 1824

The University of Manchester

National Institute for Health Research NHS

MANCHESTER CITY COUNCIL

Manchester Mental Health and Social Care Trust NHS

Efficacy and Mechanism Evaluation programme

MRC Medical Research Council

Mental Health Research Network

13. Appendix 4 – Consent form



BeneMin Research Study Team
Schizophrenia and Psychosis Research Group
School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
The Barberry National Centre for Mental Health
25 Vincent Drive
Birmingham
B15 2FG
☎ (Phone)
🖷 (Fax)
✉ (e-mail)

The Benefit of Minocycline on Negative Symptoms in Psychosis: Extent and Mechanisms

CONSENT FOR PARTICIPATION IN TRIAL

		Please circle your answers and place your initials in each box
1	I confirm that I have read and understand the information leaflet dated February 29th 2012 (version 3) for the above study and have had the opportunity to ask questions. I acknowledge that the risks mentioned in the Patient Information Leaflet have been explained to me.	YES….NO….. ☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	YES….NO…. ☐
3	I understand that sections of any of my records held by the NHS may be looked at by people who undertake to maintain confidentiality from the University of Manchester working closely with the NHS. I give permission for these individuals to have access to my records during my participation in the trial and also during the follow up period which may extend to four years. I understand that data collection, storage and destruction will be in line with the Data Protection Act 1998.	YES….NO…. ☐
4	I understand that my GP, my medical consultant and my care coordinator will be informed of my participation in the trial.	YES….NO…. ☐
5	I agree to take part in the above study.	YES….NO…. ☐

SIGNATURES

Name of Participant	Signature	Date
Researcher	Signature	Date
Name of Person taking consent (if different from researcher)	Signature	Date

1 copy for participant; 1 for researcher; 1 to be kept with hospital medical notes

14. Appendix 5 - Data cleaning proforma

Data Cleaning Record

Participant ID Number_BMN/00__

Site No./Name: _____ RA completing record: _____

Date data check started: 0/0/2015__ Date data check completed: ____/____/_____

Data Checks Completed:

	A	B	C	D	E
Screening	☐	☐	☐	☐	☐
Randomisation	☐	☐	☐	☐	☐
3-month FU	☐	☐	☐	☐	☐
6-month FU	☐	☐	☐	☐	☐
9-month FU	☐	☐	☐	☐	☐
12-month FU	☐	☐	☐	☐	☐
15-month FU	☐	☐	☐	☐	☐

DUP First positive symptom to first medication: _____ weeks.

Medication check
indicate whole or half months of continuous medication ie known interruptions

							total months
2013	Jan	Feb	Mar	Apr	May	Jun	
	Jul	Aug	Sep	Oct	Nov	Dec	
2014	Jan	Feb	Mar	Apr	May	Jun	
	Jul	Aug	Sep	Oct	Nov	Dec	
2015	Jan	Feb	Mar	Apr	May	Jun	
	Jul	Aug	Sep	Oct	Nov	Dec	
2016	Jan	Feb	Mar	Apr	May	Jun	
						Grand total	
Example	Jan	Feb	Mar	Apr	May	Jun	4.5

Section A: Check hard copy and source data; every page should have ID, date and visit type. If missing, use coloured biro to insert data and to initial and date the correction.
Check list • Full BeneMin ID now on every page? • Date of record now on every page? • Visit type now specified on every page? • Have all additions to hard copy/source data been made in coloured ink, initialled and dated?
Any uncertainties about page documentation? If so don't guess and don't assume anything! Just flag as anomalies in **Section D** for office to sort out
When this section A checklist is complete for a visit, tick appropriate Box A on front page.

Do not correct any other anomalies or mistakes on hard copy or in OpenCDMS.
Don't worry about mistakes, they happen. Just itemise below and the office will sort it out with you.

Section B. Check OpenCDMS data against hard copy source data		
Visit	Necessary corrections/comments (or state 'Checked') against each visit	
Screening		
Randomisation		
3-month FU		
6-month FU		
9-month FU		
12-month FU		
15-month FU		

Section C. Check missing value codings; do they identify the correct reason for a missing value?		
Visit	Necessary corrections (or state 'Checked') against each visit	Office action
Screening		

Randomisation		
3-month FU		
6-month FU		
9-month FU		
12-month FU		
15-month FU		

Section D. Other anomalies		
Visit	Necessary corrections (or state ‘Checked’) against each visit	Office action
Screening		
Randomisation		
3-month FU		
6-month FU		
9-month FU		
12-month FU		
15-month FU		

E – Summarise free text and file notes relevant to (a) course of illness, or (b) interruption of data	
Visit	Flag up or summarise text notes and relevance (or state ‘None relevant’) against each visit
Screening	
Randomisation	
3-month FU	
6-month FU	
9-month FU	
12-month FU	
15-month FU	

15. Appendix 6 – positive and negative syndrome scale interview (PANSS)

STRUCTURED CLINICAL INTERVIEW
FOR THE POSITIVE AND NEGATIVE SYNDROME SCALE
(SCI-PANSS)

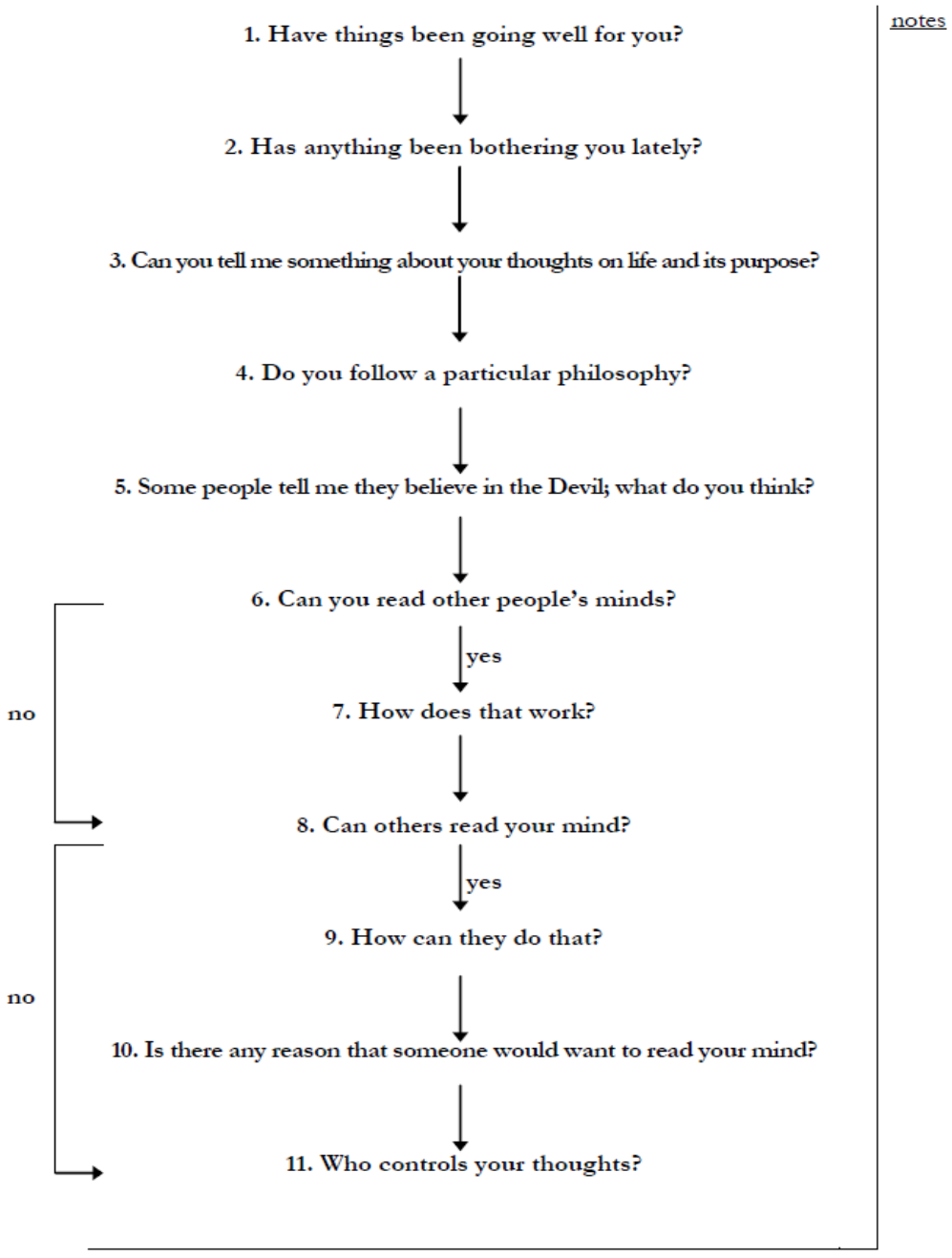
Data on **LACK OF SPONTANEITY** and **FLOW OF CONVERSATION**, **POOR RAPPORT** and **CONCEPTUAL DISORGANISATION.**

	notes
Hi, I'm ….. We're going to be spending the next 20 or 30 minutes talking about yourself and your reasons for being here. Maybe you can start by telling me something about yourself and your background? (Allow at least 5 minutes for a non-directive phase serving to establish rapport in the context of an overview before proceeding to the specific questions listed below.)	

