Inflammation and anxiety-based disorders in children and adolescents: a systematic review and meta-analysis

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Table of Contents

List of Figures……..……iii

List of Tables……...iv

Abstract……....v

Declaration……...…vi

Acknowledgements ……....vii

Chapter 1: Introduction ……..…1

1.1 Anxiety-Based Disorders: Classification, Prevalence, and Aetiology…..……...……..…1

1.2 Inflammation……4

1.2.1 Adaptive and Maladaptive Inflammation……4

1.2.2 Cytokines……..4

1.2.3 Markers of Inflammation……6

1.2.4 Inflammation and Depression…….……6

1.2.5 Inflammation and Anxiety ……...……...……7

1.3 Gaps in the Literature……...8

1.4 Summary and Research Aims ……9

Chapter 2: Method ……..……..11

2.1 Literature Search…….....11

2.2 Eligibility Criteria……..11

2.3 Data Extraction, Organisation, and Preparation…….. ……..13

2.4 Quality Assessment……15

2.5 Statistical Analysis and Interpretation……15

Chapter 3: Results…….……..…...17

3.1 Study Characteristics ……17

3.2 Participant Characteristics……...……...19

3.3 Quality Assessments of Included Studies…….. ……...21

3.4 Meta-analyses…….23

3.4.1 Cytokines …….23

3.4.2 C-Reactive Protein……...28

3.4.3 White Blood Cells…….…...28

3.5 Findings from Individual Studies……..31

Chapter 4: Discussion……....33
4.1 Key Finding……....33
4.1.1 Cytokines……...33
4.1.2 C-Reactive Protein……....34
4.1.3 White blood cells……. ……35
4.1.4 Quality Findings……36
4.1.4 Study Heterogeneity……..38
4.2 Clinical Implications and Future Research……39
4.3 Study Limitations and Strengths…….……...40
4.4 Conclusions……41

References ……..42

Appendices……..56
Appendix A—Search Strategies by Database……..……56
Appendix B—Data Extraction Sheet Example……..……..61
Appendix C— Quality Assessment Criteria……..……..63

List of Figures

Figures

Figure 1. Venn diagram of pro- and anti-inflammatory cytokines……5
Figure 2. PRISMA flowchart of study selection process…….14
Figure 3. Percentage of included studies which met reporting quality criteria based on the Qualsyst tool……..……22
Figure 4. Random effects meta-analysis of TNF-α levels in individuals with anxiety-based disorders (ABD) compared with controls……25
Figure 5. Random effects meta-analysis of IL-1β levels in individuals with anxiety-based disorders (ABD) compared with controls……25
Figure 6. Random effects meta-analysis of IL-6 levels in individuals with anxiety-based disorders (ABD) compared with controls ……...……26
Figure 7. Random effects meta-analysis of IL-17A levels in individuals with anxiety-based disorders (ABD) compared with controls……26
Figure 8. Random effects meta-analysis of IL-8 levels in individuals with anxiety-based disorders (ABD) compared with controls……27
Figure 9. Random effects meta-analysis of IL-12 levels in individuals with anxiety-based disorders (ABD) compared with controls ……...……27
Figure 10. Random effects meta-analysis of CRP levels in individuals with anxiety-based disorders (ABD) compared with controls……29
Figure 11. Random effects meta-analysis of total WBC count in individuals with anxiety-based disorders (ABD) compared with controls..……...……..29
Figure 12. Random effects meta-analysis of neutrophil levels in individuals with anxiety-based disorders (ABD) compared with controls. ……..……..30
Figure 13 Random effects meta-analysis of lymphocyte levels in individuals with anxiety-based disorders (ABD) compared with controls ……..……30
Figure 14. Hedges' g and 95% CIs for inflammatory markers investigated in individual studies……...32

List of Tables

Tables

Table 1. *Characteristics of Anxiety-Based Disorders* ……...2

Table 2. *Search Terms and Boolean Operators Used in Database Searches*……..…...…12

