The Association of Anxiety Symptom Severity and Panic Attacks with Inflammation

This thesis is submitted in partial fulfilment of the Honours degree of Bachelor of Psychological Science (Honours)

School of Psychology

Faculty of Health and Medical Sciences

University of Adelaide

September 2022

Word Count: 9492

Table of Contents

List of Tables

Abstract

As prevalence of anxiety and anxiety disorders continue to rise worldwide, increasing focus has been placed on investigating the possible biological correlates of mental disorders. Accumulating evidence has demonstrated the important role of the immune system in the pathophysiology of mental disorders. However, the relations between inflammatory markers and anxiety disorders, especially their symptomology, have been sparsely examined. Therefore, the aim of this pilot study was to assess the associations between anxiety symptom severity, panic attack history, and several pro-inflammatory markers. For this purpose, structured interviews using the DSM-5 and the HAM-A were conducted to assess anxiety symptom severity and panic attack history in 38 individuals (aged 18-76) over two time points. Blood samples were collected to assess serum levels of C-reactive protein (CRP), interferon-γ (IFN-γ), interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor (TNF)-α. Anxiety symptom severity had small associations with decreased levels of CRP and IFN-γ in Pearson's product-moment correlations. However, linear regression analyses attenuated these associations after controlling for significant covariates, specifically, BMI, age, and sex. No associations were found between other inflammatory markers and anxiety symptom severity, or panic attack history. Although, this study was limited by a small sample size, and consequently was underpowered to detect significant associations. Further research examining these associations in a larger population may be worthwhile. Interestingly, significant, large effects were found between BMI and inflammatory markers in models with anxiety symptom severity and panic attack history, indicating potential avenues for therapeutic interventions.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge, this thesis contains no material previously published except where due reference is made. I give permission for the digital version of this thesis to be made available on the web, via the University of Adelaide's digital thesis repository, the Library Search and through web search engines, unless permission has been granted by the School to restrict access for a period of time.

(October 2022)

Contribution Statement

In writing this thesis, my supervisors and I collaborated to generate research questions of interest and design the appropriate methodology. After the approval of the ethics application, I conducted the literature search, collated relevant data from the pre-existing data set, statistical analysis, and thesis write up. My supervisor assisted me with the RStudio programming. This thesis was based on a pre-existing data set from a study conducted by my supervisor (Principal Investigator), who was responsible for study design, participant recruitment, and data collection.

The Association of Anxiety Symptom Severity and Panic Attacks with Inflammation

Anxiety disorders are widespread and often associated with longstanding disability and several physiological and psychological conditions. Approximately one in four individuals are likely to have a current or remitted anxiety disorder (Wittchen et al., 2011). Psychoneuroimmunology research has led to considerable advances in understanding the reciprocal interactions between the immune system and the central nervous system (CNS) in psychological conditions (Ader et al., 1995; Bankier et al., 2008; Hou et al., 2017). Targeting the biomarkers of psychological conditions may yield specialised identification, assessment, and treatment procedures, as well as provide insight for future targets of drug and therapeutic development (Belem da Silva et al., 2017).

Anxiety and Anxiety-Based Disorders: Classification, Prevalence, and Aetiology

Stress is an adaptive response, enabling one to escape from, or avoid danger (Parsons et al., 2021). However, this innate response to stress is susceptible to dysregulation if the stressor is excessive or prolonged (Vieira et al., 2010), and can become pathological if it interferes with an individual's well-being (Won & Kim, 2020). Anxiety disorders are a cluster of mental health conditions defined by excessive fear and anxiety regarding real or perceived threat (American Psychiatric Association (APA), 2022). Although these two states coexist, fear is often associated with the emotional response to imminent threat, whereas anxiety is the anticipation of future threat (APA, 2022). The Diagnostic and Statistical Manual of Mental Disorders, 5th ed, Text Revision (DSM-5-TR) classifies the following as anxiety disorders: Separation Anxiety Disorder, Selective Mutism, Specific Phobia, Social Anxiety Disorder (SAD), Panic Disorder (PD), Agoraphobia, Generalized Anxiety Disorder (GAD), Substance/Medication-Induced Anxiety Disorder, Anxiety Disorder Due to Another Medical Condition, Other Specified Anxiety Disorder, and Unspecified Anxiety Disorder. These disorders share similar characteristics of maladaptive anxiety, physiological symptoms,

behavioural disturbances, distorted thinking, avoidance behaviours, and functional impairment (APA, 2022). Panic attacks (PA) feature prominently among anxiety disorders as part of the fear response. Panic attacks are characterised as abrupt surges of intense fear or discomfort that occurs in conjunction with physical and cognitive symptoms: including palpitations, sweating, and fear of losing control (APA, 2022). Panic attacks can also occur in the context of other mental disorders (such as depressive disorders, posttraumatic stress disorder (PTSD), and substance use disorders) and medical conditions (including cardiac, respiratory, vestibular and gastrointestinal conditions) (APA, 2022).

Originally considered part of the DSM-IV anxiety disorder group, PTSD and obsessive-compulsive disorder (OCD) have been reclassified into separate domains of trauma- and stressor-related disorders, and obsessive-compulsive and related disorders, respectively in the DSM-5. However, similarly to anxiety disorders, PTSD and OCD share the underlying features of maladaptive anxiety (Lack, 2012; Taylor et al., 1992; Williams et al., 2005), distorted thought patterns (Turner et al., 1992), and avoidance (APA, 2022; Hayes et al., 1996). This study will be discussing and referring to specific phobia, SAD, PD, Agoraphobia, and GAD collectively as anxiety disorders, and PTSD and OCD as anxiety-based disorders (see Table 1 for list of included disorders and their key features).

Anxiety and anxiety-based disorders are among the most prevalent and disabling mental health conditions (Buist-Bouwman et al., 2006; Kessler et al., 2005), with a global prevalence rate as high as 28.3% (Baxter et al., 2013). These disorders tend to persist throughout the lifetime, due to a recurrent-intermittent course involving waxing and waning symptoms (Craske et al., 2017; Kessler et al., 2009). Consequently, this can lead to negative outcomes in relation to quality of life and wellbeing, measured by physical and mental health, and psychosocial functioning (Baxter et al., 2014; Mendlowicz & Stein, 2000).

Table 1

Characteristics of Anxiety and Anxiety-Based Disorders

Anxiety and Anxiety-Based Disorder	Key Features
Specific Phobia	Fearful and anxious about, or avoidant of, specific objects or situations.
Social Anxiety Disorder	Fearful or anxious about, or avoidant of, social interactions and environments involving the potential of being scrutinised.
Panic Disorder	Experience of recurrent unexpected panic attacks and consistently worried about experiencing more panic attacks or adopts maladaptive behaviour as a result of the panic attacks.
Agoraphobia	Fearful and anxious of being in several different situations where escape may be difficult, or assistance may not be available, e.g., public transport or standing in line.
Generalised Anxiety Disorder	Persistent and excessive anxiety and worry regarding various domains that may be difficult to control.
Posttraumatic Stress Disorder	Development of characteristic symptoms after exposure to one or more traumatic event. Symptoms include fear-based recurrent recollections, anhedonia, and reactive-externalising behaviours.
Obsessive-Compulsive Disorder	Presence of obsessions, and/or compulsions. Obsessions: unwanted recurrent and persistent thoughts or urges. Compulsions: repetitive behaviours in response to obsessions or rigid rules.

Note. Adapted from American Psychiatric Association (APA) (2022). Anxiety Disorders; Obsessive-Compulsive and Related Disorders; Trauma- and Stressor-Related Disorders. In *Diagnostic and statistical manual of mental disorders* (5th ed., text revision. ed.). American Psychiatric Association Publishing.

Globally, anxiety disorders are the sixth largest contributor to non-fatal health loss and are ranked in the top ten causes of years lived with disability (World Health Organisation, 2017). These disorders contribute to the largest health burden in relation to cost (Gustavsson et al., 2011), likely due to misdiagnosis and inappropriate treatment (Furtado & Katzman, 2015). Therefore, it is imperative we understand the causes of these disorders to improve both treatment outcomes and individual wellbeing.

To achieve improved treatment outcomes and wellbeing for those affected, an increasing body of literature has focused on further investigating the aetiology of anxiety and anxiety-based disorders. These disorders encompass a diverse range of categorically distinct conditions, highlighting the unique neuropathophysiological mechanisms underlying each condition. This reflects the heterogeneous nature of anxiety and anxiety-based disorders (Craske et al., 2017). However, emerging research has indicated certain commonalities regarding the psychophysiology of anxiety and fear. Anxiety disorders have strong associations to common genetic and environmental risk factors (Faravelli et al., 2012; Kendler & Baker, 2007; Shimada-Sugimoto et al., 2015; Waszczuk et al., 2014), and similar treatment options have proven successful for several anxiety disorders (Roy-Byrne et al., 2010). Current conceptualisations of the aetiology of anxiety and anxiety-based disorders involves an interaction of biological factors (including genetics, sex differences, neurotransmission impairment, and neural abnormalities in signal transduction mechanisms), and psychosocial factors (such as trauma, stressful life events, and childhood and adolescent adversity) (Craske et al., 2017; Hettema et al., 2005).

The exposure to stress and traumatic events leads to hypothalamic-pituitary-adrenal (HPA) axis reactivity, and the subsequent activation of the immune system (Michopoulos et al., 2017). Heightened interest in the field of psychoneuroimmunology research has led to substantial advances in understanding the reciprocal relationship between the CNS and the

immune system in psychological conditions (Ader et al., 1995; Au et al., 2015). Evidence from clinical research elucidates the pivotal role of immune functioning, and its influence on the brain and behaviour (Brambilla et al., 1994; Dantzer et al., 2008). Additionally, increasing evidence has linked anxiety to neurological, vascular, respiratory, and metabolic conditions (Bankier et al., 2008; Carroll et al., 2009; Roest et al., 2010; Seldenrijk et al., 2010; Vogelzangs et al., 2010). As low-grade systemic inflammation is a key feature in the aetiology of these physiological conditions, it has been hypothesised that inflammation has an important function in anxiety and anxiety-based disorders (Vogelzangs et al., 2013). Leading to the emergence of literature exploring the interactions between the immune system and anxiety disorders.

Inflammation

Inflammation is the immune system's response to harmful stressors, such as pathogens and damaged cells. It acts by removing injurious stimuli and instigating the healing process (Ferrero-Miliani et al., 2007). As such, inflammation is a defence mechanism, which is critical to health (Nathan & Ding, 2010). Inflammation can be categorised as either acute or chronic. During the acute inflammatory response, cellular and molecular interactions effectively minimise the results of injury or infection (Chen et al., 2018). This process results in the restoration of homeostasis and the resolution of the acute inflammatory cycle.

Previously, the CNS and immune system were thought to act relatively independently of one another (Lucas et al., 2009). However, recent literature suggests that the inflammatory response can penetrate the blood-brain barrier, through humoral, neural, and cellular pathways, and interacts with various areas of the brain relating to mood regulation, neurocircuitry, and synaptic plasticity (Capuron & Miller, 2011). Consequently, prolonged, uncontrolled inflammation can become chronic, contributing to negative health outcomes such as cardiovascular disease, autoimmune conditions, and diabetes (Khansari et al., 2009),

as well as neuropsychiatric and psychological disorders (Capuron & Miller, 2011; Dantzer et al., 2008; Hou & Baldwin, 2012).

Markers of Inflammation and their Mechanism of Action

Interestingly, similar inflammatory mechanisms occur in response to traumatic and stressful events, including the exposure to fear- and anxiety-provoking stimuli. The exposure to stressful events results in HPA axis reactivity, activation of the immune system, and subsequent inflammatory response (Furtado & Katzman, 2015; Michopoulos et al., 2017). The inflammatory response is broadly orchestrated by proteins; C-reactive protein (CRP) and a cluster of proteins termed cytokines. Cytokines function within a complex network and act synergistically or antagonistically (Hou & Baldwin, 2012). These signalling proteins primarily function to activate cell-mediated and anti-body mediated immunity (Glik & Douvdevani, 2006), facilitate communication between cells, and modulate immune responses (Zhang & An, 2007). Cytokines are broadly divided into either pro- or anti-inflammatory, based upon their interaction with target cells (Furtado & Katzman, 2015). Although, some cytokines present with both pro- and anti-inflammatory properties, such as interleukin (IL)-6 (Scheller et al., 2011). Pro-inflammatory cytokines act as a guide for the inflammatory response to enhance the elimination of the illness, injury, or stressor, while anti-inflammatory cytokines enable tissue repair and dampen the synthesis of pro-inflammatory cytokines (Kronfol & Remick, 2000). Therefore, the balance between pro- and anti-inflammatory cytokines is a critical determinant in the regulation of the inflammatory response (Hou et al., 2017), and vital for normal neuropsychological functioning (Brambilla et al., 1994; Camacho, 2013).

In psychological conditions, such as anxiety disorders, anxiety-based disorders, and major depressive disorder (MDD), the delicate balance between pro- and anti-inflammatory cytokines is often altered (Furtado & Katzman, 2015). Over time, with the continued

exposure to stressors, the HPA axis and immune function become dysregulated, leading to the imbalance of cytokines (Michopoulos et al., 2017). Specifically, these disorders have been associated with a heightened inflammatory state (Martino et al., 2012).

In order to assess inflammation in psychological conditions, blood-based markers of inflammation are measured. Pro-inflammatory cytokines (such as tumour necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, and interferon (IFN)-γ) are produced predominantly by activated white blood cells called macrophages and are increasingly being implicated in the aetiology of psychological conditions (Zhang & An, 2007). CRP is manufactured in the liver and secreted into the blood ensuing injury, infection or stressful stimuli (Ballantyne & Nambi, 2005). Additionally, CRP is highly sensitive and most commonly used as a marker for the diagnosis of acute inflammation in humans (Sproston & Ashworth, 2018).