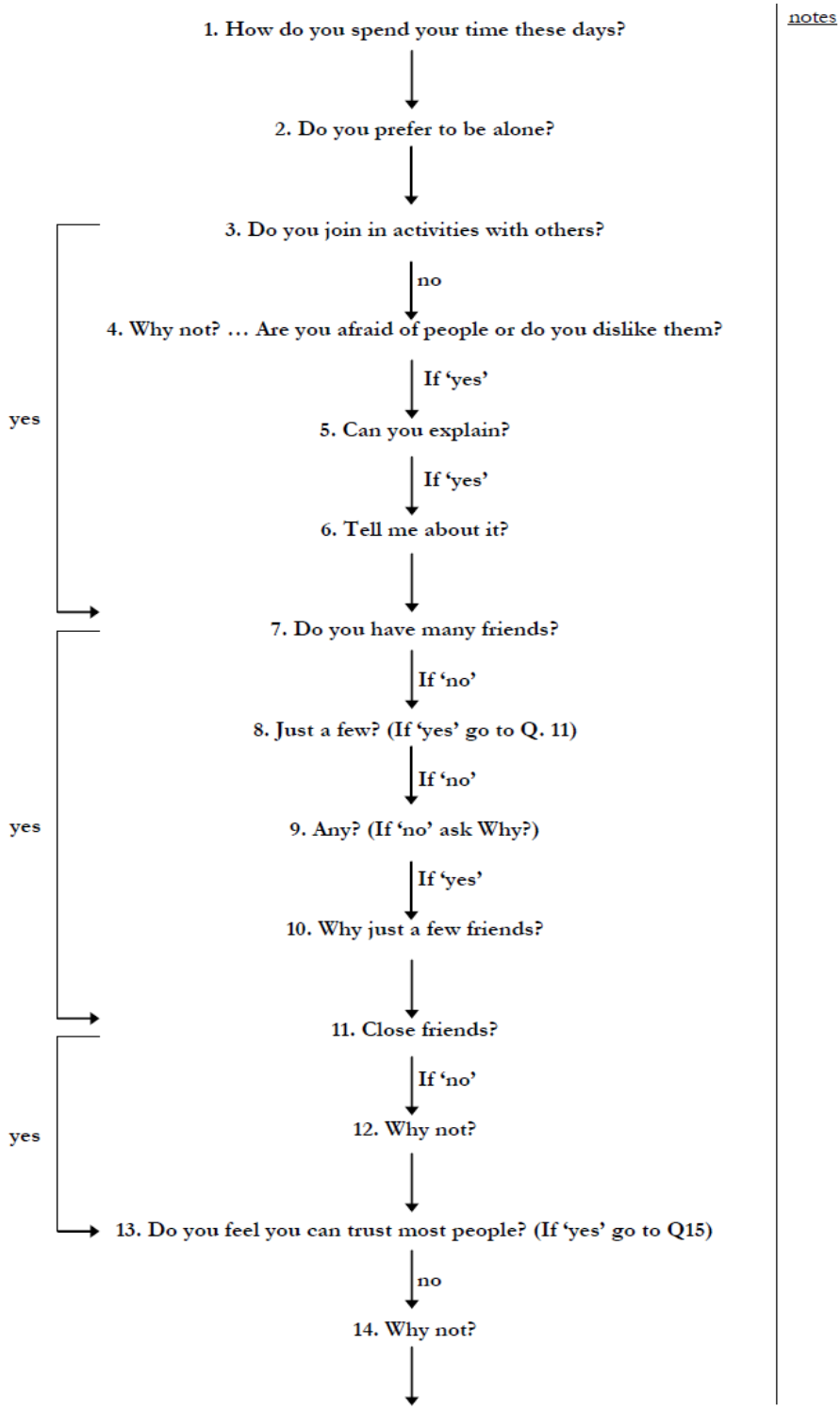
Data on **ANXIETY.**

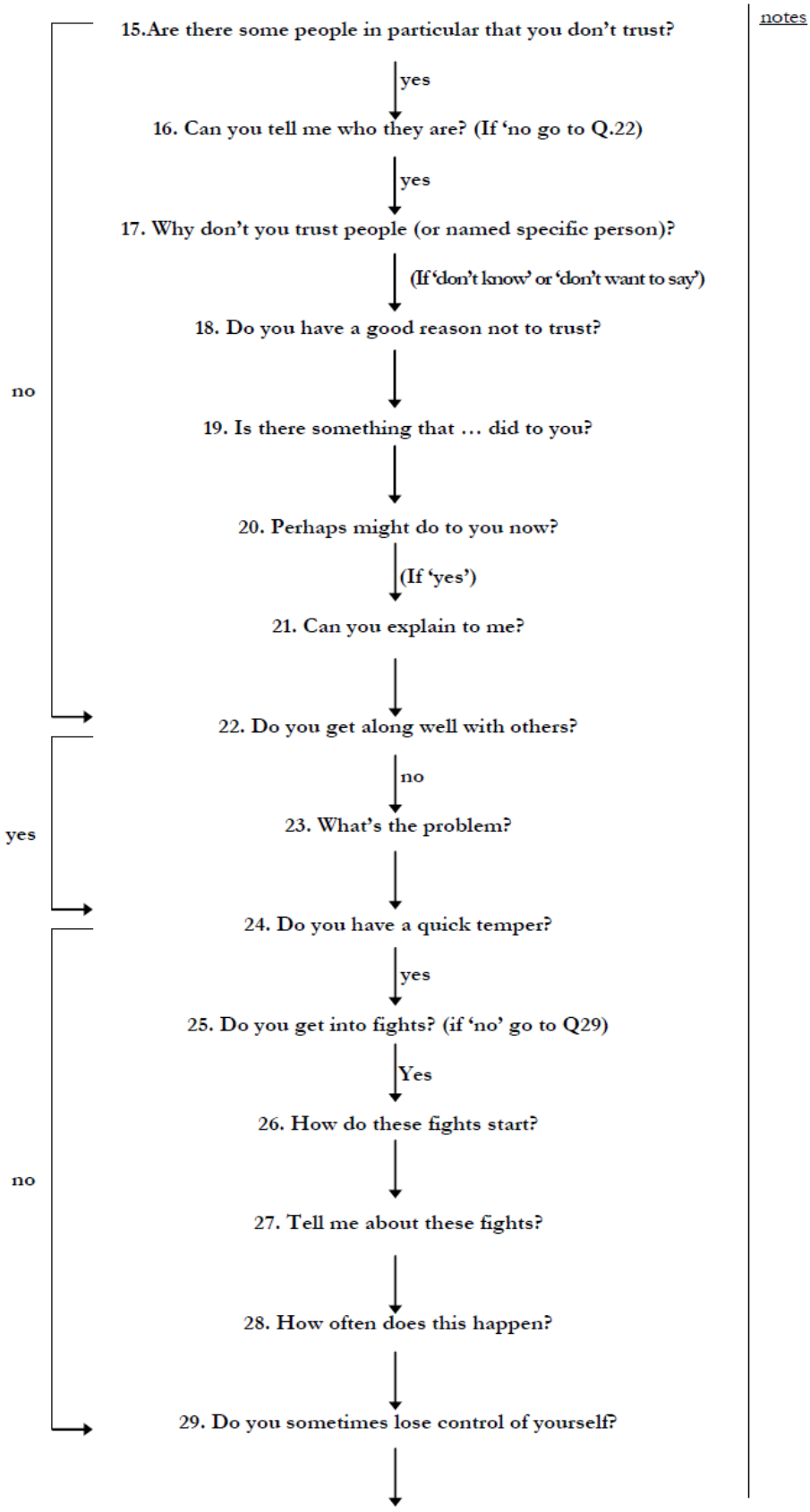
	notes
1. Have you been feeling worried or nervous in the past week? ↓ no 2. Would you say that you're usually calm and relaxed? yes → 3. What's been making you feel nervous (worried, uncalm, unrelaxed)? ↓ 4. Just how nervous (worried, etc.) have you been feeling? ↓ 5. Have you been shaking at times, or has your heart been racing? ↓ 6. Do you get into a state of panic? ↓ 7. Has your sleep, eating or participation in activities been affected?	