Table 3. *Study Characteristics*……18

Table 4. *Participant Characteristics* ……..20

Table 5. *Heterogeneity Statistics for Meta-analyses*……31

Abstract

Anxiety-based disorders are common, chronic conditions which often develop during childhood and adolescence. An emerging literature has examined the role of inflammation in these disorders by measuring blood concentrations of inflammatory markers such as cytokines, C-reactive protein, and white blood cell counts. However, results from these studies are inconsistent, with available meta-analyses only including adult populations. To date, no review has consolidated findings among the population of children and adolescents. To address this gap, this paper aimed to summarise and quantitatively evaluate the current literature on inflammation and anxiety-based disorders in children and adolescents. A systematic search of five electronic databases identified eight independent studies which compared concentrations of inflammatory markers between individuals with anxiety-based disorders and healthy controls. Quality assessments were conducted, and Hedges' g was calculated to determine the magnitude and direction of standardised between-group differences. No significant between-group differences were observed for any inflammatory marker. However, these findings were based on a small number of studies with high heterogeneity. Furthermore, quality assessments identified important limitations; primarily, small sample sizes and a lack of control over confounding variables. Further research which adheres to the recommendations provided by this review is required to disentangle the relationship between inflammation and anxiety among this population.

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 which has been 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.

1st October 2019

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Chapter 1

Introduction

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

Anxiety is an adaptive response, facilitating escape from, or avoidance of danger. However, anxiety is susceptible to dysregulation and becomes pathological when its intensity, duration, or frequency interferes with an individual's well-being. Anxiety disorders are a group of mental health conditions characterised by excessive and prolonged levels of fear and anxiety in relation to a real or perceived threat (American Psychiatric Association, 2013). The Diagnostic and Statistical Manual of Mental Disorders, 5th ed (DSM-5) classifies the following as anxiety disorders: generalised anxiety disorder (GAD), separation anxiety disorder, specific phobia, social anxiety disorder, panic disorder, agoraphobia, and selective mutism. Although categorically distinct, these disorders share the common characteristics of maladaptive anxiety, physiological symptoms, distorted thinking, avoidance behaviours, and functional impairment (American Psychiatric Association, 2013).

Recently, the DSM-5 reclassified posttraumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD) from anxiety disorders, to the separate domains of trauma- and stressor-related disorders and obsessive-compulsive and related disorders, respectively. However, they too share the key underlying characteristic of maladaptive anxiety (Lack, 2012; Taylor, Koch, & McNally, 1992; Williams, Shahar, Riskind, & Joiner, 2005) as well as distorted thought patterns (Turner, Beidel, & Stanley, 1992) and experiential avoidance (Hayes, Wilson, Gifford, Follette, & Strosahl, 1996). Furthermore, the recent reclassification means certain studies refer to and group PTSD and OCD as anxiety disorders, in line with the categorisation system of the DSM-IV (American Psychiatric Association, 2000). Subsequently, this review will refer to anxiety disorders, PTSD, and OCD under the umbrella term: anxiety-based disorders (see Table 1 for a list of included disorders and characteristics).

Combined, anxiety-based disorders are the most prevalent mental conditions (Kessler et al., 2005) and contribute the greatest health burden in terms of cost (Gustavsson et al., 2011). Childhood and adolescence represent a primary risk phase for the development of these disorders, with the combined median age of onset being 11 years old (Kessler et al., 2005).

Table 1

Characteristics of Anxiety-Based Disorders

Anxiety-Based Disorder	Key Characteristics
Generalised anxiety disorder	Excessive anxiety and worry (not focused on any trigger) which is present more days than not.
Separation anxiety disorder	Inappropriate and excessive fear and anxiety concerning separation from attachment figures.
Specific phobia	Fear or anxiety about a specific object or situation.
Social anxiety disorder	Fear or anxiety in regard to social situations.
Panic disorder	Recurring and unexpected panic attacks: the sudden onset of intense fear or discomfort which peaks within minutes.
Agoraphobia	Excessive anxiety about being in a place or situation where escape may be difficult e.g., being home alone or in a crowd.
Posttraumatic stress disorder	Recurrent, involuntary recollections of a traumatic event e.g., exposure to death, threatened death, actual or threatened violence.
Obsessive-compulsive disorder	Presence of unwanted obsessions: persistent and recurrent thoughts, urges and impulses and/or compulsions: repetitive behaviours or mental acts performed in an attempt to reduce obsessions.
Selective mutism	Disturbance caused by a consistent failure to speak in situations where speech is expected.