Inflammation and Anxiety and Anxiety-Based Disorders

Research examining the presence of inflammatory responses in psychological disorders began in the field of depression. As signs of immune disturbances in depression were reported in the early 1990s (Copeland et al., 2012; Dantzer et al., 2008; Decker et al., 1996), the role of cytokines and the immune system in MDD have been extensively researched. The current theory of inflammation and depression proposes that the imbalance of pro- and anti-inflammatory states cause pro-inflammatory state dominance, resulting in increased cytokine activity to the brain. This leads to behavioural correlates of depressive mood (such as loss of appetite, and loss of interest in physical and social environments), referred to as sickness behaviours (Dantzer et al., 2008; Kelley et al., 2003). Depression is associated with significantly increased levels of pro-inflammatory cytokines IL-6, IL-10, TNF-α, and CRP (Haapakoski et al., 2015; Howren et al., 2009; Kohler et al., 2017). The high comorbidity of anxiety and anxiety-based disorders, and MDD, and the similar treatment effects indicate shared neurobiological substrates (Hou et al., 2017). Moreover, the

pronounced response of central and peripheral cytokines to stressors has incited further interest in the role of the immune system in the aetiology of anxiety disorders.

Although associations between anxiety and anxiety-based disorders and inflammation have been less extensively studied, ongoing research suggests immune system involvement within these disorders. The established relationship between immune dysregulation and depression and the highly comorbid nature of depression and anxiety, are but one of several links associating anxiety and inflammation. As aforementioned, anxiety disorders have been linked to physical conditions associated with low grade systemic inflammation (Carroll et al., 2009; Roest et al., 2010; Seldenrijk et al., 2010; Vogelzangs et al., 2010), as well as chronic stress and exposure to stressful and traumatic events, which have been found to incite an inflammatory response (Hamer et al., 2006).

While the underlying mechanisms are not yet fully understood, it has been hypothesised that the prolonged exposure to trauma and stress facilitates HPA axis reactivity and increased immune activation in both the periphery and CNS, leading to the dysregulation of these systems and the subsequent continued release of cytokines (Michopoulos et al., 2017). Cytokine signals entering the CNS can influence the processes involved with mood regulation and behaviour, such as anxiety, arousal, and alarm (Michopoulos et al., 2017). Moreover, cytokine signalling can affect neurotransmitter systems that regulate the release of serotonin and dopamine, which may facilitate the production or maintenance of anxiety-based symptoms in these disorders (Michopoulos et al., 2017). Cytokines may also contribute to fear- and anxiety-based symptom maintenance, as they can influence the activity and connections of brain regions implicated in the aetiology of anxiety disorders, such as the amygdala, hippocampus, and medial prefrontal cortex (Michopoulos et al., 2017). However, numerous mechanisms may explain associations between immune activity and anxiety disorders, it can also be expected that immune dysregulation may be confined to specific

subgroups of individuals experiencing anxiety, rather than a generalisable phenomenon (Copeland et al., 2012; Liu et al., 2021; Renna et al., 2018; Vogelzangs et al., 2013).

Gaps in the Literature

A growing body of research has assessed levels of inflammatory markers among individuals with anxiety and anxiety-based disorders, although findings are often inconsistent. Research has primarily focused on immune dysregulation in PTSD, whereby raised levels of inflammatory markers have been observed. Specifically, increased pro-inflammatory cytokines IL-1β, IL-6, TNF-α (Gill et al., 2009; Hoge et al., 2009; Newton et al., 2014), and CRP (Eraly et al., 2014; Heath et al., 2013; Spitzer et al., 2010) were found in individuals with PTSD. Baseline CRP plasma concentrations were also found to be significant overall predictors of PTSD (Eraly et al., 2014). Contrastingly, other studies have failed to identify significant associations in IL-6 concentrations (Song et al., 2008) and CRP (von Känel et al., 2006) with PTSD.

A large cohort study found increased levels of CRP in males with anxiety, social phobia, and agoraphobia, after adjusting for sociodemographic, lifestyle, and disease factors (Vogelzangs et al., 2013). Interestingly, lower levels of CRP were found among women, indicating potential sex differences (Vogelzangs et al., 2013). Elevated levels of CRP have also been evidenced in individuals with GAD, independent of co-morbid depression (Bankier et al., 2008; Khandaker et al., 2016). A comprehensive, longitudinal study demonstrated associations between CRP and GAD, however the heightened levels of CRP were attributable to body mass index (BMI) and medication use (Copeland et al., 2012). Mixed results have been found regarding associations between cytokines and GAD. Significant increases in the serum levels of IL-6 (O'Donovan et al., 2010; Zou et al., 2020), TNF-α (Hou et al., 2017; Vieira et al., 2010), and IFN-γ (Hou et al., 2017) in individuals with GAD have been found, independent of age, sex, BMI, smoking and alcohol use, and the presence or degree of

depression. However, few studies have found no associations between anxiety disorders and IL-6 and TNF-α (Vogelzangs et al., 2013). Additionally, individuals with anxiety and rheumatoid arthritis (RA) exhibited significantly elevated concentrations of IL-6 and TNF-α compared to RA individuals without anxiety (Liu et al., 2012), indicating that disability is increased when anxiety disorders are present in combination with medical conditions (Sareen et al., 2005).

The associations between inflammation and anxiety symptom severity have been sparsely researched. Although, a few studies have indicated positive correlations between anxiety symptoms and CRP (Liukkonen et al., 2011), TNF-α, IL-6 (Pitsavos et al., 2006), and IFN-γ (Zou et al., 2020). Additionally, while the relationship between PD and inflammation has been studied, little has been reported regarding immune dysregulation and panic attacks. As panic attacks are associated with extreme levels of stress, and experienced not only as part of anxiety disorders, but in other psychological and physiological conditions, understanding its relationship to inflammation is crucial. Nonetheless, PD has been associated with elevated levels of CRP (Vogelzangs et al., 2013), IL-1β (Belem da Silva et al., 2017; Brambilla et al., 1994; Quagliato & Nardi, 2022; Zou et al., 2020), IL-6, and TNF-α (Hoge et al., 2009; Liu et al., 2021).

Inconsistencies in the literature regarding anxiety and panic disorders, as well as gaps in knowledge concerning symptom severity and panic attacks produces difficulties in evaluating the role of specific inflammatory markers in these conditions. Moreover, whether the underlying processes involved with anxiety and inflammation differ across disorders remains unclear. The inconsistencies in findings may be attributable to a failure to control for confounding variables. For example, age, BMI, medication, chronic illness, acute infection, and mental health co-morbidities have the potential to affect inflammation (Michopoulos et al., 2017). A recent meta-analysis examined moderating factors in the relationship between

inflammatory markers and anxiety disorders (Renna et al., 2018). Significant overall differences in pro-inflammatory cytokines were found between individuals with anxiety disorders and healthy controls, with IL-1β, IL-6, and TNF-α driving the effect (Renna et al., 2018). Although, type of diagnosis was revealed to have significant moderating effects, whereby individuals with PTSD showed significant differences to healthy controls (Renna et al., 2018). Overall, determining the role inflammation plays in specific anxiety disorders is pivotal to understanding their pathophysiology. This may lead us to the formulation of alternative, specified approaches to the treatment and management of these conditions.

The Present Study

Review of the extant literature indicates that immune dysregulation and inflammation are increasingly being associated with anxiety disorders and altered behaviour and mood, although findings remain inconsistent. Additionally, individuals with anxiety disorders frequently experience increased disability when there is a comorbid physical condition, leading to decreased quality of life (Sareen et al., 2005). Therefore, further investigations are required to better understand the underlying mechanisms of these disorders, leading to the development of efficacious treatments targeting these associated inflammatory markers.

First, this study aimed to investigate the association between the severity of anxiety symptoms and circulating levels of pro-inflammatory markers (CRP and cytokines IFN-γ, IL-1β, IL-6, IL-8, and TNF-α) over two time points. Based on previous research, we also aimed to control for factors commonly known to influence inflammation (including comorbid depression and autoimmune disorder, BMI, age, and sex assigned at birth) to further discriminate the relationship between anxiety symptom severity and inflammation. As stress is an innate part of anxiety symptom severity, we aimed to examine the associations between individuals perceived levels of stress and inflammatory markers to assist with the investigation of the first aim. Second, we aimed to explore the relation between a history of

panic attacks and inflammation. Given the previous findings, we hypothesised that severity of anxiety symptoms and perceived levels of stress would be positively associated with heightened pro-inflammatory markers. While there is limited research examining panic attacks and inflammation, we also hypothesised that a history of panic attacks would be positively associated with greater levels of pro-inflammatory markers.

Methods

Participants

Individuals aged 12 years and older were recruited via flyers posted around the University of Adelaide. Additional information about the study, and details on how to participate were published on the university website. Individuals who were unable to provide informed consent or, had a history of psychosis or Bipolar Affective Disorder were excluded. All participants received a $20 Coles-Myer honorarium for their participation in the study at baseline visit.

The sample of this pilot study consisted of 38 individuals aged 18 to 76 years. Participants were recruited as cases (n=20) and controls (n=18). Cases included individuals meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for current or past anxiety or anxiety-based disorder, and controls comprised of individuals with no history of: (1) an anxiety or anxiety-based disorder, (2) MDD, (3) Bipolar Affective Disorder, or (4) psychotic disorder.

Measures

The Structured Clinical Interview for DSM-5 Disorders Clinician Version

To assess the presence of anxiety and anxiety-based disorders (GAD, PD, Agoraphobia, SAD, Specific Phobia, OCD, PTSD), the Structured Clinical Interview for DSM-5, Clinician Version (SCID-5-CV) (First et al., 2016) was conducted. Additionally, the measure was used to screen for a history of MDD and psychosis. The SCID-5-CV is a semi-

structured interview for making the DSM-5 diagnoses, and is administered by a clinician, or trained mental health professional. The SCID-5-CV has been used extensively in psychiatric research and has presented excellent reliability (level of positive agreement for diagnoses >75%), high specificity and sensitivity (.70-.80), and clinical validity (Osório et al., 2019; Shabani et al., 2021).

The presence of current or remitted co-morbid MDD was examined using the SCID-5-CV, as raised levels of inflammatory markers have been associated with MDD (Dantzer et al., 2008; Haapakoski et al., 2015; Howren et al., 2009; Kohler et al., 2017). Moreover, panic attack history was assessed using the Panic Disorder subscale of the SCID-5-CV and was characterised by individuals who have experienced one or more panic attacks in their lifetime.

Anxiety Symptom Severity

Anxiety symptom severity was assessed using the Hamilton Anxiety Scale (HAM-A) (Hamilton, 1959; Hamilton, 1969). The HAM-A is a frequently used anxiety symptom rating scale in clinical research (Shear et al., 2001). The scale consists of 14 items: a 7-item "Psychic" subscale and 7-item "Somatic" subscale, whereby items for "Somatic" are further subcategorised into muscular and sensory groups. HAM-A also includes a variable evaluating the individual's behaviour during the interview. All items are rated on a 5-point Likert scale, from 0 (Absent) to 4 (Very Severe), yielding a total severity score calculated by the summation of all items (range from 0-56). The HAM-A has displayed good internal consistency (.86) and reliability (interrater = .74 – .98; test-retest = .86) (Leentjens et al., 2011; Maier et al., 1988; Shear et al., 2001), and moderate to good validity (Clark & Donovan, 1994; Shear et al., 2001; Zimmerman et al., 2017).

Perceived Stress

The Perceived Stress Scale (PSS) is a well-validated (Chiu et al., 2016; Soria-Reyes et al., 2022) and widely used measure of an individual's stress (Cohen et al., 1983). The PSS is a brief, 10-item self-report questionnaire, where participants rated the degree to which they perceived their lives to be uncontrollable, unpredictable, and overwhelming. All items are rated on a 5-point Likert scale, from 0 (never) to 4 (very often). Items 4, 5, 7, and 8 are reverse scored, and the total score is formed by the summation of all items. Higher scores indicate greater stress levels.

Inflammatory Markers

Markers of inflammation from baseline and follow-up visits were assessed after the follow-up data was collected for all individuals, as measuring all samples in the same batch minimises measurement error. Non-fasting blood samples were collected using two 8ml tubes containing gel for serum inflammatory markers (CRP, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α). The collection time of each blood sample was noted. Blood samples were processed and kept frozen at -80 degrees Celsius in the Adelaide Mental Health Biobank (managed by the University of Adelaide, Discipline of Psychiatry). For the measurement of serum inflammatory markers, the relevant samples were thawed and measured using the Millipore Human Cytokine Panel 5-plx (for cytokines IFN-γ, IL-1β, IL-6, IL-8, and TNF-α), and high sensitivity enzyme-linked immunosorbent assay (ELISA) for CRP.

Covariates

Demographic and lifestyle characteristics have been linked to both anxiety and inflammation and are commonly used as covariates in studies examining the association between anxiety and inflammation (Copeland et al., 2012; Kitahara et al., 2014; Shafiee et al., 2017; Vogelzangs et al., 2013). These characteristics included age, sex assigned at birth, BMI, medication use, smoking and alcohol use, and medical conditions. BMI was calculated from

weight and height measurements completed at both assessments (weight in kilograms divided by height in metres squared). Smoking and alcohol use were assessed during face-to-face interviews at both assessments. Additionally, several disease-related covariates were assessed through self-report measures at the initial visit (including rheumatoid arthritis, diabetes, colitis, and systemic lupus erythematosus). Medication use was self-reported at both assessments, and classified according to the Australian Medicines Handbook (Australian Medicines Handbook, 2020). The use of systemic anti-inflammatory medication, and medication with immunoregulatory effects such as statins and omega-3 (Almog, 2003; Kiecolt-Glaser et al., 2011; Yan et al., 2011) were assessed. Antidepressants were included as a covariate, as studies have shown antidepressants to produce anti-inflammatory effects (Kubera et al., 2001; Lenze et al., 2011; Talmon et al., 2018). Antidepressant medications included selective serotonin reuptake inhibitors (SSRI) and serotonin and noradrenaline reuptake inhibitors.

Procedure and Study Design

Data for this pilot study were collected by an ongoing study led by the University of Adelaide, Discipline of Psychiatry. The study began in 2019 to investigate the longitudinal relationship between inflammatory markers and anxiety disorders. Secure, password protected de-identified data was provided to the researcher following ethics approval by University of Adelaide Human Research Ethics Committee (HREC; H-2019-092).