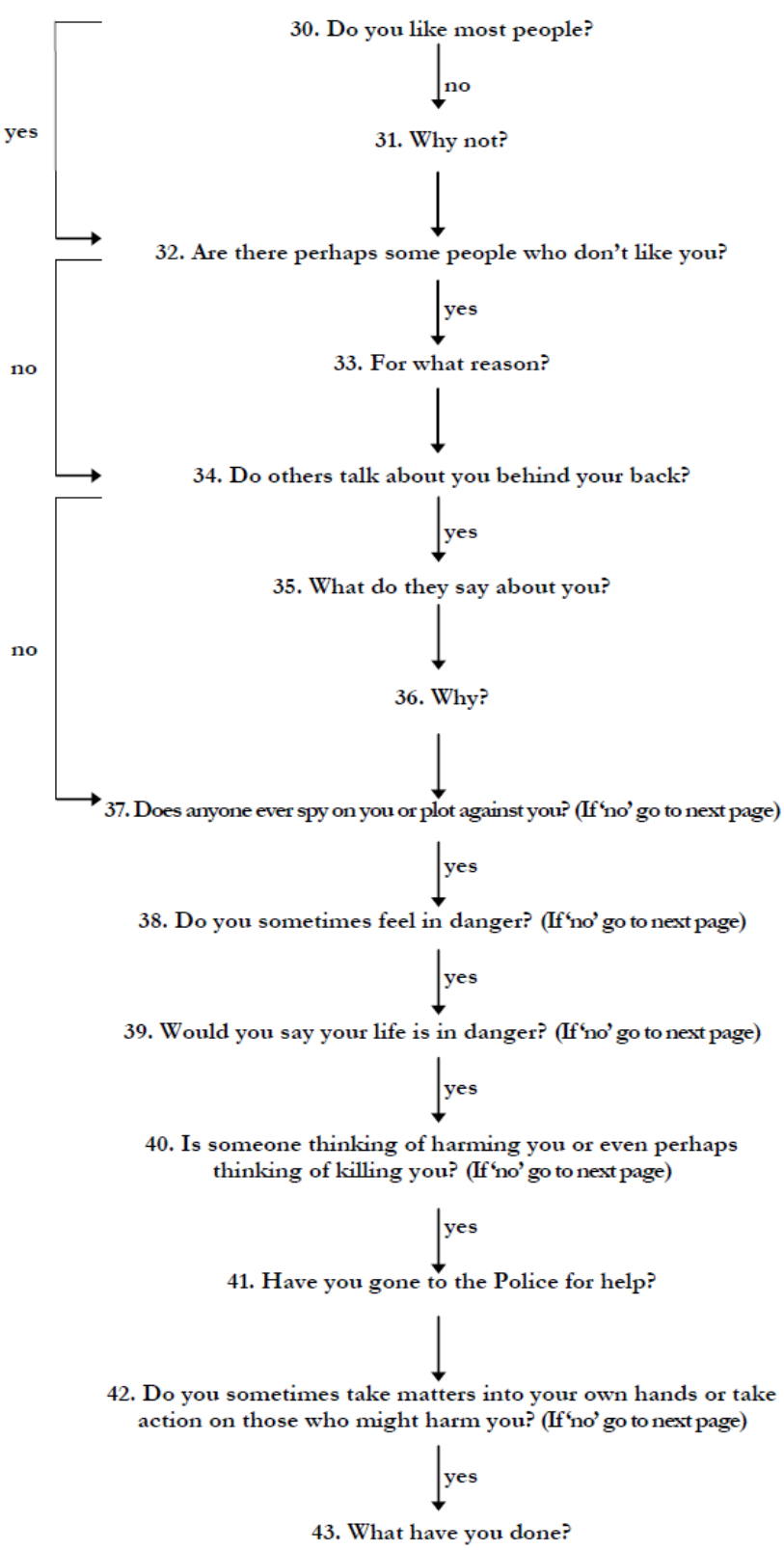
Data on **DELUSIONS (GENERAL)** and **UNUSUAL THOUGHT CONTENT.**



Data on SUSPICIOUSNESS/PERSECUTION, PASSIVE/APATHETIC SOCIAL WITHDRAWAL, ACTIVE SOCIAL AVOIDANCE and POOR IMPULSE CONTROL.