Note. Adapted from American Psychiatric Association. (2013). Anxiety Disorders. In *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: Author.

More specifically, selective mutism, social anxiety disorder, separation anxiety disorder, and specific phobia tend to develop early in childhood, whereas panic disorder, agoraphobia, and GAD have a later and more dispersed age of onset distribution (Kessler, Ruscio, Shear, & Wittchen, 2010; Wong, 2010). OCD has two peaks of onset, occurring in childhood and early adulthood (De Mathis et al., 2008). PTSD has the latest and most variable age of onset distribution, as trauma exposure can occur at any age (Kessler et al., 2010). These disorders tend to follow a recurrent intermittent course throughout the lifetime (Kessler et al., 2010), leading to negative outcomes in terms of quality of life, as measured by psychosocial functioning, physical, and emotional health (Baxter, Vos, Scott, Ferrari, & Whiteford, 2014; Mendlowicz & Stein, 2000). Globally, anxiety disorders are the sixth greatest contributor to non-fatal health loss (World Health Organisation, 2017); subsequently, early intervention in childhood and adolescence is crucial in preventing and reducing the severity of adult psychological illness (McGorry, Killackey, & Yung, 2008).

In order to improve treatment outcomes, ongoing research has focused on understanding the aetiology of anxiety-based disorders. Anxiety-based disorders are a diverse range of conditions, and the exact mechanisms underlying each condition likely reflects this heterogeneity (Craske et al., 2017). However, research suggests that certain processes underlying anxiety and fear may be similar across disorders. For example, they share genetic and environmental risk factors (Tambs et al., 2009) and similar treatment options have proven successful among multiple anxiety-based disorders (Roy-Byrne et al., 2010). Current conceptualisations of the aetiology of anxiety-based disorders implicates an interaction of biological factors (e.g., genetics, impairment in neurotransmission, and abnormalities in signal transduction mechanisms) and psychosocial factors (e.g., childhood adversity, stress and trauma) (Salim, Chugh, & Asghar, 2012). Developing an anxiety-based disorder has been linked with the experience of stress, and stressful life events, which may have nonspecific effects across the disorders (Hettema, Prescott, Myers, Neale, & Kendler, 2005). Exposure to stressful and traumatic events leads to hypothalamic-pituitary-adrenal (HPA) axis reactivity and activation of the immune system (Padgett & Glaser, 2003). Recently, research in the field of psychoneuroimmunology has found an interaction between the immune system and the central nervous system (CNS), identifying mechanisms through which immune functioning can influence our brain and behaviour (Salim et al., 2012; Dantzer, O'Conner, Freund, Johnson, & Kelley, 2008). A central feature of immune functioning is the inflammatory response (Chen et al., 2017). Subsequently, an emerging literature has begun to explore the role of inflammation in anxiety-based disorders.

1.2 Inflammation

1.2.1 Adaptive and Maladaptive Inflammation

Inflammation is a central feature of immune functioning which can be categorised as acute or chronic. Acute inflammation in response to illness or injury is temporary and adaptive—cellular and molecular interactions act to minimise the effect of injury or infection—promoting the healing process (Chen et al., 2017). Following repair, the inflammatory response should end, returning the body to a state of homeostasis. However, when immune response becomes chronic or dysregulated, prolonged inflammation can lead to negative health outcomes including diabetes, cardiovascular disease, and autoimmune conditions (Khansari, Shakiba, & Mahmoudi, 2009).