An observational, longitudinal design was employed. All potential participants were provided information sheets. Informed consent for the use of participants' de-identifiable data for the current study – with the option of consenting to the use of the data in future studies – was obtained at the initial visit, prior to the administration of study interviews, questionnaires, and blood collection. An optional consent to release information form was given to participants, and if signed, allowed the research team to contact treating health

practitioners in circumstances such as elevated scores from psychological measures where the treating practitioner may be unaware.

The study involved an initial (baseline) visit and follow-up visit after 6 months. During baseline visit, structured interviews were conducted using all measures and scales to assess severity of anxiety symptoms (HAM-A) and panic attack history (SCID-5-CV). Other psychometric scales used in the overall study were also administered. Participants completed a self-report questionnaire evaluating their perceived level of stress (PSS). Demographic information and additional factors that may confound the relationship between inflammation and anxiety disorders (BMI, smoking and alcohol use, medical conditions, and current medications) were collected. Blood samples were then taken and stored until use for measurement of inflammatory markers.

All interviews, questionnaires, and phlebotomy were administered by trained researcher staff, during visits to the Clinical Research Facility at the University of Adelaide. Additional contact information of mental health care services was provided to all participants. During the follow up visit 6 months later, repeat clinical and biological measures were collected, including information regarding the confounding factors.

Data Analytic Strategy

After appropriate data for the pilot study were cleaned, analyses were conducted using R statistical software (RStudioTeam, 2022). All variables were tested for normality, and logarithmic transformations were applied to all inflammatory markers to normalise distributions. Log-transformed inflammatory markers were used for all analyses. To address the association between anxiety symptom severity and inflammation, Pearson's product-moment correlations were computed for each inflammatory marker and HAM-A and PSS scores. Further examination of the differences in significant inflammatory markers between participants who scored above the median HAM-A score and those who scored below were

examined using t-tests. Additional t-tests were calculated to assess mean group differences in each inflammatory marker between males and females, and individuals with a history of panic attacks and those with no history. Cohen's *d* was used to measure the effect sizes for each t-test. The strength of these relationships was further analysed using linear regressions, controlling for age, sex, BMI, medication use, smoking and alcohol use, presence of autoimmune disorder, and co-morbid depression. As the study was underpowered to add all covariates into the model, a systematic approach to the inclusion of specific covariates was taken. Each model was run with each individual covariate – the covariate that contributed in a statistically significant way to the model was included first. This method continued until there were no other statistically significant contributors. To assess the first aim controlling for the covariates, HAM-A scores were added to the larger model to assess any significant contributions. Repeat procedures were conducted with data from both time points. To contribute to the investigation of the first aim, PSS scores were added into the linear regression models which included only the significant covariates. The second aim assessing the relationship of panic attack history and inflammatory markers were calculated using similar method, whereby panic attack history was added to the model that included only significant covariates. Effect sizes for linear regression models were calculated by Cohen's f^2: R^2 divided by 1 minus R^2 (Selya et al., 2012). A post hoc analysis to compute the achieved power given the sample size was conducted using G*Power 3.1 (Faul et al., 2009). Assuming a small to moderate effect size would be of interest, the study was underpowered (.39) to detect such effect for multiple linear regression with four predictors. Although, a moderate effect size (f^2=.25) satisfied sufficient power of .62 using an alpha of .05.

There were 36 valid measures of IFN-γ data for both time points, and 1 missing measurement for follow up CRP. Antidepressant and anti-inflammatory medication were combined as one variable, "Medication Use", as there were insufficient data when considered

separately (see Table 2 for number of participants using antidepressants or anti-inflammatories).

Results

Descriptive Statistics

Baseline and follow-up characteristics of the total sample (N=38) are shown in Tables 2 and 3. Mean age of the pilot study sample at baseline was 32.2 (SD=14.9) years and 76.3% were women. The sample consisted of 7 backgrounds: Asian, Caucasian, Indian, Italian, Saudi Arabian, South American, and Swedish. On average, participants scored on the upper limit of normal, approaching mild anxiety symptom severity at baseline (mean=12.8, SD=7.5), according to National Institute of Neurological Disorders and Stroke: Common Data Elements (2022) (HAM-A scores between 0 and 13 are classified as normal). Around half of the participants at baseline and follow-up (50% and 52.6%, respectively) had experienced at least one panic attack in their lifetime; 4 individuals recorded experiencing panic attacks within the month of the initial visit, and 11 individuals recorded experiencing panic attacks over a month prior to initial visit.

Correlations and Comparisons

To observe the relationship between inflammatory markers and anxiety symptom severity before controlling for covariates, Pearson's correlations were conducted, and all other assumptions of Pearson's correlations were met. Associations between inflammatory markers and continuous covariates were also examined. The correlations are presented in Table 2. HAM-A scores had significant small, negative correlations with CRP at baseline (r=-.36, p=.03). Significant group differences in CRP levels at baseline were additionally observed between participants who scored above the median HAM-A score (mean CRP=.8 mg/l) and those who scored below (mean CRP=1.9 mg/l) (t(24.3)=-2.10, p=.05).

Table 2

Descriptive Statistics and Correlations[a] *for Continuous Study Variables*

Variable	Time Point	*M(SD)*	*Range*	CRP (mg/l)	IFN-γ (pg/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
Covariates									
Age (years)	1	32.2 (14.9)	18 – 76	.41**	-.06	.01	.06	.11	.15
	2	34.2 (15.0)	18 – 77	.23	-.18	-.09	-.01	.11	.13
BMI, kg/m^2	1	24.7 (4.4)	18 – 36	.48**	-.15	.16	.09	.10	.12
	2	25.1 (5.0)	18 – 39	.52**	-.23	.17	-.04	.11	.08
Alcohol use (standard drinks /week)	1	0.6 (1.8)	0 – 10	-.04	.07	.01	-.07	.04	.11
	2	0.3 (1.3)	0 – 7	.10	-.09	-.13	-.13	-.05	-.04
Primary Anxiety Symptom Severity (HAM-A)	1	12.8 (7.5)	0 – 35	-.36*	-.31	-.27	-.17	-.18	-.23
	2	10.2 (6.3)	0 – 27	.08	-.01	-.05	-.08	-.04	-.06
Perceived Stress (PSS)	1	16.4 (8.2)	2 – 33	-.24	-.27	-.13	-.19	-.29	-.27
	2	14.4 (7.0)	0 – 29	-.02	-.03	-.10	-.16	-.11	-.14

Note. N = 38. HAM-A = Hamilton Anxiety Scale; PSS = Perceived Stress Scale; *M(SD)* = mean (standard deviation); BMI = Body Mass Index; CRP = C-reactive protein; IFN-γ = interferon-γ; TNF-α = tumour necrosis factor-α; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-8 = interleukin-8.

[a] All correlations use Pearson's product-moment correlations between log-transformed inflammatory markers and other variables.

*$p < .05$. **$p < .01$.

HAM-A scores had a small, negative relationship, approaching significance with IFN-γ (r=-.31, p=.07). Correlations between other inflammatory markers and HAM-A scores were non-significant, negative, and negligible. Similarly, PSS scores had negative, negligible correlations with all inflammatory markers, although there was a small correlation with IL-6 at baseline approaching significance (r=-.29, p=.07). Moreover, age and BMI had significant, moderate, positive correlations with CRP at baseline and follow-up (r=.41–.52, p<.01).

Group differences in inflammatory marker levels for panic attack history and sex are presented in Table 4. There were no significant group differences in inflammatory marker concentrations between individuals who have experienced panic attacks and those who have not. Although, small to moderate effect sizes (Cohen's d=-0.22– -0.53) were observed in mostly all inflammatory markers between individuals with a history of panic attacks and those without. Interestingly, individuals who have never experienced a panic attack in their lifetime had greater levels of inflammatory markers compared to individuals who have experienced panic attacks. For example, IFN-γ in individuals with panic attack history=5.2pg/ml; IFN-γ in individuals with no history=9.1pg/ml. Additionally, there were no significant group differences in inflammatory marker concentrations between females and males. However, small to moderate effect sizes (Cohen's d=-0.21– -0.68) were observed in most inflammatory markers between females and males, with baseline IFN-γ indicating large effects (Cohen's d=-0.83). Moreover, males tended to exhibit greater levels of inflammatory markers than females (for example, IFN-γ in males=11.7pg/ml; IFN-γ in females=5.7pg/ml).

Table 3

Descriptive Statistics for Categorical Study Variables[a]

Variables	Time Point	Total Sample	
		N	%
Primary			
History of Panic Attack	1	19	50.0
	2	20	52.6
Covariates			
Sex, female	–	29	76.3
Medication use	1	15	39.5
	2	12	31.6
Antidepressant	1	6	15.8
	2	9	23.7
Anti-inflammatory	1	9	23.7
	2	4	10.5
Smoking status, current	1	0	0
	2	2	5.3
Autoimmune/ inflammatory disease	1	11	29.0
	2	11	29.0
Diabetes Type 2	–	2	5.3
Psoriasis	–	1	2.6
Colitis	–	3	7.9
Rheumatoid Arthritis	–	2	5.3
Systemic Lupus Erythematosus	–	1	2.6
Celiac	–	1	2.6
PCOS	–	1	2.6
Eczema	–	3	7.9
MDD	1	16	42.1
	2	16	42.1

Note. N = 38. HAM-A = Hamilton Anxiety Scale; PSS = Perceived Stress Scale; MDD = major depressive disorder; PCOS = polycystic ovarian syndrome.

[a] Reflects the number and percentage of participants answering “yes” to this question.

Table 4

Descriptive Statistics and Mean Group Comparisons[a] *between Categorical Study Variables*

Outcome variable	Time Point	History of Panic Attack		No History of Panic Attack		*t*	*p*	*d*
		M	*SD*	*M*	*SD*			
CRP (mg/l)	1	1.1	1.6	1.6	1.6	-0.87	.39	-0.28
	2	1.5	1.8	1.4	1.3	0.24	.81	0.08
IFN-γ (pg/ml)	1	5.2	7.0	9.1	16.4	-1.58	.12	-0.53
	2	8.7	7.8	10.9	11.3	-0.47	.65	-0.15
TNF-α (pg/ml)	1	11.4	3.9	18.5	4.8	-1.57	.13	-0.51
	2	13.0	19.3	26.0	47.0	-1.17	.26	-0.40
IL-1β (pg/ml)	1	1.7	1.6	2.7	6.2	-0.92	.37	-0.30
	2	2.9	4.6	5.3	14.7	-0.65	.53	-0.22
IL-6 (pg/ml)	1	31.8	43.0	40.6	42.9	-0.58	.57	-0.19
	2	31.9	50.7	47.0	57.7	-0.91	.37	-0.30
IL-8 (pg/ml)	1	45.7	69.0	50.7	54.1	-0.23	.82	-0.07
	2	39.6	71.6	62.8	82.1	-1.01	.32	-0.34
Outcome variable	Time Point	Female		Male		*t*	*p*	*d*
		M	*SD*	*M*	*SD*			
CRP (mg/l)	1	1.2	1.5	1.9	2.3	-0.84	.42	-0.40
	2	1.5	1.6	1.4	0.9	0.08	.94	-0.02
IFN-γ (pg/ml)	1	5.7	6.4	11.7	9.4	-1.78	.10	-0.83
	2	9.0	14.7	12.0	12.7	-0.60	.56	-0.21
TNF-α (pg/ml)	1	14.2	16.0	17.3	5.3	-0.90	.37	-0.22
	2	20.0	37.5	16.4	4.5	0.50	.62	-0.11
IL-1β (pg/ml)	1	2.0	3.7	2.9	2.6	-0.85	.41	0.27
	2	4.5	12.5	2.5	2.4	0.82	.42	0.18
IL-6 (pg/ml)	1	29.1	41.9	59.0	55.9	-1.48	.17	-0.66
	2	32.5	43.2	60.2	67.3	-1.17	.27	-0.56
IL-8 (pg/ml)	1	38.6	58.4	78.8	94.7	1.20	.26	-0.59
	2	39.6	50.9	85.6	106.2	-1.25	.24	-0.68

Note. N = 38. *M* = mean; *SD* = standard deviation; CRP = C-reactive protein; IFN-γ = interferon-γ; TNF-α = tumour necrosis factor-α; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-8 = interleukin-8.

[a] All analyses use t-tests between log-transformed inflammatory markers and variables.

Analyses of Anxiety Symptom Severity and Inflammatory Markers

HAM-A scores and Inflammatory Markers

After systematically assessing the significant contributions of each covariate, sex ultimately had the greatest significant association with most inflammatory markers (IFN-γ, IL-1β, IL-6, IL-8, and TNF-α at baseline). BMI, age, and medication use were also found to significantly contribute to CRP, IFN-γ at baseline, and TNF-α at follow-up. As shown in Table 5, the first set of regression models present associations between CRP and HAM-A scores at each time point. This includes BMI and age as covariates for baseline, and BMI as a covariate for follow-up. The overall model for baseline CRP was significant ($F(3,34)=7.12$, $p<.001$, $R^2=.33$), and had a large effect size (Cohen's $f^2=.49$). HAM-A scores, BMI and age accounted for 33.2% of the variance in CRP levels. BMI ($t(34)=2.80$, $p=.008$) had significant associations with CRP, with age ($t(34)=1.89$, $p=.068$) approaching significance. However, following the inclusion of BMI and age, associations with HAM-A became non-significant ($t(34)=-1.76$, $p=.088$). Comparatively, CRP at follow-up produced similar results, whereby the overall model was significant ($F(2,34)=6.28$, $p=.004$, $R^2=.23$) and had a large effect size (Cohen's $f^2=.30$). HAM-A scores and BMI accounted for 23.7% of the variance in CRP levels. Although, like baseline, BMI ($t(34)=3.50$, $p=.001$) remained significantly associated with CRP, while associations between CRP and HAM-A ($t(34)=-0.10$, $p=.918$) became non-significant following the inclusion of BMI in the model.

The second set of regression models presented in Table 5 assess associations between IFN-γ and HAM-A scores at each time point. Including sex and medication use as covariates for baseline, and sex as a covariate for follow-up. The overall model for baseline IFN-γ was significant ($F(3,32)=3.54$, $p=.025$, $R^2=.18$), with a moderate effect size (Cohen's $f^2=.22$). HAM-A scores, sex, and medication accounted for 17.9% of the variance in IFN-γ levels.