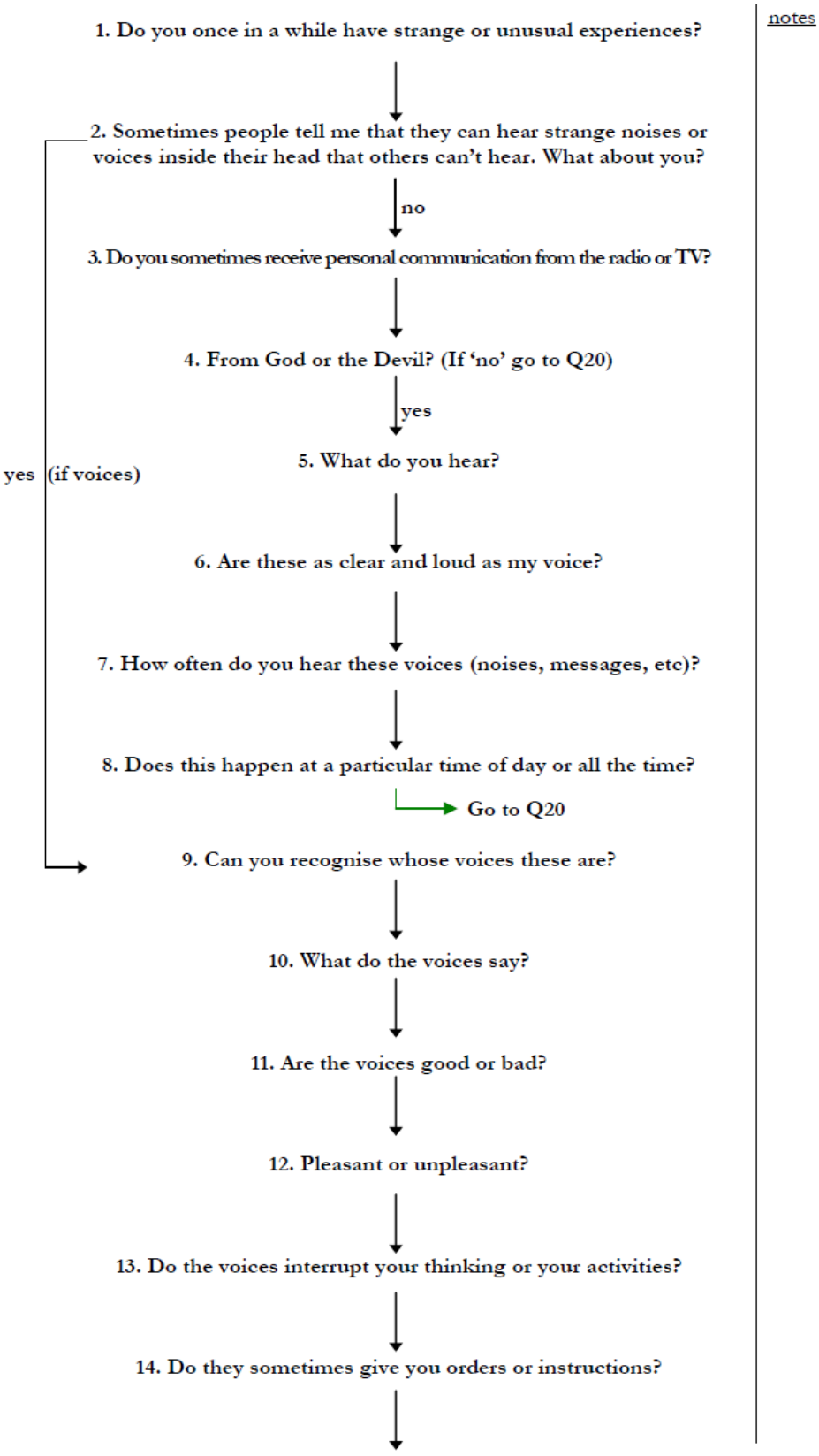


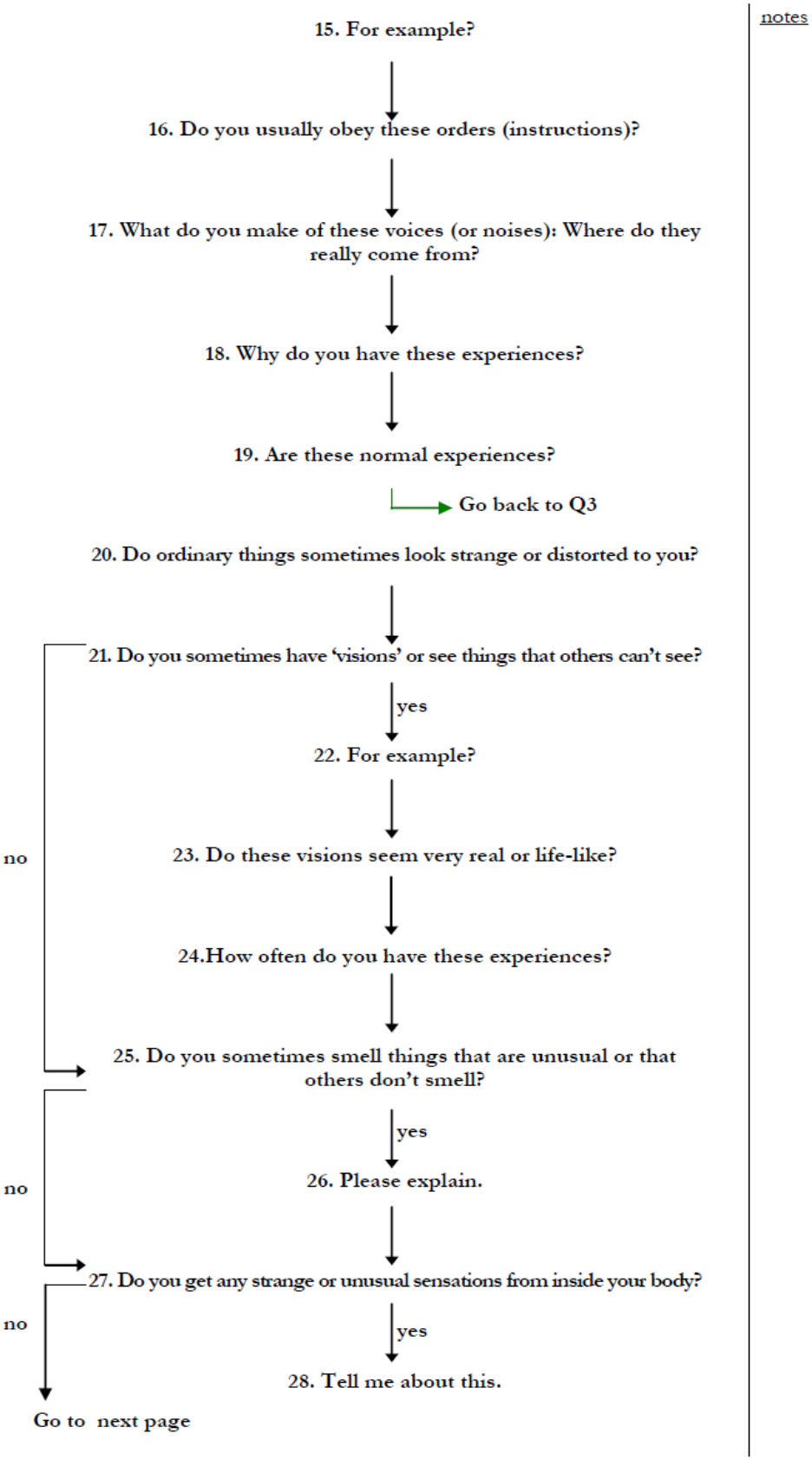

notes
15.Are there some people in particular that you don't trust?
yes
16. Can you tell me who they are? (If 'no go to Q.22)
yes
17. Why don't you trust people (or named specific person)?
(If 'don't know' or 'don't want to say')
18. Do you have a good reason not to trust?
no
19. Is there something that … did to you?
20. Perhaps might do to you now?
(If 'yes')
21. Can you explain to me?
22. Do you get along well with others?
no
yes
23. What's the problem?
24. Do you have a quick temper?
yes
25. Do you get into fights? (if 'no' go to Q29)
Yes
26. How do these fights start?
no
27. Tell me about these fights?
28. How often does this happen?
29. Do you sometimes lose control of yourself?


30. Do you like most people?
no
yes
31. Why not?
32. Are there perhaps some people who don't like you?
yes
no
33. For what reason?
34. Do others talk about you behind your back?
yes
35. What do they say about you?
no
36. Why?
37. Does anyone ever spy on you or plot against you? (If 'no' go to next page)
yes
38. Do you sometimes feel in danger? (If 'no' go to next page)
yes
39. Would you say your life is in danger? (If 'no' go to next page)
yes
40. Is someone thinking of harming you or even perhaps thinking of killing you? (If 'no' go to next page)
yes
41. Have you gone to the Police for help?
42. Do you sometimes take matters into your own hands or take action on those who might harm you? (If 'no' go to next page)
yes
43. What have you done?

notes

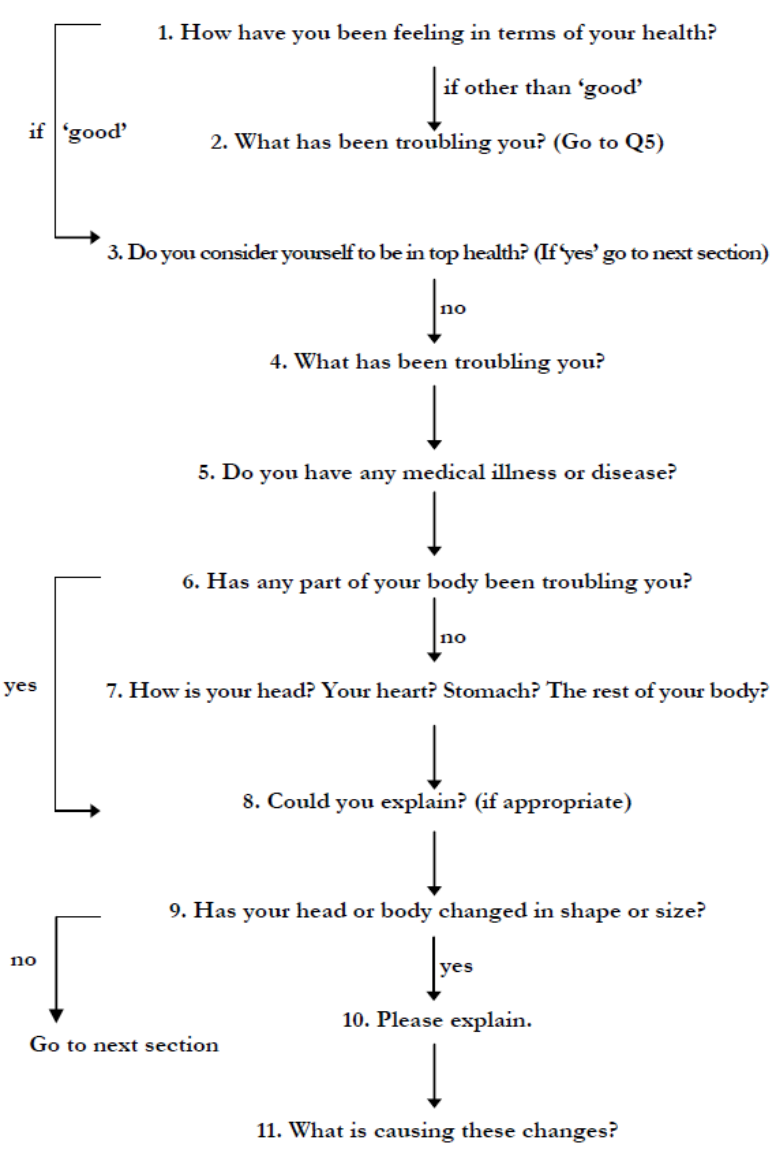
Data on **HALLUCINATORY BEHAVIOUR** and **ASSOCIATED DELUSIONS.**




15. For example?
16. Do you usually obey these orders (instructions)?
17. What do you make of these voices (or noises): Where do they really come from?
18. Why do you have these experiences?
19. Are these normal experiences?
Go back to Q3
20. Do ordinary things sometimes look strange or distorted to you?
21. Do you sometimes have 'visions' or see things that others can't see?
yes
22. For example?
23. Do these visions seem very real or life-like?
no
24.How often do you have these experiences?
25. Do you sometimes smell things that are unusual or that others don't smell?
yes
26. Please explain.
no
27. Do you get any strange or unusual sensations from inside your body?
yes
28. Tell me about this.
no
Go to next page

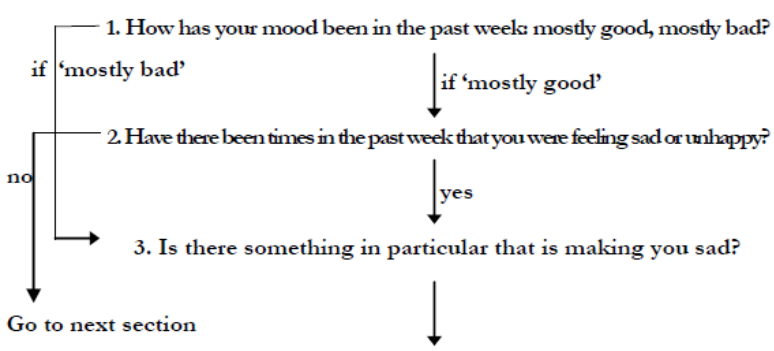
notes

Data on **SOMATIC CONCERN.**



notes

Data on **DEPRESSION.**



notes

notes

4. How often do you feel sad?

5. Just how sad have you been feeling?

6. Have you been crying lately?

7. Has your mood in any way affected your sleep?

8. Has it affected your appetite?

9. Do you participate less in activities on account of your mood?

10. Have you had any thoughts of harming yourself?

11. Any thoughts about ending your life?

yes

12. Have you attempted suicide?

no

Go to next section

Data on **GUILT FEELINGS** and **GRANDIOSITY.**

notes

same (go to Q5) ← 1. If you were to compare yourself to the average person, how would you come out: a little better, maybe a little worse, or about the same?