1.2.2 Cytokines

Central to the inflammatory response are proteins called cytokines. Cytokines are secreted by various cells in the periphery (parts of the body distinct from the CNS), as well as in the CNS (Hou & Baldwin, 2012). These signalling molecules play a crucial part in immunity and inflammation, facilitating the interaction and communication between cells and the modulation of immune responses (Zhang & An, 2007). Cytokines are categorised as either pro-inflammatory or anti-inflammatory depending on their interaction with target cells (Steinke & Borish, 2006). Pro-inflammatory cytokines direct the inflammatory response to illness, injury, or stress, whereas anti-inflammatory cytokines reduce or abate the immune response and synthesis of pro-inflammatory cytokines (Kronfol & Remick, 2000). As such, adaptive immune functioning and inflammatory responses rely on a delicate balance of pro- and anti-inflammatory cytokines. Heightened levels of pro-inflammatory cytokines are indicative of an inflammatory state, whereas increased levels of anti-inflammatory cytokines may signify dysregulation of the immune system (Zhang & An, 2007). However, the distinction between pro- and anti-inflammatory cytokines is not always clear; some cytokines, including interleukin (IL)-6, have both pro- and anti-inflammatory properties (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011) (see Figure 1 for a list of pro- and anti-inflammatory cytokines).

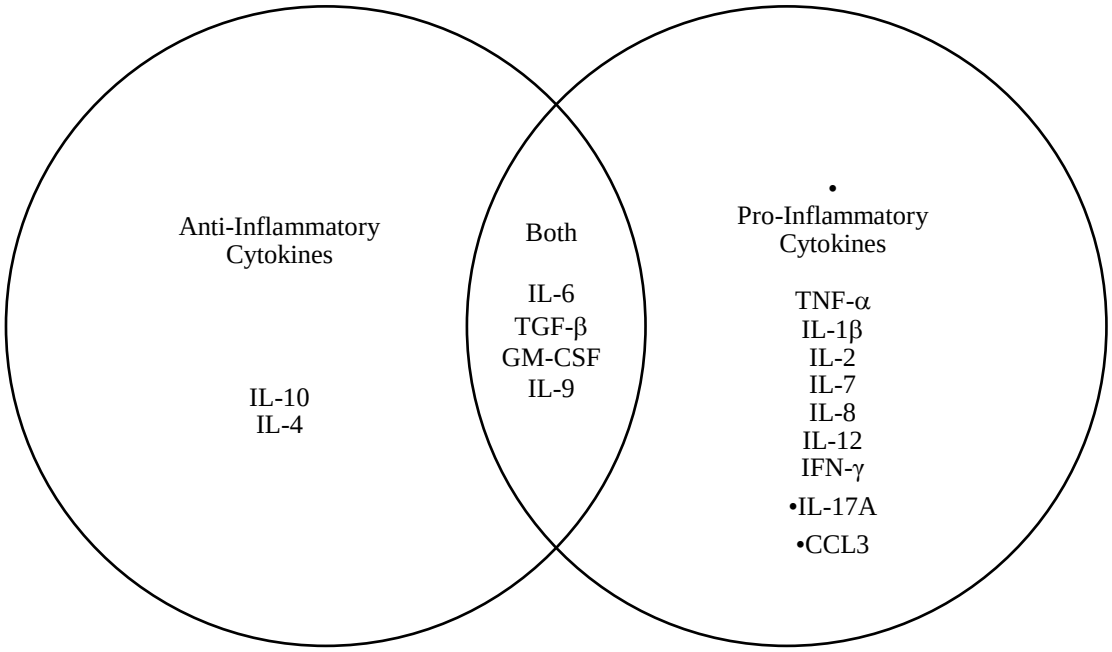

Figure 1. Venn diagram of pro- and anti-inflammatory cytokines.
IL = interleukin; TGF = transforming growth factor; TNF = tumour necrosis factor; IFN = interferon; GM-CSF = granulocyte macrophage colony stimulating factor; CCL = chemokine ligand.