Table 5

Associations[a] between Inflammatory Markers[b] and Anxiety Symptom Severity at each time point

				95% CIs			
	Covariate	Estimate	*SE*	*LL*	*UL*	*p*	*Adjusted R^2*
CRP T1							
	HAM-A	-0.04	0.03	-0.09	0.01	.088	.33
	BMI	0.12	0.04	0.03	0.21	.008	
	Age	0.02	0.01	-0.01	0.05	.068	
CRP T2							
	HAM-A	-0.01	0.03	-0.06	0.06	.918	.23
	BMI	0.15	0.04	0.06	0.24	.001	
IFN-γ T1							
	HAM-A	-0.02	0.03	-0.08	0.04	.534	.18
	Sex	-1.00	0.47	-1.95	-0.05	.040	
	Medication use	-0.82	0.42	-1.68	0.04	.062	
IFN-γ T2							
	HAM-A	0.01	0.04	-0.08	0.09	.924	-.04
	Sex	-0.45	0.55	-1.56	0.67	.423	
IL-1β T1							
	HAM-A	-0.01	0.02	-0.05	0.03	.535	.05
	Sex	-0.53	0.31	-1.16	0.10	.098	
IL-1β T2							
	HAM-A	-0.01	0.03	-0.07	0.05	.702	-.05
	Sex	-0.15	0.41	-0.99	0.69	.721	
IL-6 T1							
	HAM-A	-0.03	0.04	-0.11	0.06	.496	.06
	Sex	-1.29	0.74	-2.79	0.19	.086	
IL-6 T2							
	HAM-A	0.01	0.05	-0.10	0.10	.940	.03
	Sex	-1.24	0.71	-2.68	0.21	.092	
IL-8 T1							
	HAM-A	-0.03	0.03	-0.08	0.03	.296	.06
	Sex	-0.70	0.46	-1.64	0.24	.137	
IL-8 T2							
	HAM-A	-0.01	0.03	-0.07	0.07	.961	.01
	Sex	-0.68	0.47	-1.64	0.28	.160	
TNF-α T1							
	HAM-A	-0.02	0.01	-0.04	0.01	.209	.09
	Sex	-0.33	0.21	-0.75	0.09	.121	
TNF-α T2							
	HAM-A	-0.01	0.02	-0.04	0.02	.624	.01
	BMI	0.03	0.02	-0.01	0.07	.170	

Note. CRP = C-reactive protein; IFN-γ = interferon-γ; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumour necrosis factor-α; *SE* = standard error; CI = confidence interval; *LL* = lower limit; *UL* = upper limit; HAM-A, Hamilton Anxiety Rating Scale; T1 = baseline visit; T2 = follow up visit.

[a] Based on linear regression analyses.

[b] Inflammatory markers were log-transformed to normalize distributions.

Sex ($t(32)=-2.14$, $p=.040$) had significant associations with IFN-γ, while medication use ($t(32)=-1.93$, $p=.062$) was approaching significance. Similarly to the CRP models, following the inclusion of the covariates, associations between HAM-A and IFN-γ became non-significant ($t(32)=-0.63$, $p=.534$). At follow-up, the overall model of IFN-γ was non-significant ($F(2,33)=0.33$, $p=.721$, $R^2=-.04$), with a small effect size (Cohen's $f^2=-.04$), whereby HAM-A scores and sex accounted for 4% of the variance in IFN-γ levels.

The proceeding regression models shown in Table 5 measures the associations between IL-1β, IL-6, IL-8, and TNF-α and HAM-A scores, respectively. There were no statistically significant overall models, or individual associations within each model, nevertheless, these associations had small effect sizes, ranging from Cohen's f^2 of .002 to .094.

PSS scores and Inflammatory Markers

As the significant associations between each covariate and inflammatory markers had been previously assessed, covariates that had the greatest significant associations were included in the models. As shown in Table 6, the first set of regression models examine associations between CRP and PSS scores at each time point. The overall model for baseline CRP was significant ($F(3,34)=5.84$, $p=.003$, $R^2=.28$), with a large effect size (Cohen's $f^2=.39$). PSS scores, BMI and age accounted for 28.2% of the variance in CRP levels. BMI

(t(34)=2.88, p=.007) had significant associations with CRP, while age (t(34)=1.74, p=.091) and PSS scores (t(34)=-0.71, p=.485) had non-significant associations. Follow-up CRP produced similar results, whereby the overall model was significant (F(2,34)=6.39, p=.004, R^2=.23), and had a large effect size (Cohen's f^2=.30). PSS scores and BMI accounted for 23% of the variance in CRP levels. Similarly to baseline, BMI (t(34)=3.57, p=.001) had significant associations with CRP, while PSS (t(34)=-0.41, p=.684) had non-significant associations.

The second set of regression models presented in Table 6 assess associations between IFN-γ and PSS scores at each time point. The overall model for baseline IFN-γ was significant (F(3,32)=3.38, p=.030, R^2=.17), with a moderate effect size (Cohen's f^2=.21). PSS scores, sex, and medication use accounted for 17% of the variance in IFN-γ levels. Both sex (t(32)=-2.22, p=.034) and medication use (t(32)=-2.07, p=.047) had significant associations with IFN-γ, although PSS (t(32)=-0.20, p=.843) had non-significant associations. At follow-up, overall IFN-γ model was non-significant (F(2,33)=0.33, p=.722, R^2=-.04), with a small effect size (Cohen's f^2=-.04), whereby PSS scores and sex accounted for 4% of the variance in IFN-γ levels.

The fourth set of regression models in Table 6 depict associations between IL-6 and PSS scores, with sex as a covariate for each time point. The overall model for baseline IL-6 was approaching significance (F(2,35)=2.94, p=.066, R^2=.10), with a moderate effect size (Cohen's f^2=.11). PSS scores and sex accounted for 9.5% of the variance in IL-6 levels. However, with the inclusion of sex in the model, associations with both variables became non-significant (PSS: t(35)=-1.33, p=.193; sex: t(35)=-1.56, p=.128). The overall model for follow-up IL-6 was non-significant (F(2,35)=2.22, p=.124, R^2=-.02), with a small effect size (Cohen's f^2=-.02), whereby PSS scores and sex accounted for merely 2.4% of the variance in IL-6 levels. While PSS scores (t(35)=-0.68, p=.498) had non-significant associations with IL-6, sex (t(35)=-1.91, p=.065) had associations approaching significance.

Table 6

Associations[a] between Inflammatory Markers[b] and Perceived Stress at each time point

				95% CIs			
	Covariate	Estimate	*SE*	*LL*	*UL*	*p*	*Adjusted R^2*
CRP T1							
	PSS	-0.02	0.03	-0.07	0.03	.485	.28
	BMI	0.13	0.04	0.04	0.22	.007	
	Age	0.03	0.01	-0.01	0.05	.091	
CRP T2							
	PSS	-0.01	0.03	-0.07	0.04	.684	.23
	BMI	0.15	0.04	0.06	0.23	.001	
IFN-γ T1							
	PSS	-0.01	0.03	-0.06	0.05	.843	.17
	Sex	-1.06	0.48	-2.03	-0.09	.034	
	Medication use	-0.89	0.43	-1.76	-0.02	.047	
IFN-γ T2							
	PSS	-0.01	0.03	-0.07	0.07	.944	-.04
	Sex	-0.43	0.54	-1.54	0.67	.433	
IL-1β T1							
	PSS	-0.01	0.02	-0.05	0.02	.505	.05
	Sex	-0.51	0.31	-1.15	0.13	.112	
IL-1β T2							
	PSS	-0.02	0.03	-0.08	0.03	.344	-.02
	Sex	-0.14	0.40	-0.96	0.68	.731	
IL-6 T1							
	PSS	-0.05	0.04	-0.13	0.03	.193	.10
	Sex	-1.14	0.73	-2.63	0.34	.128	
IL-6 T2							
	PSS	-0.03	0.04	-0.12	0.06	.498	-.02
	Sex	-1.37	0.72	-2.83	0.09	.065	
IL-8 T1							
	PSS	-0.03	0.02	-0.08	0.02	.211	.08
	Sex	-0.65	0.47	-1.59	0.29	.171	
IL-8 T2							
	PSS	-0.02	0.03	-0.08	0.04	.470	.02
	Sex	-0.65	0.46	-1.59	0.29	.170	
TNF-α T1							
	PSS	-0.01	0.01	-0.03	0.02	.777	.05
	Sex	-0.37	0.21	-0.81	0.06	.089	
TNF-α T2							
	PSS	-0.01	0.01	-0.04	0.02	.540	.01
	BMI	0.03	0.02	-0.01	0.07	.186	

Note. CRP = C-reactive protein; IFN-γ = interferon-γ; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumour necrosis factor-α; *SE* = standard error;

CI = confidence interval; *LL* = lower limit; *UL* = upper limit; PSS = perceived stress scale; T1 = baseline visit; T2 = follow up visit.

[a] Based on linear regression analyses.

[b] Inflammatory markers were log-transformed to normalize distributions.

The additional regression models presented in Table 6 examine the associations between IL-1β, IL-8, and TNF-α and PSS scores, respectively. There were no significant overall models, or individual associations within each model, although these inflammatory makers had small effect sizes, ranging from Cohen's f^2 of 005 to .081.

Analyses of Panic Attack History and Inflammatory Markers

The relationship between a history of panic attacks and inflammatory markers, controlling for confounding factors were examined, and results are presented in Table 7. As the significant contributions of each covariate to inflammatory markers had been previously assessed, covariates that had the greatest significant associations were included in the models. The first set of regression models assess associations between CRP and panic attack history at each time point. The overall model for baseline CRP was significant ($F(3,34)=6.06$, $p=.002$, $R^2=.29$), with a large effect size (Cohen's $f^2=.41$). Panic attack history, BMI, and age accounted for 29.1% of the variance in CRP levels. Both BMI ($t(34)=2.95$, $p=.006$) and age ($t(34)=2.10$, $p=.043$) had significant associations with CRP, while panic attack history ($t(34)=-0.97$, $p=.337$) had non-significant associations. Follow-up CRP produced similar results, whereby the overall model was significant ($F(2,34)=6.28$, $p=.005$, $R^2=.23$), with a large effect size (Cohen's $f^2=.29$). Panic attack history and BMI accounted for 22.7% of the variance in CRP levels. BMI ($t(34)=3.54$, $p=.001$) had significant associations with CRP, however panic attack history ($t(34)=0.06$, $p=.954$) had non-significant associations.

The second set of regression models in Table 7 examine associations between IFN-γ and panic attack history at each time point. The overall model for baseline IFN-γ was significant ($F(3,32)=3.83$, $p=.019$, $R^2=.20$), with a large effect size (Cohen's $f^2=.24$). Panic attack history, sex, and medication use accounted for 19.5% of the variance in IFN-γ levels. Both sex ($t(32)=-2.21$, $p=.034$) and medication use ($t(32)=-2.15$, $p=.039$) had significant associations with IFN-γ, although panic attack history ($t(32)=-1.03$, $p=.311$) had non-significant associations. At follow-up, the overall IFN-γ model was non-significant ($F(2,33)=0.74$, $p=.487$, $R^2=-.02$), with a small effect size (Cohen's $f^2=-.02$), whereby panic attack history and sex accounted for 1.5% of the variance in IFN-γ levels.

The fourth set of regression models in Table 7 assess associations between IL-6 and panic attack history at each time point. The overall model for baseline IL-6 was non-significant ($F(2,35)=1.98$, $p=.153$, $R^2=.05$), with a small effect size (Cohen's $f^2=.05$), whereby panic attack history and sex accounted for 5% of the variance in IL-6 levels. Although sex ($t(35)=-1.98$, $p=.055$) had significant associations with IL-6, panic attack history ($t(35)=0.21$, $p=.836$) had non-significant associations. Competitively, the overall model for follow-up IL-6 was non-significant ($F(2,35)=2.05$, $p=.144$, $R^2=.05$), with a small effect size (Cohen's $f^2=-.06$), whereby panic attack history and sex accounted for 5.4% of the variance in IL-6 levels. Yet, similar to baseline, sex ($t(35)=-2.03$, $p=.051$) had significant associations with IL-6, while panic attack history ($t(35)=0.42$, $p=.680$) had non-significant associations.

The subsequent regression models shown in Table 7 measure associations between IL-1β, IL-8, and TNF-α and panic attack history, respectively. There were no significant overall models, and individual associations within each model, nonetheless these inflammatory markers had small effect sizes, ranging from Cohen's f^2 of .021 to .094.

Table 7

Associations[a] between Inflammatory Markers[b] and Panic Attack History[c] at each time point

				95% CIs			
	Covariate	Estimate	*SE*	*LL*	*UL*	*p*	*Adjusted R^2*
CRP T1							
	Panic attack history	-0.36	0.04	-1.11	0.39	.337	.29
	BMI	0.13	0.04	0.04	0.22	.006	
	Age	0.03	0.01	0.01	0.05	.043	
CRP T2							
	Panic attack history	0.02	0.37	-0.73	0.77	.954	.23
	BMI	0.15	0.04	0.06	0.23	.001	
IFN-γ T1							
	Panic attack history	-0.40	0.39	-1.19	0.39	.311	.20
	Sex	-0.99	0.45	-1.90	-0.08	.034	
	Medication use	-0.84	0.39	-1.64	-0.04	.039	
IFN-γ T2							
	Panic attack history	-0.43	0.48	-1.39	0.54	.377	-.02
	Sex	-0.33	0.55	-1.44	0.79	.556	
IL-1β T1							
	Panic attack history	-0.03	0.26	-0.56	0.50	.915	.04
	Sex	-0.57	0.31	-1.19	0.06	.074	
IL-1β T2							
	Panic attack history	-0.19	0.35	-0.91	0.52	.586	-.04
	Sex	-0.13	0.41	-0.97	0.71	.754	
IL-6 T1							
	Panic attack history	0.13	0.62	-1.13	1.39	.836	.05
	Sex	-1.45	0.73	-2.93	0.03	.055	
IL-6 T2							
	Panic attack history	0.26	0.63	-1.01	1.53	.680	.05
	Sex	-1.49	0.73	-2.98	0.01	.051	
IL-8 T1							
	Panic attack history	-0.08	0.39	-0.88	0.72	.846	.03
	Sex	-0.80	0.46	-1.74	0.14	.092	
IL-8 T2							
	Panic attack history	-0.30	0.40	-1.12	0.51	.454	.02
	Sex	-0.61	0.47	-1.57	0.35	.206	
TNF-α T1	Panic attack history	-0.22	0.17	-0.57	0.13	.208	.09
	Sex	-0.34	0.20	-0.76	0.07	.101	
TNF-α T2	Panic attack history	-0.31	0.19	-0.69	0.07	.106	.07
	BMI	0.03	0.02	-0.01	0.07	.153	

Note. CRP = C-reactive protein; IFN-γ = interferon-γ; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumour necrosis factor-α; *SE* = standard error;

CI = confidence interval; *LL* = lower limit; *UL* = upper limit; T1 = baseline visit; T2 = follow up visit.