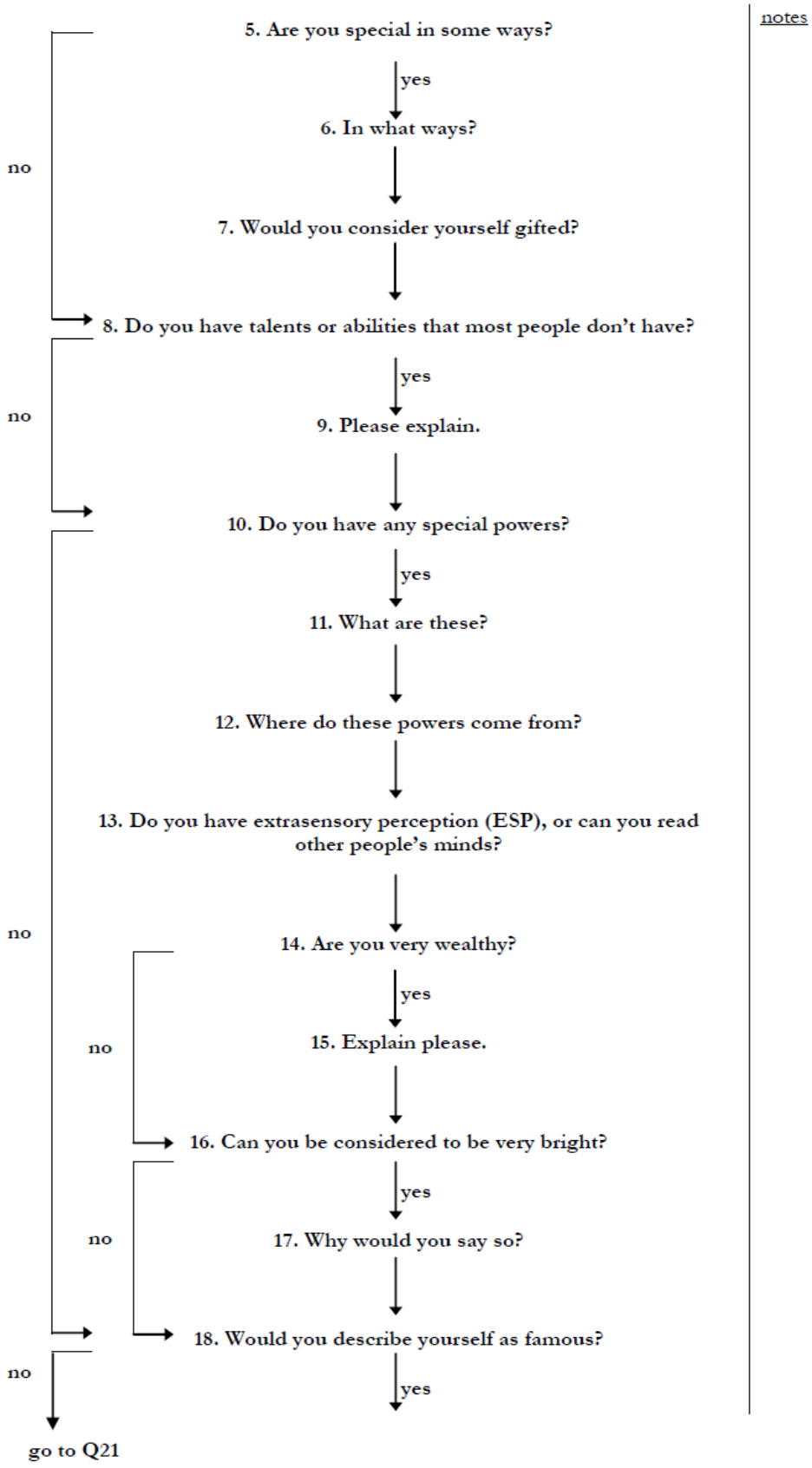
worse

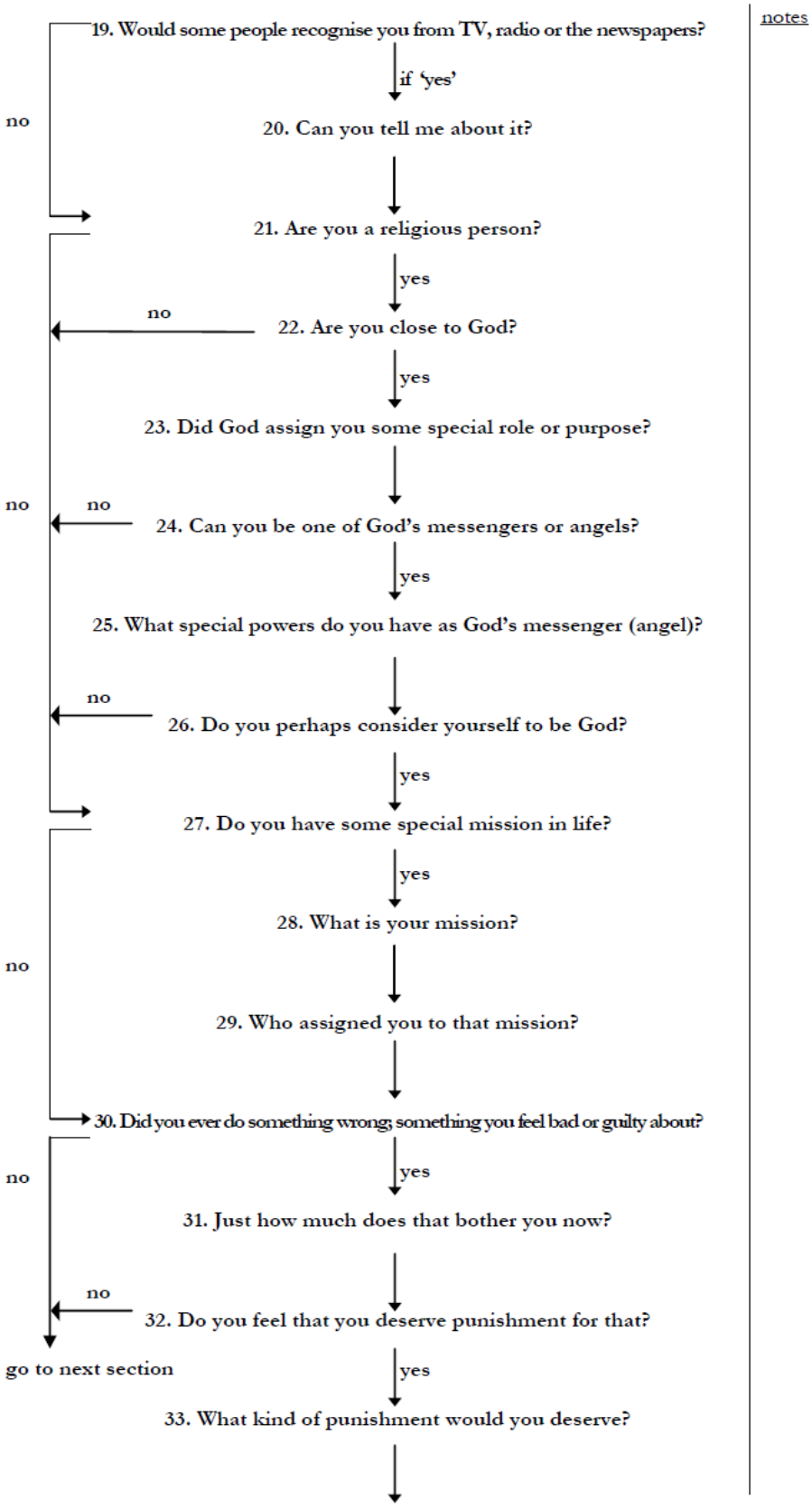
2. Worse in what ways?

3. Just how do you feel about yourself? (go to Q5)

better

4. Better in what ways? (go to Q5)


notes
5. Are you special in some ways?
yes
6. In what ways?
no
7. Would you consider yourself gifted?
8. Do you have talents or abilities that most people don't have?
yes
no
9. Please explain.
10. Do you have any special powers?
yes
11. What are these?
12. Where do these powers come from?
13. Do you have extrasensory perception (ESP), or can you read other people's minds?
no
14. Are you very wealthy?
yes
no
15. Explain please.
16. Can you be considered to be very bright?
yes
no
17. Why would you say so?
18. Would you describe yourself as famous?
no
yes
go to Q21


notes
19. Would some people recognise you from TV, radio or the newspapers?
if 'yes'
no
20. Can you tell me about it?
21. Are you a religious person?
yes
no
22. Are you close to God?
yes
23. Did God assign you some special role or purpose?
no
no
24. Can you be one of God's messengers or angels?
yes
25. What special powers do you have as God's messenger (angel)?
no
26. Do you perhaps consider yourself to be God?
yes
27. Do you have some special mission in life?
yes
28. What is your mission?
no
29. Who assigned you to that mission?
30. Did you ever do something wrong; something you feel bad or guilty about?
yes
no
31. Just how much does that bother you now?
no
32. Do you feel that you deserve punishment for that?
go to next section
yes
33. What kind of punishment would you deserve?

no

34. Have you at times thought of punishing yourself?

notes

go to next section

yes

35. Have you ever acted upon these thoughts?

Data on **DISORIENTATION.**

notes

1. Can you tell me what is today's date (i.e. the day, the month, the year)?

2. What is the name of the place you are in now?

3. (If hospitalised) What ward are you on?

4. (If hospitalised) What is the address of where you now stay?

5. If someone had to reach you by phone, what number would they call?

6. What is the name of the doctor who is treating you?

7. (If hospitalised) Can you tell me who else in on the staff and what they do?

8. Do you know who is now the Prime Minister?

9. Who was the Prime Minister before?

10. Who is the leader of the opposition party?

Data on **DIFFICULTY IN ABSTRACT THINKING.**

notes

1. I'm going to now say a pair of words, and I'd like you to tell me in what important way they are alike. Lets start for example, with the words … see Appendix A. How are they alike- what do they have in common?

if any other answer

if correct answer

2. Good. Now what about….? (Select three other items from the similarities list at varying levels of difficulty and based upon the interview stage and record the responses. Then go to Q4)

(If we use 'apple & banana' as the items) The incorrect answer will be either CONCRETE ('they both have skins' or 'you can eat them'), TANGENTIAL ('they're small), or IDIOSYNCHRATIC ('monkeys like them') – record this in the notes section to the left.

3. Okay, but they're both… give answer. Now how about … : how are these alike? (Select three other items from the similarities list at varying levels of difficulty and based upon the interview stage and record the responses.)

4. You've probably heard the expression… select proverb from Appendix B. What does this really mean?

5. What about when people say… select second proverb from Appendix B. What do they mean?

6. There's a very old saying… see Appendix B for third proverb. What is the deeper meaning of this proverb? (Select 1 other proverb from the final set in Appendix B)

Data on **LACK OF JUDGEMENT and INSIGHT.**

notes

1. How long have you been in the hospital (clinic etc)?

2. Why did you come to the hospital (Clinic, etc)?

yes

3. Do you need to be in the hospital (Clinic, etc)?

go to Q5

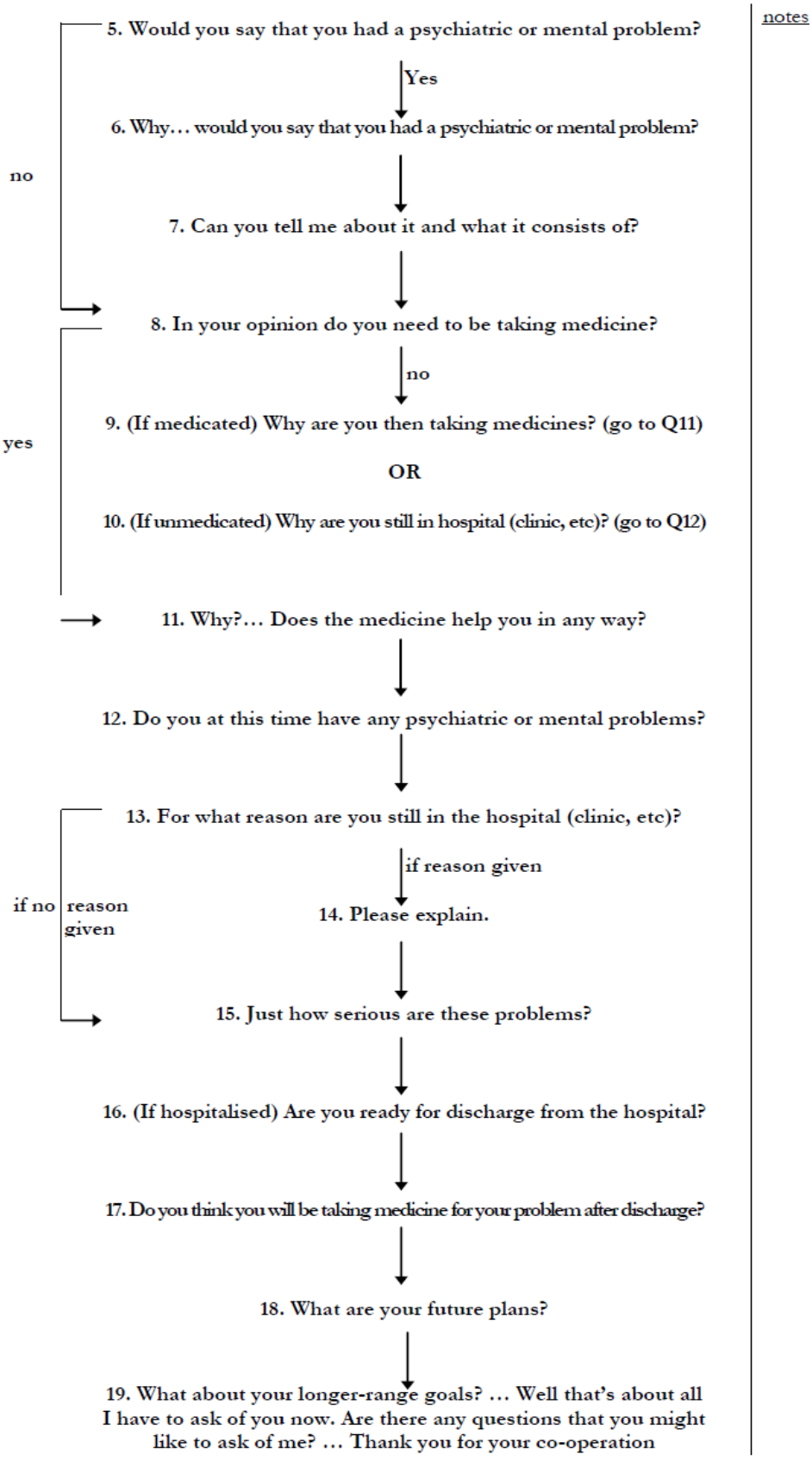
No

no

4. Did you have a problem that needed treatment?

go to Q8

Yes


notes
5. Would you say that you had a psychiatric or mental problem?
Yes
6. Why… would you say that you had a psychiatric or mental problem?
no
7. Can you tell me about it and what it consists of?
8. In your opinion do you need to be taking medicine?
no
yes
9. (If medicated) Why are you then taking medicines? (go to Q11)
OR
10. (If unmedicated) Why are you still in hospital (clinic, etc)? (go to Q12)
11. Why?… Does the medicine help you in any way?
12. Do you at this time have any psychiatric or mental problems?
13. For what reason are you still in the hospital (clinic, etc)?
if reason given
if no reason given
14. Please explain.
15. Just how serious are these problems?
16. (If hospitalised) Are you ready for discharge from the hospital?
17. Do you think you will be taking medicine for your problem after discharge?
18. What are your future plans?
19. What about your longer-range goals? … Well that's about all I have to ask of you now. Are there any questions that you might like to ask of me? … Thank you for your co-operation

Appendix A: Items for assessing SIMILARITIES in the evaluation of **DIFFICULTY IN ABSTRACT THINKING.**

2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	1. How are a ball and orange alike? 2. Apple and banana? 3. Pencil and pen? 4. Nickel and dime?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	5. Table and chair? 6. Tiger and elephant? 7. Hat and shirt? 8. Bus and train?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	9. Arm and leg? 10. Rose and tulip? 11. Uncle and cousin? 12. The sun and the moon?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	13. Painting and poem? 14. Hilltop and valley? 15. Air and water? 16. Peace and prosperity?

Appendix B: Items for assessing PROVERB INTERPRETATION in the evaluation of **DIFFICULTY IN ABSTRACT THINKING.**

What does the saying mean:

2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	1. "Plain as the nose on you face"? 2. "Carrying a chip on your shoulder"? 3. "Two heads are better than one"? 4. "Two many cooks spoil the broth"?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	5. "Don't judge a book by its cover"? 6. "One man's food is another man's poison"? 7. "All that glitters is not gold"? 8. "Don't cross the bridge until you come to it"?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	9. "What's good for the goose is good for the gander"? 10. "The grass always looks greener on the other side"? 11. "Don't keep all your eggs in one basket"? 12. "One swallow does not make a Summer"?
2-year assessment **Baseline/6mth assessment** **1-year/Discharge/18mth assessment** **Acute ward assessment**	13. "A stitch in time saves nine"? 14. "A rolling stone gathers no moss"? 15. "The acorn never falls far from the tree"? 16. "People who live in glass houses should not throw stones at others"?

16. Appendix 7 – Calgary Depression in Schizophrenia Scale

Interviewer: Ask the first question as written. Use follow up probes or qualifiers at your discretion. Time frame refers to last two weeks unless stipulated. N.B. The last item, #9, is based on observations of the entire interview.

1. DEPRESSION: How would you describe your mood over the last two weeks? Do you keep reasonably cheerful or have you been very depressed or low spirited recently? In the last two weeks how often have you (own words) every day? All day?

0. Absent
1. Mild - Expresses some sadness or discouragement on questioning.
2. Moderate - Distinct depressed mood persisting up to half the time over last 2 weeks: present daily.
3. Severe - Markedly depressed mood persisting daily over half the time interfering with normal motor and social functioning.

2. HOPELESSNESS: How do you see the future for yourself? Can you see any future? - or has life seemed quite hopeless? Have you given up or does there still seem some reason for trying?

0. Absent
1. Mild - Has at times felt hopeless over the last two weeks but still has some degree of hope for the future.
2. Moderate - Persistent, moderate sense of hopelessness over last week. Can be persuaded to acknowledge possibility of things being better.
3. Severe - Persisting and distressing sense of hopelessness.

3. SELF DEPRECIATION: What is your opinion of your self-compared to other people? Do you feel better, not as good, or about the same as others? Do you feel inferior or even worthless?

0. Absent
1. Mild - Some inferiority; not amounting to feeling of worthlessness.
2. Moderate - Subject feels worthless, but less than 50% of the time.
3. Severe - Subject feels worthless more than 50% of the time. May be challenged to acknowledge otherwise.

4. GUILTY IDEAS OF REFERENCE: Do you have the feeling that you are being blamed for something or even wrongly accused? What about? (Do not include justifiable blame or accusation. Exclude delusions of guilt.)

0. Absent
1. Mild - Subject feels blamed but not accused less than 50% of the time.
2. Moderate - Persisting sense of being blamed, and/or occasional sense of being accused.
3. Severe - Persistent sense of being accused. When challenged,
0. acknowledges that it is not so.

5. PATHOLOGICAL GUILT: Do you tend to blame yourself for little things you may have done in the past? Do you think that you deserve to be so concerned about this?