[a] Based on linear regression analyses.

[b] Inflammatory markers were log-transformed to normalize distributions.

[c] Reflects participants who have experienced at least one panic attack in their lifetime.

Discussion

Previous research suggests that chronic exposure to stressors may alter the HPA axis response, resulting in an increase in the production of inflammatory markers (Furtado & Katzman, 2015; Liu et al., 2021; Michopoulos et al., 2017; Passos et al., 2015; Renna et al., 2018; Won & Kim, 2020). In order to investigate the role of inflammation in anxiety disorder symptomology, specifically symptom severity and panic attacks, concentrations of circulatory inflammatory markers were assessed in 38 individuals. We hypothesised that anxiety symptom severity and panic attack history is positively associated with elevated levels of inflammatory markers. A series of linear regression analyses of five cytokines and CRP were conducted. Although significant associations were initially found between anxiety symptom severity, CRP, and IFN-γ, these relationships were attenuated to non-significance after controlling for confounding factors. Additionally, we found no significant associations between perceived stress, individuals with a history of panic attacks and inflammatory markers. However, the study was powered to only detect large effects. Further research examining these relationships utilising a larger population may be valuable. This chapter will critically discuss findings from this study, with reference to their implications in clinical and research settings.

Anxiety Symptom Severity

Results from linear regression analyses of CRP and five cytokines – TNF-α, IFN-γ, IL-1β, IL-6, and IL-8 – showed no significant associations with anxiety symptom severity or perceived stress after controlling for significant covariates. Although heightened CRP and IFN-γ (at baseline only) had significant correlations with reduced anxiety symptom severity, this was attenuated by BMI, age, and medication use. Additionally, the results for all inflammatory markers indicated a negative relationship between inflammation and anxiety symptom severity and perceived stress, after controlling for covariates. The results from the study do not support the hypothesis that anxiety symptom severity is positively associated with inflammatory marker concentrations. This is in concordance with previous findings by McCanlies et al. (2011) who observed no significant associations of CRP and IL-6 levels with PTSD symptomology. Comparatively to our findings, police officers with high PTSD symptomology had lower mean levels of CRP and IL-6 compared to those with low PTSD symptomology, although these group differences were not significant (McCanlies et al., 2011). It was suggested that differences in observed study populations and small sample size may have resulted in negative associations between PTSD symptomology and inflammatory markers (McCanlies et al., 2011). Likewise, Vogelzangs et al. (2013) and Duivis et al. (2013) examined participants from the Netherlands Study of Depression and Anxiety. While Vogelzangs et al. (2013) found no significant associations between anxiety symptom severity and inflammation, similar negative relationships were observed, with negative beta values reported for symptom severity. Duivis et al. (2013) however, found associations between somatic anxiety symptoms with elevated concentrations of CRP, IL-6, and TNF-α, and cognitive anxiety symptoms with elevated levels of CRP. Yet, similarly to our findings, lifestyle factors, specifically BMI, played an important role in the relationship (Duivis et al., 2013).

In contrast to our findings, several studies have reported clear positive associations between inflammatory markers and severity of anxiety symptomology after adjusting for confounding factors. Liukkonen et al. (2011) observed that males reporting anxiety symptoms had greater CRP levels compared to those who did not report symptoms of anxiety. Our results became non-significant when controlling for factors that affect inflammation, with BMI incurring the strongest effect on the association. Whereas their findings remained unaltered when adjusted for a range of covariates including BMI. Moreover, van Eeden et al. (2021) found positive associations between inflammatory marker concentrations and levels of anxiety, specifically regarding somatic anxiety symptoms and symptoms of agoraphobia. Studies by Fonkoue et al. (2020) and Lindqvist et al. (2017) reported similar associations with increased inflammation and symptom severity of PTSD, which were mostly maintained following the adjustment of confounding factors MDD and BMI, respectively. Specifically, elevated levels of TNF-α, IL-1β, and IL-6 were found in severe PTSD (Fonkoue et al., 2020).

The discrepancies observed between our findings and results of others may be a result of differences in the studied populations, sample sizes, or clinical and biological differences between specific anxiety disorder symptomologies. The negative associations may be explained by less severe symptom presentations, since participants were recruited in community settings. Additionally, a lack of association may occur if inflammation is related to specific symptoms of anxiety disorders. Thus, the role of inflammation in anxiety symptoms may be better gleaned by examining the individual symptoms of anxiety disorders, as an alternative to total number of symptoms or anxiety in general. Further research examining the role of the immune system in a range of anxiety disorders and specific symptomology may aid in disentangling these relationships.

Panic Attack History

The linear regression analyses of CRP and the five cytokines reported no significant associations with panic attack history. However, this finding should be interpreted with caution as it is limited by the categorisation of history of panic attacks. To our knowledge, this is the first study to examine the relationship between panic attacks and several pro-inflammatory markers. As such, results can only be compared to those of PD studies. Although comparisons made should also be cautiously inferred, as these studies have recruited from clinically diagnosed patients with PD. Therefore, it is likely that they will have greater symptom severity. Nonetheless, several studies investigating PD have found significant associations with inflammatory markers. Similarly to the negative associations found in this study, Tükel et al. (2012) reported that mean concentrations of IFN-γ were significantly lower in patients with PD compared to healthy controls. Additionally, they found no significant associations in IL-1β, IL-6, and TNF-α levels between patients with PD and healthy controls. Though, the authors acknowledge that their study was underpowered, which may indicate issues regarding the interpretation of their findings. Low statistical power can lead to increased risks that a statistically significant finding is a Type I error – the rejection of a true null hypothesis (Christley, 2010). Moreover, the lack of associations may have resulted from the absence of sufficient power to detect such differences.

Conversely, findings from a meta-analysis of fourteen studies indicated that only CRP, IL-6, and TNF-α were significantly elevated in PD patients compared to healthy controls (Liu et al., 2021). The meta-analysis reported moderate effect sizes between CRP and PD (Liu et al., 2021), unlike the large effects found in our study, however, small effect sizes were reported between TNF-α and PD, similarly to our study's findings. Although, no significant differences in IL-1β and IFN-γ were identified.

Comparably to results in this study, Belem da Silva et al. (2017) found strong associations between inflammation and BMI. Although the relationship found was between IL-6 and BMI, unlike CRP in this study, this finding remains an important avenue for future research to examine further. Assessing the effects of BMI on panic attack prevalence and recency would be pivotal regarding therapeutic interventions.

Overall, as this is one of the first studies to evaluate the association between panic attacks and inflammatory markers, this study and its findings highlight the lack of understanding and research regarding the role of the immune system in panic attacks, indicating a need for future research in this area. It is important that future studies consider panic attacks in not only anxiety disorders, but several other psychological and physiological conditions, as it may assist in understanding whether inflammation plays a significant role in its aetiology.

Confounders

Correspondingly to previous research (Copeland et al., 2012; Duivis et al., 2013; Farris et al., 2016; Shafiee et al., 2017; Vogelzangs et al., 2013), this study signifies that demographic and lifestyle factors are significant contributors to the relationship between anxiety and inflammation. Particularly, as found in this study and previous research, BMI explained a considerable amount (or all) of the relationship between CRP and anxiety symptom severity (Copeland et al., 2012; Duivis et al., 2013; Farris et al., 2016). A higher BMI has been linked to increased inflammation (Khaodhiar et al., 2004; Kitahara et al., 2014; O'Connor et al., 2009). Research suggests that adipose tissue acts as a source to produce pro-inflammatory cytokines IL-6 and TNF-α, and with increases in the severity of obesity, CRP production is elevated in response to the stimulation of IL-6 (Khaodhiar et al., 2004). This is further evidenced by the significant associations between CRP and BMI in this study. Moreover, Hartmann et al. (2021) found that positive lifestyle changes, such as exercise, can

have significant effects on reducing anxiety symptom severity. As aerobic capacity had negative correlations with adiposity and anxiety, it can be suggested that enhancing exercise to reduce adipose tissue in the body can decrease the severity of anxiety symptoms. The specific causal mechanisms underlying inflammatory responses in anxiety symptom severity are yet to be determined, although exploring therapeutic interventions that target BMI would be an important direction for future research.

This study found considerable differences in concentrations of inflammatory markers between females and males. Although the differences were not significant, sex as a confounding factor had the greatest level of association with IFN-γ, IL-1β, IL-6, IL-8, and TNF-α. Specifically, males had greater inflammatory marker concentrations in this study. These results can be further evidenced by studies that have found significant sex differences in the relationship between inflammation and anxiety, whereby males had significant positive associations between inflammation and anxiety symptom severity (Duivis et al., 2013; Liukkonen et al., 2011; Shafiee et al., 2017; Vogelzangs et al., 2013). Sex differences in inflammation and anxiety symptom severity may be attributable to hormonal changes. Pitsavos et al. (2006) found significant associations between both sexes, although had a sample with individuals of a greater age (mean age 45 years, 18-89 years), compared to Liukkonen et al. (2011) (all 31 years old), and our study (mean age 34 years, 18-76 years). It is likely that sex differences become less distinguishable with increasing age, resulting from hormonal changes across the lifespan of women, which influence inflammation levels (Cushman et al., 1999). Understanding specific mechanisms which differentiate males and females regarding inflammation and anxiety symptom severity is crucial to uncovering the immune systems role in the aetiology of anxiety.

Study Limitations and Strengths

A number of limitations should be considered when interpreting findings from this pilot study. The main limitation was the small sample size, resulting in an underpowered study. Low statistical power can lead to increased risks of Type II errors – failure to reject a false null hypothesis – minimising the likelihood of observing significant associations in the data, where associations exist (Christley, 2010). This is problematic as this study was underpowered to detect significant, small to moderate effect sizes, where previous literature on inflammation in adults with depression (Howren et al., 2009) and anxiety-based disorders (Renna et al., 2018) demonstrated effects of small to moderate sizes. Therefore, the results from this study cannot conclusively dismiss any potential associations between inflammation and anxiety symptom severity or panic attacks.

In addition, the study was underpowered to adequately adjust for potential confounders. A maximum of three variables were included into each linear regression analysis, despite obtaining measurements of eight different confounding factors (BMI, age, sex, medication, presence of autoimmune disorder, comorbid MDD, smoking and alcohol use) that have been associated with increased systemic inflammation (Baumeister et al., 2016; Carroll et al., 2009; Haapakoski et al., 2015; Kiecolt-Glaser et al., 2011; Kitahara et al., 2014; Kubera et al., 2001). Recommendations from other research and reviews have indicated a need to assess all confounders, therefore, recruiting samples of a sufficient size to control for the effects of the aforementioned variables may increase study quality (Parsons et al., 2021).

Moreover, the limited sample size restricted the ability to differentiate symptom severity among specific anxiety disorders. Therefore, the study was not able to discern potential specific effects of inflammation on the symptom severity of GAD, PD, SAD, or agoraphobia. Future studies would benefit from an adequate sample size in order to determine

whether associations between inflammation and anxiety disorder are universal or specific to a condition or symptom.

Furthermore, while the overall study was longitudinal in design, the pilot sample size was underpowered to examine the longitudinal relationship of anxiety symptom severity and inflammation. Instead, the second time point was utilised to further evidence the relationships occurring at baseline. Therefore, no inferences can be made on the direction of the relationship of anxiety symptom severity with inflammation.

Finally, participants were categorised into two groups when examining the relationship between panic attacks and inflammatory markers: one including individuals who have experiences at least one panic attack in their lifetime, and the other including those who have never. This classification may have led to a considerable amount of variability in the "yes" group, as individuals experiencing severe and/or often panic attacks were grouped with individuals who may have experienced few attacks in the past. Therefore, the exact relationship between panic attacks was unable to be determined.

Despite these limitations, the current study also contains numerous strengths. First, it represents the first study to examine the relationship between panic attacks and several inflammatory markers. Hence, significant relationships were identified between inflammatory markers and covariates including BMI and sex in relation to panic attacks. These results highlight important avenues of study that future research can build upon.

Second, several widely utilised pro-inflammatory markers and their associations with anxiety symptom severity and panic attacks were examined. Additionally, all data collection (blood sampling and psychometric measures) were performed in a clinical setting by trained staff members and blood samples were processed at a laboratory using standardised methods.

Finally, the study sample was recruited from the general population and included individuals from seven different backgrounds. While statistically significant associations

between anxiety symptom severity, stress, or panic attacks and inflammation were not found, this study has the potential to be generalisable to other samples, or the broader population of individuals with anxiety and anxiety-based disorders. A recent review indicated limitations regarding the generalisability of extant literature, as the vast majority of studies recruited participants from hospital settings (Parsons et al., 2021). Therefore, it is also unlikely that the study has biases regarding severity and acuity of clinical presentations (Carr et al., 2002).

Clinical Implications and Future Research

Based on the results of this pilot study, and the limitations to their interpretation, the association between inflammation and anxiety symptom severity or panic attacks cannot be concluded. However, there remains important, potential clinical implications in psychoneuroimmunology research, primarily regarding therapeutic interventions. While the findings of this study were statistically underpowered, clinically significant relationships were observed between CRP and BMI, in relation to both symptom severity and panic attack history. As aforementioned, BMI is a notable confounding factor to the relationship between anxiety and anxiety-based disorders and inflammation (Copeland et al., 2012; Duivis et al., 2013; Farris et al., 2016). The strong association with BMI and inflammation in the symptom severity of anxiety and anxiety-based disorders highlights a potential novel avenue for therapeutic research. Future research targeting BMI reducing strategies in conjunction with psychotherapies may aid in improving treatment outcomes.