0. Absent
1. Mild - Subject sometimes feels over guilty about some minor offense, but less than 50% of time.
2. Moderate - Subject usually (over 50% of time) feels guilty about past actions the significance of which he exaggerates.
3. Severe - Subject usually feels s/he is to blame for everything that has gone wrong, even when not his/her fault.

6. MORNING DEPRESSION: When you have felt depressed over the last 2 weeks have you noticed the depression being worse at any particular time of day?

0. Absent - No depression.
1. Mild - Depression present but no diurnal variation.
2. Moderate - Depression spontaneously mentioned to be worse in a.m.
3. Severe - Depression markedly worse in a.m., with impaired functioning which improves in p.m.

7. EARLY WAKENING: Do you wake earlier in the morning than is normal for you? How many times a week does this happen?

0. Absent - No early wakening.
1. Mild - Occasionally wakes (up to twice weekly) 1 hour or more before normal time to wake or alarm time.
2. Moderate - Often wakes early (up to 5 times weekly) 1 hour or more before normal time to wake or alarm.
3. Severe - Daily wakes 1 hour or more before normal time.

8. SUICIDE: Have you felt that life wasn't worth living? Did you ever feel like ending it all? What did you think you might do? Did you actually try?

0. Absent

1. Mild - Frequent thoughts of being better off dead, or occasional
    i. thoughts of suicide.
2. Moderate - Deliberately considered suicide with a plan, but made no attempt.
3. Severe - Suicidal attempt apparently designed to end in death (i.e.: accidental discovery or inefficient means).

9. OBSERVED DEPRESSION: Based on interviewer's observations during the entire interview. The question "Do you feel like crying?" used at appropriate points in the interview, may elicit information useful to this observation.

0. Absent
1. Mild - Subject appears sad and mournful even during parts of the interview, involving affectively neutral discussion.
2. Moderate - Subject appears sad and mournful throughout the interview, with gloomy monotonous voice and is tearful or close to tears at times.
3. Severe - Subject chokes on distressing topics, frequently sighs deeply and cries openly, or is persistently in a state of frozen misery if examiner is sure that this is present.

17. Appendix 8 - Simpson-Angus Scale

1. GAIT:

The patient is examined as s/he walks into the examining room, his gait, the swing of his arms, his general posture, all form the basis for an overall score for this item. This is rated as follows:

0. Normal
1. Diminution in swing while the patient is walking
2. Marked diminution in swing with obvious rigidity in the arm
3. Stiff gait with arms held rigidly before the abdomen
4. Stopped shuffling gait with propulsion and retropulsion

2. ARM DROPPING:

The patient and the examiner both raise their arms to shoulder height and let them fall to their sides. In a normal subject, a stout slap is heard as the arms hit the sides. In the patient with extreme Parkinson's syndrome, the arms fall very slowly:

0. Normal, free fall with loud slap and rebound
1. Fall slowed slightly with less audible contact and little rebound
2. Fall slowed, no rebound
3. Marked slowing, no slap at all
4. Arms fall as though against resistance; as though through glue

3. SHOULDER SHAKING:

The subject's arms are bent at a right angle at the elbow and are taken one at a time by the examiner who grasps one hand and also clasps the other around the patient's elbow. The subject's upper arm is pushed to and fro and the humerus is externally rotated. The degree of resistance from normal to extreme rigidity is scored as follows.

0. Normal
1. Slight stiffness and resistance
2. Moderate stiffness and resistance
3. Marked rigidity with difficulty in passive movement
4. Extreme stiffness and rigidity with almost a frozen shoulder

4. ELBOW RIGIDITY:

The elbow joints are separately bent at right angles and passively extended and flexed, with the subject's biceps observed and simultaneously palpated. The resistance to this procedure is rated. (The presence of cogwheel rigidity is noted separately.)

0. Normal
1. Slight stiffness and resistance
2. Moderate stiffness and resistance
3. Marked rigidity with difficulty in passive movement
4. Extreme stiffness and rigidity with almost a frozen shoulder

5. WRIST RIGIDITY or Fixation of position:

The wrist is held in one hand and the fingers held by the examiner's other hand, with the wrist n1oved to extension, flexion and ulner and radial deviation:

0. Normal
1. Slight stiffness and resistance
2. Moderate stiffness and resistance
3. Marked rigidity with difficulty in passive movement
4. Extreme stiffness and rigidity with almost a frozen shoulder

6. LEG PENDULOUSNESS:

The patient sits on a table with his legs hanging down and swinging free. The ankle is grasped by the examiner and raised until the knee is partially extended. It is then allowed to fall. The resistance to falling and the Jack of swinging form the basis for the score on this item:

0. The legs swing freely
1. Slight diminution in the swing of the legs
2. Moderate resistance to swing
3. Marked resistance and damping of swing
4. Complete absence of swing

7. HEAD DROPPING:

The patient lies on a well-padded examining table and his head is raised by the examiner's hand. The hand is then withdrawn and the head allowed to drop. In the normal subject the head will fall upon the table. The movement is delayed in extrapyramidal system disorder and in extreme parkinsonism it is absent. The neck muscles are rigid and the head docs not reach the examining table. Scoring is as follows:

0. The head falls completely with a good thump as it hits the table
1. Slight slowing in fall, mainly noted by lack of slap as head meets the table
2. Moderate slowing in the fall quite noticeable to the eye
3. Head falls stiffly and slowly
4. Head does not reach the examining table

8. GLABELLA TAP:

Subject is told to open eyes wide and not to blink. The glabella region is tapped at a steady, rapid speed. The number of times patient blinks in succession is noted:

0. 0 - 5 blinks
1. 6 – 10 blinks
2. 11 – 15 blinks
3. 16 - 20 blinks
4. 21 and more blinks

9. TREMOR:

Patient is observed walking into examining room and is then re-examined for this item:

0. Normal
1. Mild finger tremor, obvious to sight and touch
2. Tremor of hand or arm occurring spasmodically
3. Persistent tremor of one or more limbs
4. Whole body tremor

10. SALIVATION:

Patient is observed while talking and then asked to open his mouth and elevate his tongue. The following ratings are given:

0. Normal
1. Excess salivation to the extent that pooling takes place if the mouth is open and the tongue raised.
2. When excess salivation is present and might occasionally result in difficulty in speaking
3. Speaking with difficulty because of excess salivation
4. Frank drooling

18. Appendix 9 –Regression analysis

Linear regression of avolition-apathy and depression at baseline

	B	SE B	β
Constant	6.01	.76	
CDSS Depression	.11	.04	.21*
Simpson-Angus scale	.09	.07	.08
PANSS positive symptoms	.03	.04	.06

F(203) = 4.09, *R = .24, R^2= .058; *p = .005*

Linear regression of expressive deficits and depression at baseline

	B	SE B	β
Constant	23.37	18.29	
CDSS Depression	.71	1.09	.04
Simpson-Angus scale	-1.22	2.03	-.04
PANSS positive symptoms	-.82	1.06	-.05

F(203) = .38, *R = .07, R^2=.005*

Linear regression of total PANSS negative symptoms and depression at baseline

	B	SE B	β
Constant	14.61	1.47	
CDSS Depression	.17	.08	.14*
Simpson-Angus scale	.21	.16	.09
PANSS positive symptoms	.08	.08	.06

F(203) = 2.66, *R = .19, R^2= .038; *p < .05*

Linear regression between total negative symptom, depression and sex at 2 months.

	B	SE B	β
Constant	14.82	.57	
2 month depression	.28	.11	.19*
Sex	.00	.00	-.04

$F(166) = 3.29$, $p < .01$. R = .19, $R^2 = .03$; *$p < .01$

Linear regression between total negative symptom, depression and EPSE at 6 months.

	B	SE B	β
Constant	14.44	.68	
6 month depression	.23	.13	.14
EPSE	.67	.29	.19*

$F(135) = 4.33$, $p < .01$. R = .24, $R^2 = .06$; *$p < .05$

Linear regression between total negative symptoms and depression at 9 months

	B	SE B	β
Constant	14.03	.66	
6 month depression	.33	.12	.25*

$F(129) = 6.82$, $p < .01$ (R = .22, $R^2 = .05$); *$p < .05$

Linear regression between total negative symptom, depression and EPSE at 12 months.

	B	SE B	β
Constant	13.81	64	
12 month depression	.31	.12	.22*
Sex	.53	.24	.19*

$F(125) = 6.19$, $p < .01$. $R = .30$, $R^2 = .09$; *$p < .01$

Linear regression of the avolition-apathy sub-domain at two months after controlling for positive symptoms and depression.

	B	SE B	β
Constant	3.46	.85	
2 month positive symptoms	.07	.06	1.12
2 months depression	.33	.07	.35*

$F(167) = 16.02$, $p < .001$. $R = .40$, $R^2 = .16$ $p < .001$; *$p < .001$

Linear regression of the avolition-apathy sub-domain at 6 months after controlling for EPSE, depression, and positive symptoms.

	B	SE B	β
Constant	1.04	.86	
6 months positive symptoms	.12	.06	.16*
6 months depression	.35	.08	.33**
6 months EPSE	.66	.16	.29**

$F(135) = 17.36$, $p < .001$; $R = .53$, $R^2 = .28$ $p < .001$; *$p < .05$, **$p < .001$

Linear regression of the avolition-apathy sub-domain after controlling for depression and positive symptoms at 9 months.