Moreover, anxiety and anxiety-based disorder have been linked to inflammatory conditions including metabolic syndrome (Carroll et al., 2009), atherosclerosis (Seldenrijk et al., 2010), and coronary heart disease (Roest et al., 2010). Further associations with elevated levels of CRP (Di Angelantonio et al., 2010) and IL-6 (Sarwar et al., 2012) and the development of cardiovascular disease in individuals with anxiety-based disorders have been

established. Therefore, early treatment may reduce the risks of developing these co-morbidities and improve overall health and wellbeing of these individuals.

Furthermore, our study observed significant relationships between medication use, sex, and IFN-γ, with implications to both panic attack history and anxiety symptom severity. Antidepressants and antipsychotics in conjunction with psychotherapies have demonstrated anti-inflammatory effects and better treatment outcomes in anxiety disorders (Koh & Lee, 2004; Lenze et al., 2011). Similarly, anti-inflammatory drugs in combination with antidepressants or antipsychotics have demonstrated favourable effects on symptom reduction in patients with schizophrenia (Cho et al., 2019) and MDD (Faridhosseini et al., 2014). Thus, if inflammation truly plays an integral part in the aetiology or severity of anxiety and anxiety-based disorders, similar treatment options may prove beneficial.

Taking into consideration the implications and limitations found in this study, we highlight the need for further research in this field. First, studies should aim to recruit large sample sizes that are adequately powered to detect small to moderate effects. The Australian Department of Health provide suggestions for the recruitment of clinical participants, including: raising awareness amid health care professionals of clinical research prior to the commencement of the study, devoting research staff to recruitment procedures, and highlighting the benefits of research regarding therapeutic treatments to potential participants (Ernst & Young, 2016).

Second, understanding the associations between panic attacks and inflammation may have important novel implications to therapeutic research. Future studies investigating panic attacks would be recommended to examine the relationship regarding recency of attack or prevalence. It is crucial that research examining panic attacks encompass several mental health conditions without limiting investigations to PD specifically. This will aid to mitigate

the exclusion of many individuals who do not satisfy the high severity and prevalence associated with PD diagnosis.

Third, the continuation of comparative examinations between healthy controls and individuals with anxiety would aid in establishing clear associations between inflammation and anxiety symptom severity. Additional within group differences is recommended to discern the specific relationships between inflammatory markers and normal, low, medium, and high levels anxiety symptom severity.

Finally, as aforementioned, van Eeden et al. (2021) and Duivis et al. (2013) found that inflammatory markers were significantly associated with somatic symptoms of anxiety and agoraphobia symptoms. Somatic symptoms of anxiety include hot flushes, heart palpitations, shaking hands, and difficulty breathing. These findings indicate potentially pivotal links between inflammation and anxiety. Future studies would be recommended to examine the relationship between inflammatory markers and specific symptoms. Understanding if inflammation plays a role in the specific symptomology of anxiety disorders could have implications for therapeutic developments.

Conclusions

This pilot study provides a steppingstone towards the continued investigation of the role inflammation plays in anxiety and anxiety-based disorders. Although no significant associations were observed in relation to any inflammatory marker, the limited sample size and associated consequences for power render these findings provisional. Research in psychoneuroimmunology has the potential for significant clinical implications regarding therapeutic interventions. This study demonstrates the need for further research encompassing sufficiently powered samples and adequate inclusion of confounding factors within a broad range of anxiety and anxiety-based disorders. Implementing the recommendations provided in this study may aid in obtaining a greater understanding of the

potential associations between the symptomology of anxiety and anxiety-based disorders and inflammation.

References

Ader, R., Cohen, N., & Felten, D. (1995). Psychoneuroimmunology: interactions between the nervous system and the immune system. *The Lancet (British edition)*, *345*(8942), 99-103. https://doi.org/10.1016/S0140-6736(95)90066-7

Almog, Y. (2003). Statins, inflammation, and sepsis: hypothesis. *Chest*, *124*(2), 740-743.

American Psychiatric Association (APA). (2022). *Diagnostic and statistical manual of mental disorders: DSM-5-TR* (5th edition, text revision. ed.). American Psychiatric Association Publishing. https://doi.org/https://doi.org/10.1176appi.books.9780890425787

Au, B., Smith, K. J., Gariépy, G., & Schmitz, N. (2015). The longitudinal associations between C-reactive protein and depressive symptoms: evidence from the English Longitudinal Study of Ageing (ELSA). *International journal of geriatric psychiatry*, *30*(9), 976-984. https://doi.org/10.1002/gps.4250

Australian Medicines Handbook. (2020). *Australian medicines handbook*. Australian Medicines Handbook Pty Ltd. https://amhonline.amh.net.au/

Ballantyne, C. M., & Nambi, V. (2005). Markers of inflammation and their clinical significance. *Atherosclerosis Supplements*, *6*(2), 21-29. https://doi.org/10.1016/j.atherosclerosissup.2005.02.005

Bankier, B., Barajas, J., Martinez-Rumayor, A., & Januzzi, J. L. (2008). Association between C-reactive protein and generalized anxiety disorder in stable coronary heart disease patients. *European heart journal*, *29*(18), 2212-2217. https://doi.org/10.1093/eurheartj/ehn326

Baumeister, D., Ciufolini, S., & Mondelli, V. (2016). Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric

treatment? *Psychopharmacology*, *233*(9), 1575-1589. https://doi.org/10.1007/s00213-015-4044-5

Baxter, A. J., Scott, K. M., Vos, T., & Whiteford, H. A. (2013). Global prevalence of anxiety disorders: a systematic review and meta-regression. *Psychological medicine*, *43*(5), 897-910. https://doi.org/10.1017/S003329171200147X

Baxter, A. J., Vos, T., Scott, K. M., Ferrari, A. J., & Whiteford, H. A. (2014). The global burden of anxiety disorders in 2010. *Psychological medicine*, *44*(11), 2363-2374. https://doi.org/10.1017/S0033291713003243

Belem da Silva, C. T., Costa, M. d. A., Bortoluzzi, A., Pfaffenseller, B., Vedana, F., Kapczinski, F., & Manfro, G. G. (2017). Cytokine Levels in Panic Disorder: Evidence for a Dose-Response Relationship. *Psychosomatic medicine*, *79*(2), 126-132. https://doi.org/10.1097/PSY.0000000000000384

Brambilla, F., Bellodi, L., Perna, G., Bertani, A., Panerai, A., & Sacerdote, P. (1994). Plasma interleukin-1 beta concentrations in panic disorder. *Psychiatry research*, *54*(2), 135-142. https://doi.org/10.1016/0165-1781(94)90002-7

Buist-Bouwman, M. A., De Graaf, R., Vollebergh, W. A. M., Alonso, J., Bruffaerts, R., & Ormel, J. (2006). Functional disability of mental disorders and comparison with physical disorders: a study among the general population of six European countries. *Acta psychiatrica Scandinavica*, *113*(6), 492-500. https://doi.org/10.1111/j.1600-0447.2005.00684.x

Camacho, A. (2013). Is anxious-depression an inflammatory state? *Medical hypotheses*, *81*(4), 577-581. https://doi.org/10.1016/j.mehy.2013.07.006

Capuron, L., & Miller, A. H. (2011). Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & Therapeutics*, *130*(2), 226-238. https://doi.org/10.1016/j.pharmthera.2011.01.014

Carr, V. J., Lewin, T. J., Barnard, R. E., Walton, J. M., Allen, J. L., Constable, P. M., & Chapman, J. L. (2002). Comparisons between schizophrenia patients recruited from Australian general practices and public mental health services. *Acta psychiatrica Scandinavica*, *105*(5), 346-355. https://doi.org/10.1034/j.1600-0447.2002.1o156.x

Carroll, D., Phillips, A. C., Thomas, G. N., Gale, C. R., Deary, I., & Batty, G. D. (2009). Generalized Anxiety Disorder Is Associated with Metabolic Syndrome in the Vietnam Experience Study. *Biological psychiatry (1969)*, *66*(1), 91-93. https://doi.org/10.1016/j.biopsych.2009.02.020

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204-7218. https://doi.org/10.18632/oncotarget.23208

Chiu, Y.-H., Lu, F. J.-H., Lin, J.-H., Nien, C.-L., Hsu, Y.-W., & Liu, H.-Y. (2016). Psychometric properties of the Perceived Stress Scale (PSS): measurement invariance between athletes and non-athletes and construct validity. *PeerJ 4*, e2790-e2790. https://doi.org/10.7717/peerj.2790

Cho, M., Lee, T. Y., Kwak, Y. B., Yoon, Y. B., Kim, M., & Kwon, J. S. (2019). Adjunctive use of anti-inflammatory drugs for schizophrenia: A meta-analytic investigation of randomized controlled trials. *Australian & New Zealand Journal of Psychiatry*, *53*(8), 742-759. https://doi.org/10.1177/0004867419835028

Christley, R. M. (2010). Power and error: increased risk of false positive results in underpowered studies. *The Open Epidemiology Journal*, *3*, 16-19. https://doi.org/10.2174/1874297101003010016

Clark, D. B., & Donovan, J. E. (1994). Reliability and validity of the Hamilton anxiety rating scale in an adolescent sample. *Journal of the American Academy of Child &*

Adolescent Psychiatry, *33*(3), 354-360. https://doi.org/https://doi.org/10.1097/00004583-199403000-00009

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of health and social behavior*, *24*(4), 385-396. https://doi.org/10.2307/2136404

Copeland, W. E., Shanahan, L., Worthman, C., Angold, A., & Costello, E. J. (2012). Generalized anxiety and C-reactive protein levels: a prospective, longitudinal analysis. *Psychological medicine*, *42*(12), 2641-2650. https://doi.org/10.1017/S0033291712000554

Craske, M. G., Stein, M. B., Eley, T. C., Milad, M. R., Holmes, A., Rapee, R. M., & Wittchen, H.-U. (2017). Anxiety disorders. *Nature Reviews Disease Primers*, *3*(1), 17024-17024. https://doi.org/10.1038/nrdp.2017.24

Cushman, M., Legault, C., Barrett-Connor, E., Stefanick, M. L., Kessler, C., Judd, H. L., Sakkinen, P. A., & Tracy, R. P. (1999). Effect of postmenopausal hormones on inflammation-sensitive proteins: The Postmenopausal Estrogen/Progestin Interventions (PEPI) study. *Circulation*, *100*(7), 717-722. https://doi.org/10.1161/01.CIR.100.7.717

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*, *9*(1), 46-56. https://doi.org/10.1038/nrn2297

Decker, D., Schöndorf, M., Bidlingmaier, F., Hirner, A., & von Ruecker, A. A. (1996). Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma *Surgery*, *119*(3), 316-325.

Di Angelantonio, E., Thompson, S. G., Collins, R., Ford, C. E., Pressel, S. L., Jungner, I., Folsom, A. R., Ballantyne, C. M., Knuiman, M. W., Tanne, D., Morris, R. W., Willeit, J., Schett, G., Wald, N., Gallacher, J., Casiglia, E., Bachman, D. L., Cushman, M., Psaty, B. M., . . . Alexander, M. (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *The Lancet (British edition)*, *375*(9709), 132-140. https://doi.org/10.1016/S0140-6736(09)61717-7

Duivis, H. E., Vogelzangs, N., Kupper, N., de Jonge, P., & Penninx, B. W. J. H. (2013). Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: Findings from the Netherlands Study of Depression and Anxiety (NESDA). *Psychoneuroendocrinology*, *38*(9), 1573-1585. https://doi.org/10.1016/j.psyneuen.2013.01.002

Eraly, S. A., Nievergelt, C. M., Maihofer, A. X., Barkauskas, D. A., Biswas, N., Agorastos, A., O'Connor, D. T., & Baker, D. G. (2014). Assessment of Plasma C-Reactive Protein as a Biomarker of Posttraumatic Stress Disorder Risk. *JAMA Psychiatry 71*(4), 423-431. https://doi.org/10.1001/jamapsychiatry.2013.4374

Ernst & Young. (2016). *Scoping and analysis of issues in recruitment and retention in Australian clinical trials*. Retrieved from https://www1.health.gov.au/internet/main/publishing.nsf/Content/EE207D978A44E4B8CA257FA90081B212/$File/EY%20Final%20Report%20-%20Recruitment%20and%20retention%20in%20Australian%20clinical%20trials%2030%20June%202016.pdf

Faravelli, C., Sauro, C. L., Lelli, L., Pietrini, F., Lazzeretti, L., Godini, L., Benni, L., Fioravanti, G., Talamba, G. A., Castellini, G., & Ricca, V. (2012). The Role of Life Events and HPA Axis in Anxiety Disorders: A Review *Current Pharmaceutical Design*, *18*, 5663–5674.

Faridhosseini, F., Sadeghi, R., Farid, L., & Pourgholami, M. (2014). Celecoxib: a new augmentation strategy for depressive mood episodes. A systematic review and metaanalysis of randomized placebo-controlled trials. *Human Psychopharmacology: Clinical and experimental immunology*, *29*(3), 216-223. https://doi.org/10.1002/hup.2401

Farris, S. G., Paulus, D. J., Gonzalez, A., Mahaffey, B. L., Bromet, E. J., Luft, B. J., Kotov, R., & Zvolensky, M. J. (2016). Posttraumatic Stress Symptoms and Body Mass Index among World Trade Center Disaster-Exposed Smokers: A Preliminary Examination of the Role of Anxiety Sensitivity. *Psychiatry research*, *241*, 135-140. https://doi.org/10.1016/j.psychres.2016.04.074

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods*, *41*, 1149-1160. https://doi.org/10.3758/BRM.41.4.1149

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., & Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clinical and experimental immunology*, *147*(2), 227-235. https://doi.org/10.1111/j.1365-2249.2006.03261.x

First, M. B., Williams, J. B. W., Karg, R. S., & Spitzer, R. L. (2016). *Structured Clinical Interview for DSM-5* (Vol. Clinician Version (SCID-5-CV)). American Psychiatric Association Publishing.