	B	SE B	β
Constant	1.82	.87	
9 months positive symptoms	.05	.06	.06
9 months depression	.48	.07	.04*

$F(130) = 23.84$, $p < .001$; R = .52, $R^2 = .27$ $p < .001$; *$p < .001$

Linear regression of avolition-apathy and depression controlling for positive symptoms and EPSE at 12 months.

	B	SE B	β
Constant	1.28	.80	
12 months positive symptoms	.08	.05	.12
12 months depression	.30	.07	.33*
12 months EPSE	.48	.14	.26*

$F(126) = 13.59$, $p < .001$; R = .49, $R^2 = .24$; *$p < .001$

Linear regression of expressive deficits controlling for depression at 2 months.

	B	SE B	β
Constant	4.84	.34	

6 months depression	.21	.06	.23*

$F(187) = 10.37$, $p = .002$, $R = .23$, $R^2 = .05$; $^*p < .01$

Linear regression of the expressive deficits sub-domain after controlling for depression and EPSE at 6 months.

	B	SE B	β
Constant	2.66	.36	
6 months depression	.20	.07	.20*
6 months EPSE	.95	.15	.42**

$F(165) = 24.06$, $p < .001$, $R = .47$, $R^2 = .22$. $^*p < .01$ $^{**}p < .001$

Linear regression of expressive deficits controlling for depression at 9 months,

	B	SE B	β
Constant	4.36	.37	
6 months depression	.25	.07	.25*

$F(158) = 11.06$, $p < .001$, $R = .27$, $R^2 = .06$; $^*p < .001$

Linear regression of expressive deficits and depression, controlling for EPSE at 12 months.

	B	SE B	β

Constant	2.65	.38	
12 months depression	.21	.07	.21*
12 months EPSE	.67	.14	.34**

*F(153) = 16.22, p < .001, R = .42, R2 = .17. *p = .001 **p < .001*

19. Appendix 10 – Factor analysis and correlations between inflammatory markers, CDSS and PANSS

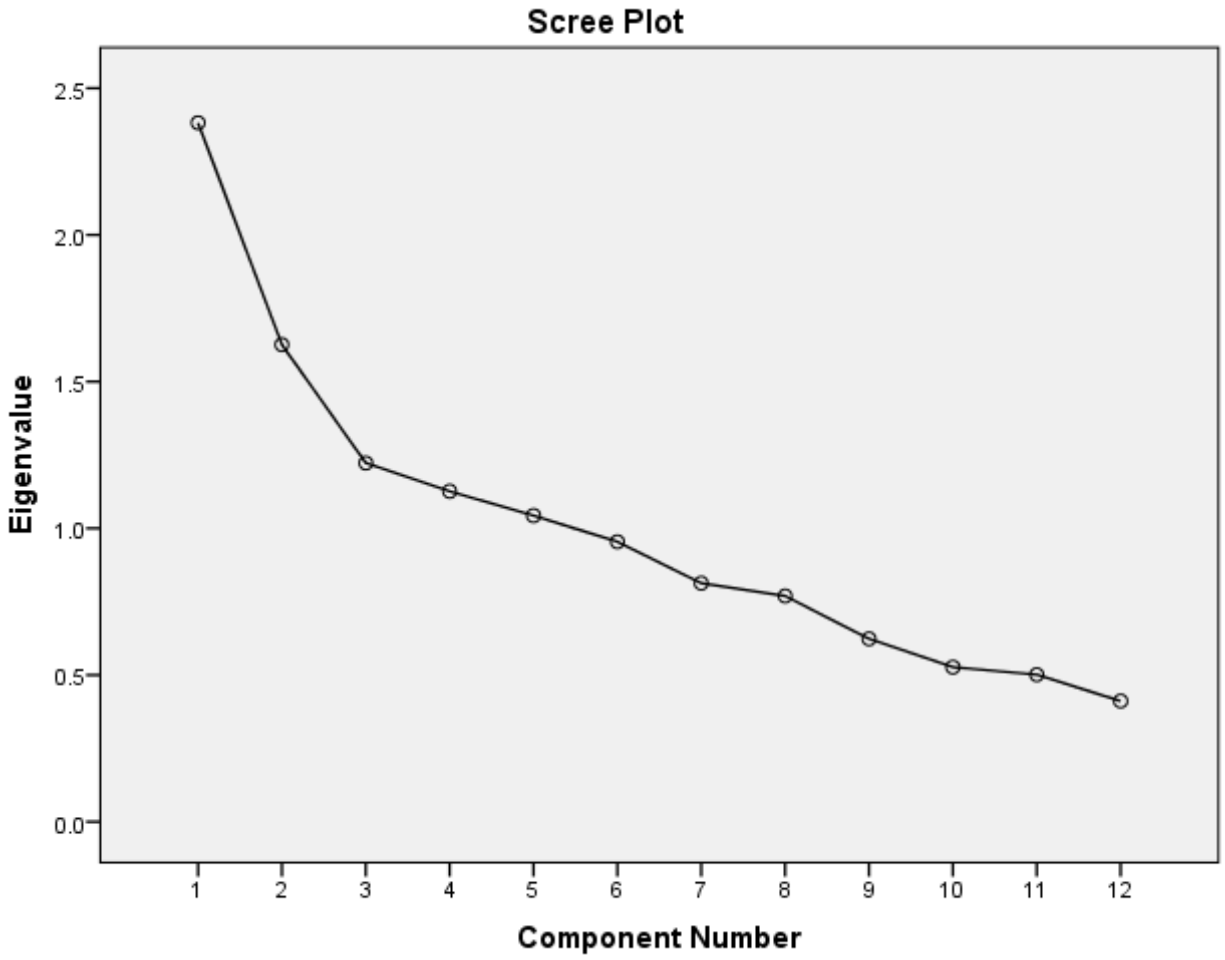
Factor analysis of inflammatory markers

Ten of the inflammatory markers were subjected to principle components analysis (PCA) using SPSS (v.22). Prior to performing PCA, the suitability of data for factor analysis was assessed. Inspection of the correlation matrix revealed the presence of many coefficients of .3 and above. The Kaiser-Meyer-Oklin value was .634 and Bartlett's Test of Sphericity reached statistical significance, supporting the factorability of the correlation matrix.

Principle components analysis revealed the presence of five components with eigenvalues exceeding one. The five-component solution cumulatively explaining 61% of the variance, with component one (IL-6) contributing 20%, component two (TNF-α) contributing 14%, component three (CRP) contributing 10%, component four (IFN-G) contributing 9% and component five (IL-1RA) also contributing 9%. The interpretation of was that IL-6, CRP and TNF-α contributed to the most amount of variance.

	Component				
	1	2	3	4	5
IL-6	.794				
TNF-α	.651				

CRP	.620	-.387			
IFN-G	.588			-.423	
IL-1RA	.574	-.377			
IL-13		.687		.396	
IL-2		.621	-.300		.328
IL-8			.604		-.466
IL-4		.441	.532		
IL-12p70		.357		.585	
IL-1B			.524	-.340	.536
IL-10				-.341	-.526



Correlations between inflammatory markers, CDSS and PANSS scores at baseline.

	Total PANSS positive	Total PANSS negative	expressive deficits	avolition-apathy	BMI	CRP	IL-6	TNF-α	CDSS	SAS
Total PANSS positive		.07	-.06	.11	-.12	.06	-.02	.09	.28**	-.04
Total PANSS negative			.84**	.75**	.02	.01	-.01	.08	.12	-.00
expressive deficits				.44**	.03	-.04	-.03	.05	.00	-.03
avolition-apathy					.04	.09	-.01	.11	.20**	.01
BMI						.46**	.39**	.22**	.05	.03
CRP							.44**	.28**	.04	.21*
IL-6								.34**	.14*	.16*
TNF-α									.14*	.14*
CDSS										-.00
SAS										

***p <.001. *p<.005.*

Correlations between inflammatory markers, CDSS and PANSS scores at 6 months.

	Total PANSS positive	Total negative symptoms	expressive deficits	avolition-apathy	CRP	IL-6	TNF-α	CDSS
Total PANSS positive		.42**	.18*	.36**	-.01	.01	-.13	.43**
Total negative symptoms			.82**	.83**	.11	-.03	-.00	.20*
expressive deficits				.85**	.09	-.05	-.02	.33**

Avolition-apathy					.12	.10	-.05	.46**
CRP						.42**	.34**	.15
IL-6							.28**	.08
TNF-α								.04
CDSS								

***p <.001. *p<.005.*

Correlations between inflammatory markers, CDSS and PANSS scores at 12 months.

	PANSS positive	Total negative symptoms	expressive deficits	avolition-apathy	BMI	CRP	IL-6	TNF-α	CDSS	SAS
PANSS positive		.40**	.08	.34*	.11	.10	.03	-.04	.45**	.03
Total negative symptoms			.85**	.83**	.07	.12	.13	.17	.30**	.266**
expressive deficits				.88**	.03	.09	.08	.25*	.35**	.50**
avolition-apathy					.06	.07	.14	.04	.45**	.44**
BMI						.40**	.44**	.21*	.02	.04
CRP							.48**	.29**	.13	.15
IL-6								.34**	.07	.10
TNF-α									.03	.17
CDSS										.21**
SAS										

***p <.001. *p<.05.*