Fonkoue, I. T., Marvar, P. J., Norrholm, S., Li, Y., Kankam, M. L., Jones, T. N., Vemulapalli, M., Rothbaum, B., Bremner, J. D., Le, N.-A., & Park, J. (2020). Symptom severity impacts sympathetic dysregulation and inflammation in post-traumatic stress disorder (PTSD). *Brain, behavior, and immunity*, *83*, 260-269. https://doi.org/10.1016/j.bbi.2019.10.021

Furtado, M., & Katzman, M. A. (2015). Neuroinflammatory pathways in anxiety, posttraumatic stress, and obsessive compulsive disorders. *Psychiatry research*, *229*(1), 37-48. https://doi.org/10.1016/j.psychres.2015.05.036

Gill, J. M., Saligan, L., Woods, S., & Page, G. (2009). PTSD is Associated With an Excess of Inflammatory Immune Activities. *Perspectives in psychiatric care*, *45*(4), 262-277. https://doi.org/10.1111/j.1744-6163.2009.00229.x

Glik, A., & Douvdevani, A. (2006). T lymphocytes: the "cellular" arm of acquired immunity in the peritoneum. *Peritoneal Dialysis International*, *26*(4), 438-448.

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., Gannon, B., Jones, D. H., Jennum, P., Jordanova, A., Jönsson, L., Karampampa, K., Knapp, M., Kobelt, G., Kurth, T., . . . Olesen, J. (2011). Cost of disorders of the brain in Europe 2010. *European neuropsychopharmacology*, *21*(10), 718-779. https://doi.org/10.1016/j.euroneuro.2011.08.008

Haapakoski, R., Mathieu, J., Ebmeier, K. P., Alenius, H., & Kivimaki, M. (2015). Cumulative meta-analysis of interleukins 6 and 1beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. *Brain, behavior, and immunity*, *49*, 206-215. https://doi.org/10.1016/j.bbi.2015.06.001

Hamer, M., Gibson, E. L., Vuononvirta, R., Williams, E., & Steptoe, A. (2006). Inflammatory and hemostatic responses to repeated mental stress: individual stability and habituation over time. *Brain, behavior, and immunity*, *20*(5), 456-459. https://doi.org/10.1016/j.bbi.2006.01.001

Hamilton, M. (1959). The assessment of anxiety states by rating. *The British Journal of Medical Psychology*, *32*(1), 50-55. https://doi.org/https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

Hamilton, M. (1969). Diagnosis and rating of anxiety. *The British Journal of Psychiatry*, *3*, 76–79.

Hartmann, T. E., Robertson, C. V., Miller, T. D., Hunter, J. R., & Skein, M. (2021). Associations between exercise, inflammation and symptom severity in those with mental health disorders. *Cytokine*, *146*, 155648-155648. https://doi.org/10.1016/j.cyto.2021.155648

Hayes, S. C., Wilson, K. G., Gifford, E. V., Follette, V. M., & Strosahl, K. (1996). Experiential Avoidance and Behavioral Disorders: A Functional Dimensional Approach to Diagnosis and Treatment. *Journal of consulting and clinical psychology*, *64*(6), 1152-1168. https://doi.org/10.1037/0022-006X.64.6.1152

Heath, N. M., Chesney, S. A., Gerhart, J. I., Goldsmith, R. E., Luborsky, J. L., Stevens, N. R., & Hobfoll, S. E. (2013). Interpersonal violence, PTSD, and inflammation: Potential psychogenic pathways to higher C-reactive protein levels. *Cytokine*, *63*(2), 172-178. https://doi.org/10.1016/j.cyto.2013.04.030

Hettema, J. M., Prescott, C. A., Myers, J. M., Neale, M. C., & Kendler, K. S. (2005). The Structure of Genetic and Environmental Risk Factors for Anxiety Disorders in Men and Women. *Archives of general psychiatry*, *62*(2), 182-189. https://doi.org/10.1001/archpsyc.62.2.182

Hoge, E. A., Brandstetter, K., Moshier, S., Pollack, M. H., Wong, K. K., & Simon, N. M. (2009). Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. *Depression and anxiety*, *26*(5), 447-455. https://doi.org/10.1002/da.20564

Hou, R., & Baldwin, D. S. (2012). A neuroimmunological perspective on anxiety disorders. *Human psychopharmacology*, *27*(1), 6-14. https://doi.org/10.1002/hup.1259

Hou, R., Garner, M., Holmes, C., Osmond, C., Teeling, J., Lau, L., & Baldwin, D. S. (2017). Peripheral inflammatory cytokines and immune balance in Generalised Anxiety Disorder: case-controlled study. *Brain, behavior, and immunity*, *62*, 212-218. https://doi.org/10.1016/j.bbi.2017.01.021

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic medicine*, *71*(2), 171-186. https://doi.org/10.1097/PSY.0b013e3181907c1b

Kelley, K. W., Bluthé, R.-M., Dantzer, R., Zhou, J.-H., Shen, W.-H., Johnson, R. W., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, behavior, and immunity*, *17*(1), 112-118. https://doi.org/10.1016/S0889-1591(02)00077-6

Kendler, K. S., & Baker, J. H. (2007). Genetic influences on measures of the environment: a systematic review. *Psychological medicine*, *37*(5), 615-626. https://doi.org/10.1017/S0033291706009524

Kessler, R. C., Chiu, W. T., Demler, O., & Walters, E. E. (2005). Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of general psychiatry*, *62*(6), 617-627. https://doi.org/10.1001/archpsyc.62.6.617

Kessler, R. C., Ruscio, A. M., Shear, K., & Wittchen, H. U. (2009). Epidemiology of Anxiety Disorders. *Behavioral Neurobiology of Anxiety and Its Treatment*, 21-35. https://doi.org/10.1007/7854_2009_9 (Current Topics in Behavioral Neurosciences)

Khandaker, G. M., Zammit, S., Lewis, G., & Jones, P. B. (2016). Association between serum C-reactive protein and DSM-IV generalized anxiety disorder in adolescence: Findings from the ALSPAC cohort. *Neurobiology of stress*, *4*(C), 55-61. https://doi.org/10.1016/j.ynstr.2016.02.003

Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation and Allergy Drug Discoveries*, *3*(1), 73-80. https://doi.org/10.2174/187221309787158371

Khaodhiar, L., Ling, P.-R., Blackburn, G. L., & Bistrian, B. R. (2004). Serum Levels of Interleukin-6 and C-Reactive Protein Correlate With Body Mass Index Across the Broad Range of Obesity. *JPEN. Journal of parenteral and enteral nutrition*, *28*(6), 410-415. https://doi.org/10.1177/0148607104028006410

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., & Glaser, R. (2011). Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial. *Brain, behavior, and immunity*, *25*(8), 1725-1734. https://doi.org/10.1016/j.bbi.2011.07.229

Kitahara, C. M., Trabert, B., Katki, H. A., Chaturvedi, A. K., Kemp, T. J., Pinto, L. A., Moore, S. C., Purdue, M. P., Wentzensen, N., Hildesheim, A., & Shiels, M. S. (2014). Body mass index, physical activity, and serum markers of inflammation, immunity, and insulin resistance. *Cancer Epidemiology, Biomarkers & Prevention*, *23*(12), 2840-2849. https://doi.org/10.1158/1055-9965.EPI-14-0699-T

Koh, K. B., & Lee, Y. (2004). Reduced Anxiety Level by Therapeutic Interventions and Cell-Mediated Immunity in Panic Disorder Patients. *Psychotherapy and psychosomatics*, *73*(5), 286-292. https://doi.org/10.1159/000078845

Kohler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C. L., Miller, B. J., Lanctot, K. L., & Carvalho, A. F. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta psychiatrica Scandinavica*, *135*(5), 373-387. https://doi.org/10.1111/acps.12698

Kronfol, Z., & Remick, D. G. (2000). Cytokines and the Brain: Implications for Clinical Psychiatry. *The American journal of psychiatry*, *157*(5), 683-694. https://doi.org/10.1176/appi.ajp.157.5.683

Kubera, M., Lin, A.-H., Kenis, G., Bosmans, E., Van Bockstaele, D., & Maes, M. (2001). Anti-inflammatory effects of antidepressants through suppression of the interferon-γ/interleukin-10 production ratio. *Journal of clinical psychopharmacology*, *21*(2), 199-206. https://doi.org/10.1097/00004714-200104000-00012

Lack, C. W. (2012). Obsessive-compulsive disorder: Evidence-based treatments and future directions for research. *World journal of psychiatry*, *2*(6), 86-90. https://doi.org/10.5498/wjp.v2.i6.86

Leentjens, A. F. G., Dujardin, K., Marsh, L., Richard, I. H., Starkstein, S. E., & Martinez-Martin, P. (2011). Anxiety rating scales in Parkinson's disease: A validation study of the Hamilton anxiety rating scale, the Beck anxiety inventory, and the hospital anxiety and depression scale: Validation of anxiety rating scales in PD. *Movement Disorders*, *26*(3), 407-415. https://doi.org/https://doi.org/10.1002/mds.23184

Lenze, E. J. M. D., Mantella, R. C. P. D., Shi, P. M. S., Goate, A. M. P. D., Nowotny, P. P. D., Butters, M. A. P. D., Andreescu, C. M. D., Thompson, P. A. P. D., & Rollman, B. L. M. D. M. P. H. (2011). Elevated Cortisol in Older Adults With Generalized Anxiety Disorder Is Reduced by Treatment: A Placebo-Controlled Evaluation of Escitalopram. *The American journal of geriatric psychiatry*, *19*(5), 482-490. https://doi.org/10.1097/JGP.0b013e3181ec806c

Lindqvist, D., Mellon, S. H., Dhabhar, F. S., Yehuda, R., Grenon, S. M., Flory, J. D., Bierer, L. M., Abu-Amara, D., Coy, M., Makotkine, I., Reus, V. I., Aschbacher, K., Bersani, F. S., Marmar, C. R., & Wolkowitz, O. M. (2017). Increased circulating blood cell

counts in combat-related PTSD: Associations with inflammation and PTSD severity. *Psychiatry research*, *258*, 330-336. https://doi.org/10.1016/j.psychres.2017.08.052

Liu, C.-H., Hua, N., & Yang, H.-Y. (2021). Alterations in Peripheral C-Reactive Protein and Inflammatory Cytokine Levels in Patients with Panic Disorder: A Systematic Review and Meta-Analysis. *Neuropsychiatric disease and treatment*, *17*, 3539-3558. https://doi.org/10.2147/NDT.S340388

Liu, Y., Ho, R. C.-M., & Mak, A. (2012). The role of interleukin (IL)-17 in anxiety and depression of patients with rheumatoid arthritis. *International journal of rheumatic diseases*, *15*(2), 183-187. https://doi.org/10.1111/j.1756-185X.2011.01673.x

Liukkonen, T., Räsänen, P., Jokelainen, J., Leinonen, M., Järvelin, M. R., Meyer-Rochow, V. B., & Timonen, M. (2011). The association between anxiety and C-reactive protein (CRP) levels: Results from the Northern Finland 1966 Birth Cohort Study. *European psychiatry*, *26*(6), 363-369. https://doi.org/10.1016/j.eurpsy.2011.02.001

Lucas, S.-M., Rothwell, N. J., & Gibson, R. M. (2009). The role of inflammation in CNS injury and disease: The role of inflammation in CNS. *British journal of pharmacology*, *147*(S1), S232-S240. https://doi.org/10.1038/sj.bjp.0706400

Maier, W., Buller, R., Philipp, M., & Heuser, I. (1988). The Hamilton Anxiety Scale: Reliability, validity and sensitivity to change in anxiety and depressive disorders. *Journal of Affective Disorders*, *14*(1), 61-68. https://doi.org/https://doi.org/10.1016/0165-0327(88)90072-9

Martino, M., Rocchi, G., Escelsior, A., & Fornaro, M. (2012). Immunomodulation Mechanism of Antidepressants: Interactions between Serotonin/Norepinephrine Balance and Th1/Th2 Balance. *Current neuropharmacology*, *10*(2), 97-123. https://doi.org/10.2174/157015912800604542

McCanlies, E. C., Araia, S. K., Joseph, P. N., Mnatsakanova, A., Andrew, M. E., Burchfiel, C. M., & Violanti, J. M. (2011). C-reactive protein, Interleukin-6, and posttraumatic stress disorder symptomology in urban police officers. *Cytokine*, *55*(1), 74-78. https://doi.org/10.1016/j.cyto.2011.03.025

Mendlowicz, M. V., & Stein, M. B. (2000). Quality of Life in Individuals With Anxiety Disorders. *The American journal of psychiatry*, *157*(5), 669-682. https://doi.org/10.1176/appi.ajp.157.5.669

Michopoulos, V., Powers, A., Gillespie, C. F., Ressler, K. J., & Jovanovic, T. (2017). Inflammation in Fear- and Anxiety-Based Disorders: PTSD, GAD, and Beyond. *Neuropsychopharmacology*, *42*(1), 254-270. https://doi.org/10.1038/npp.2016.146

Nathan, C., & Ding, A. (2010). Nonresolving Inflammation. *Cell*, *140*(6), 871-882. https://doi.org/10.1016/j.cell.2010.02.029

National Institute of Neurological Disorders and Stroke: Common Data Elements. (2022). *Hamilton Anxiety Rating Scale (HAM-A)*. N. I. o. N. D. a. S. C. D. Elements. https://www.commondataelements.ninds.nih.gov/report-viewer/24660/Hamilton%20Anxiety%20Rating%20Scale%20%28HAM-A%29

Newton, T. L., Fernandez-Botran, R., Miller, J. J., & Burns, V. E. (2014). Interleukin-6 and soluble interleukin-6 receptor levels in posttraumatic stress disorder: Associations with lifetime diagnostic status and psychological context. *Biological psychology*, *99*, 150-159. https://doi.org/10.1016/j.biopsycho.2014.03.009

O'Donovan, A., Hughes, B. M., Slavich, G. M., Lynch, L., Cronin, M. T., O'Farrelly, C., & Malone, K. M. (2010). Clinical anxiety, cortisol and interleukin-6: evidence for specificity in emotion-biology relationships. *Brain, behavior, and immunity*, *24*(7), 1074-1077. https://doi.org/10.1016/j.bbi.2010.03.003

O'Connor, M.-F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M. E., Hoyt, M. A., Martin, J. L., Robles, T. F., Sloan, E. K., Thomas, K. S., & Irwin, M. R. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, behavior, and immunity*, *23*(7), 887-897. https://doi.org/10.1016/j.bbi.2009.04.005

Osório, F. L., Loureiro, S. R., Hallak, J. E. C., Machado‐de‐Sousa, J. P., Ushirohira, J. M., Baes, C. V. W., Apolinario, T. D., Donadon, M. F., Bolsoni, L. M., Guimarães, T., Fracon, V. S., Silva‐Rodrigues, A. P. C., Pizeta, F. A., Souza, R. M., Sanches, R. F., dos Santos, R. G., Martin‐Santos, R., & Crippa, J. A. S. (2019). Clinical validity and intrarater and test‐retest reliability of the Structured Clinical Interview for DSM‐5‐Clinician Version (SCID‐5‐CV). *Psychiatry and clinical neurosciences*, *73*(12), 754-760. https://doi.org/10.1111/pcn.12931

Parsons, C., Roberts, R., & Mills, N. T. (2021). Review: Inflammation and anxiety-based disorders in children and adolescents - a systematic review and meta-analysis. *Child and Adolescent Mental Health*, *26*(2), 143-156. https://doi.org/10.1111/camh.12434

Passos, I. C., Vasconcelos-Moreno, M. P., Costa, L. G., Kunz, M., Brietzke, E., Quevedo, J., Salum, G., Magalhaes, P. V., Kapczinski, F., & Kauer-Sant'Anna, M. (2015). Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *Lancet Psychiatry*, *2*(11), 1002-1012. https://doi.org/10.1016/S2215-0366(15)00309-0

Pitsavos, C., Panagiotakos, D. B., Papageorgiou, C., Tsetsekou, E., Soldatos, C., & Stefanadis, C. (2006). Anxiety in relation to inflammation and coagulation markers, among healthy adults: The ATTICA Study. *Atherosclerosis*, *185*(2), 320-326. https://doi.org/10.1016/j.atherosclerosis.2005.06.001

Quagliato, L. A., & Nardi, A. E. (2022). A cross-sectional healthy-control study of serum inflammatory biomarkers interleukin (IL)-1B and IL-2R in panic disorder patients and their offspring. *Journal of Psychiatric Research*, *149*, 260-264. https://doi.org/10.1016/j.jpsychires.2022.03.004

Renna, M. E., O'Toole, M. S., Spaeth, P. E., Lekander, M., & Mennin, D. S. (2018). The association between anxiety, traumatic stress, and obsessive‐compulsive disorders and chronic inflammation: A systematic review and meta‐analysis. *Depression and anxiety*, *35*(11), 1081-1094. https://doi.org/10.1002/da.22790

Roest, A. M., Martens, E. J., de Jonge, P., & Denollet, J. (2010). Anxiety and Risk of Incident Coronary Heart Disease: A Meta-Analysis. *Journal of the American College of Cardiology*, *56*(1), 38-46. https://doi.org/10.1016/j.jacc.2010.03.034

Roy-Byrne, P., Craske, M. G., Sullivan, G., Rose, R. D., Edlund, M. J., Lang, A. J., Bystritsky, A., Welch, S. S., Chavira, D. A., Golinelli, D., Campbell-Sills, L., Sherbourne, C. D., & Stein, M. B. (2010). Delivery of Evidence-Based Treatment for Multiple Anxiety Disorders in Primary Care: A Randomized Controlled Trial. *JAMA : Journal of the American Medical Association*, *303*(19), 1921-1928. https://doi.org/10.1001/jama.2010.608

RStudioTeam. (2022). *Rstudio: Integrated Development Environment for R*. In (Version 4.2.0) RStudio, PBC. http://www.rstudio.com/

Sareen, J., Cox, B. J., Clara, I., & Asmundson, G. J. G. (2005). The relationship between anxiety disorders and physical disorders in the U.S. National Comorbidity Survey. *Depression and anxiety*, *21*(4), 193-202. https://doi.org/10.1002/da.20072

Sarwar, N., Butterworth, A. S., Freitag, D. F., Gregson, J., Willeit, P., Gorman, D. N., Gao, P., Saleheen, D., Rendon, A., Nelson, C. P., Braund, P. S., Hall, A. S., Chasman, D. I., Tybjærg-Hansen, A., Chambers, J. C., Benjamin, E. J., Franks, P. W., Clarke, R.,

Wilde, A. A. M., . . . Kastelein, J. J. P. (2012). Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *The Lancet (British edition)*, *379*(9822), 1205-1213. https://doi.org/10.1016/S0140-6736(11)61931-4

Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1813*(5), 878-888. https://doi.org/10.1016/j.bbamcr.2011.01.034

Seldenrijk, A., Vogelzangs, N., van Hout, H. P. J., van Marwijk, H. W. J., Diamant, M., & Penninx, B. W. J. H. (2010). Depressive and anxiety disorders and risk of subclinical atherosclerosis Findings from the Netherlands Study of Depression and Anxiety (NESDA). *Journal of psychosomatic research*, *69*(2), 203-210. https://doi.org/10.1016/j.jpsychores.2010.01.005

Selya, A. S., Rose, J. S., Dierker, L. C., Hedeker, D., & Mermelstein, R. J. (2012). A Practical Guide to Calculating Cohen's f2, a Measure of Local Effect Size, from PROC MIXED. *Frontiers in psychology*, *3*. https://doi.org/10.3389/fpsyg.2012.00111

Shabani, A., Masoumian, S., Zamirinejad, S., Hejri, M., Pirmorad, T., & Yaghmaeezadeh, H. (2021). Psychometric properties of Structured Clinical Interview for DSM‐5 Disorders‐Clinician Version (SCID‐5‐CV). *Brain and behavior*, *11*(5), e01894-n/a. https://doi.org/10.1002/brb3.1894

Shafiee, M., Tayefi, M., Hassanian, S. M., Ghaneifar, Z., Parizadeh, M. R., Avan, A., Rahmani, F., Khorasanchi, Z., Azarpajouh, M. R., Safarian, H., Moohebati, M., Heidari-Bakavoli, A., Esmaeili, H., Nematy, M., Safarian, M., Ebrahimi, M., Ferns, G. A., Mokhber, N., & Ghayour-Mobarhan, M. (2017). Depression and anxiety symptoms are associated with white blood cell count and red cell distribution width: A

sex-stratified analysis in a population-based study. *Psychoneuroendocrinology*, *84*, 101-108. https://doi.org/10.1016/j.psyneuen.2017.06.021

Shear, M. K., Vander Bilt, J., Rucci, P., Endicott, J., Lydiard, B., Otto, M. W., Pollack, M. H., Chandler, L., Williams, J., Ali, A., & Frank, D. M. (2001). Reliability and validity of a structured interview guide for the Hamilton anxiety rating scale (SIGH-A). *Depression and anxiety*, *13*(4), 166-178. https://doi.org/https://doi.org/10.1002/da.1033

Shimada-Sugimoto, M., Otowa, T., & Hettema, J. M. (2015). Genetics of anxiety disorders: Genetic epidemiological and molecular studies in humans: Genetics of anxiety disorders. *Psychiatry and clinical neurosciences*, *69*(7), 388-401. https://doi.org/10.1111/pcn.12291

Song, Y., Zhou, D., Guan, Z., & Wang, X. (2008). Disturbance of Serum Interleukin-2 and Interleukin-8 Levels in Posttraumatic and Non-Posttraumatic Stress Disorder Earthquake Survivors in Northern China. *Neuroimmunomodulation*, *14*(5), 248-254. https://doi.org/10.1159/000112050

Soria-Reyes, L. M., Cerezo, M. V., Alarcón, R., & Blanca, M. (2022). Psychometric properties of the Perceived Stress Scale (pss-10) with breast cancer patients. *Stress and health*. https://doi.org/10.1002/smi.3170

Spitzer, C., Barnow, S., Volzke, H., Wallaschofski, H., John, U., Freyberger, H. J., Lowe, B., & Grabe, H. J. (2010). Association of posttraumatic stress disorder with low-grade elevation of C-reactive protein: evidence from the general population. *Journal of Psychiatric Research*, *44*(1), 15-21. https://doi.org/10.1016/j.jpsychires.2009.06.002

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in immunology*, *9*, 754-754. https://doi.org/10.3389/fimmu.2018.00754

Talmon, M., Rossi, S., Pastore, A., Cattaneo, C. I., Brunelleschi, S., & Fresu, L. G. (2018). Vortioxetine exerts anti‐inflammatory and immunomodulatory effects on human monocytes/macrophages. *British journal of pharmacology*, *175*(1), 113-124. https://doi.org/10.1111/bph.14074

Taylor, S., Koch, W. J., & McNally, R. J. (1992). How does anxiety sensitivity vary across the anxiety disorders? *Journal of anxiety disorders*, *6*(3), 249-259. https://doi.org/10.1016/0887-6185(92)90037-8

Tükel, R., Arslan, B. A., Ertekin, B. A., Ertekin, E., Oflaz, S., Ergen, A., Kuruca, S. E., & İsbir, T. (2012). Decreased IFN-γ and IL-12 levels in panic disorder. *Journal of psychosomatic research*, *73*(1), 63-67. https://doi.org/10.1016/j.jpsychores.2012.04.012

Turner, S. M., Beidel, D. C., & Stanley, M. A. (1992). Are obsessional thoughts and worry different cognitive phenomena? *Clinical psychology review*, *12*(2), 257-270. https://doi.org/10.1016/0272-7358(92)90117-Q

van Eeden, W. A., El Filali, E., van Hemert, A. M., Carlier, I. V. E., Penninx, B. W. J. H., Lamers, F., Schoevers, R., & Giltay, E. J. (2021). Basal and LPS-stimulated inflammatory markers and the course of anxiety symptoms. *Brain, behavior, and immunity*, *98*, 378-387. https://doi.org/10.1016/j.bbi.2021.09.001

Vieira, M. M. M., Ferreira, T. B., Pacheco, P. A. F., Barros, P. O., Almeida, C. R. M., Araújo-Lima, C. F., Silva-Filho, R. G., Hygino, J., Andrade, R. M., Linhares, U. C., Andrade, A. F. B., & Bento, C. A. M. (2010). Enhanced Th17 phenotype in individuals with generalized anxiety disorder. *Journal of neuroimmunology*, *229*(1), 212-218. https://doi.org/10.1016/j.jneuroim.2010.07.018

Vogelzangs, N., Beekman, A. T., de Jonge, P., & Penninx, B. W. (2013). Anxiety disorders and inflammation in a large adult cohort. *Translational Psychiatry*, *3*, e249. https://doi.org/10.1038/tp.2013.27

Vogelzangs, N., Seldenrijk, A., Beekman, A. T. F., van Hout, H. P. J., de Jonge, P., & Penninx, B. W. J. H. (2010). Cardiovascular disease in persons with depressive and anxiety disorders. *Journal of Affective Disorders*, *125*(1-3), 241-248. https://doi.org/10.1016/j.jad.2010.02.112

von Känel, R., Hepp, U., Kraemer, B., Traber, R., Keel, M., Mica, L., & Schnyder, U. (2006). Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder. *Journal of Psychiatric Research*, *41*(9), 744-752. https://doi.org/10.1016/j.jpsychires.2006.06.009

Waszczuk, M. A., Zavos, H. M. S., Gregory, A. M., & Eley, T. C. (2014). The Phenotypic and Genetic Structure of Depression and Anxiety Disorder Symptoms in Childhood, Adolescence, and Young Adulthood. *JAMA Psychiatry*, *71*(8), 905-916. https://doi.org/10.1001/jamapsychiatry.2014.655

Williams, N. L., Shahar, G., Riskind, J. H., & Joiner, T. E. (2005). The looming maladaptive style predicts shared variance in anxiety disorder symptoms: further support for a cognitive model of vulnerability to anxiety. *Journal of anxiety disorders*, *19*(2), 157-175. https://doi.org/10.1016/j.janxdis.2004.01.003

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., Olesen, J., Allgulander, C., Alonso, J., Faravelli, C., Fratiglioni, L., Jennum, P., Lieb, R., Maercker, A., van Os, J., Preisig, M., Salvador-Carulla, L., Simon, R., & Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European neuropsychopharmacology*, *21*(9), 655-679. https://doi.org/10.1016/j.euroneuro.2011.07.018

Won, E., & Kim, Y.-K. (2020). Neuroinflammation-Associated Alterations of the Brain as Potential Neural Biomarkers in Anxiety Disorders. *International journal of molecular sciences*, *21*(18), 6546. https://doi.org/10.3390/ijms21186546

World Health Organisation. (2017). *Depression and Other Common Mental Disorders: Global Health Estimates*. Geneva, Switzerland: WHO Document Production Services Retrieved from https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf

Yan, J., Xu, Y., Zhu, C., Zhang, L., Wu, A., Yang, Y., Xiong, Z., Deng, C., Huang, X.-F., Yenari, M. A., Yang, Y.-G., Ying, W., & Wang, Q. (2011). Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. *PloS one*, *6*(6), e20945-e20945. https://doi.org/10.1371/journal.pone.0020945

Zhang, J.-M., & An, J. (2007). Cytokines, inflammation, and pain. *International anesthesiology clinics*, *45*(2), 27-37. https://doi.org/10.1097/AIA.0b013e318034194e

Zimmerman, M., Martin, J., Clark, H., McGonigal, P., Harris, L., & Holst, C. G. (2017). Measuring anxiety in depressed patients: A comparison of the Hamilton anxiety rating scale and the DSM-5 Anxious Distress Specifier Interview. *Journal of Psychiatric Research*, *93*, 59-63. https://doi.org/https://doi.org/10.1016/j.jpsychires.2017.05.014

Zou, Z., Zhou, B., Huang, Y., Wang, J., Min, W., & Li, T. (2020). Differences in cytokines between patients with generalised anxiety disorder and panic disorder. *Journal of psychosomatic research*, *133*, 109975-109975. https://doi.org/10.1016/j.jpsychores.2020.109975