Cytokines have receptors—cell surface glycoproteins—which they bind to in order to transduce their signals. For example, the cytokine tumour necrosis factor α (TNF-α) exerts many of its functions through binding to tumour necrosis factor-receptor type 1 (TNFR1) and TNFR2 (Wajant, Pfizenmaier, & Scheurich, 2003). Measuring the soluble receptors (sTNFR1 and sTNFR2) provides a reliable marker of TNF-α activity and inflammation (Idriss & Naismith, 2000).

Previously, the blood-brain barrier was thought to prevent cytokines from reaching the brain, leaving the immune system and CNS to act relatively independently of each other (Lucas, Rothwell, & Gibson, 2006). However, research now suggests that cytokine signals from the periphery can travel to the brain through humoral, neural, and cellular pathways (Capuron & Miller, 2011). Once these signals reach the brain, they can interact with various areas related to mood regulation, neurogenesis, neurocircuitry, and synaptic plasticity. Consequently, prolonged immune activation and inflammation is thought to contribute to acute and chronic CNS, neuropsychiatric, and psychological disorders (Capuron & Miller, 2011; Dantzer et al., 2008; Hou & Baldwin, 2012).

1.2.3 Markers of Inflammation

The measurement of blood-based markers of inflammation—biological substances released as part of the immune response—is one means of assessing for systemic inflammation in these disorders. Common markers of inflammation include cytokines and C-reactive protein (CRP). CRP is produced in the liver and secreted into the blood following acute injury, infection, or other inflammatory stimuli (Ballantyne & Nambi, 2005). It is the most sensitive and commonly used diagnostic tool for the acute phase inflammatory response in humans, with plasma levels rising up to 1000-fold in response to an inflammatory stimulus (Sproston & Ashworth, 2018). Once the inflammatory stimulus has subsided, CRP levels should decrease exponentially over the following 24 hours. Increased levels of CRP are therefore indicative of acute infection or chronic inflammatory conditions.

Another clinical marker of inflammation and immune dysregulation is white blood cell (WBC) counts (Willems et al., 2010). WBCs (also called leukocytes) are a crucial part of the immune system, protecting the body against disease and foreign invaders. Of particular interest are the sub-categories: neutrophils, lymphocytes, and monocytes. Neutrophils are the most numerous WBC and the first to be activated when infection arises (Mayadas, Cullere, & Lowell, 2014). Monocytes aid in destroying invading bacteria, while also facilitating healing and repair (Karlmark, Tacke, & Dunay, 2012). Lymphocytes produce antibodies which fight against viruses, bacteria, and other invaders. There are two main types of lymphocytes, namely: (1) T cells, which play a direct role in destroying problem cells in the body; and (2) B cells, which provide signals for T cell activation and ensure that antibodies are produced against the appropriate targets (Cano & Lopera, 2013). White blood cells can also be defined based on the cluster of differentiation (CD) system; which identifies cells based on the molecules present on their surface. Common CD molecules include $CD4^+$ and $CD8^+$, which are involved in the adaptive immune response against infections, pathogens, and viruses (Nascimbeni, Shin, Chiriboga, Kleiner, & Rehermann, 2004). In the absence of current infection or disease, increased levels of lymphocytes, neutrophils, monocytes, neutrophil-lymphocyte ratio (NLR) as well as the total WBC count are recognised as important cellular markers of systemic inflammation (Li, Manwani, & Leng, 2011).

1.2.4 Inflammation and Depression

The majority of research investigating inflammation and psychological disorders has focused on depression (Irwin & Miller, 2007). The inflammation theory of depression proposes that activation of the peripheral immune system and cytokine signalling to the brain

results in sickness behaviour, such as: nausea, loss of appetite, loss of interest in physical and social environments, and irritability (Dantzer et al., 2008). Although adaptive in the short term—allowing one to use all necessary resources to heal—when immune signalling continues unabated, these symptoms become chronic, leading to depression in vulnerable individuals (Dantzer et al., 2008). In support of this theory, meta-analyses have found significantly increased levels of pro-inflammatory cytokines IL-6, IL-10, TNF-α (Dowlati et al., 2010; Kohler et al., 2017) and CRP (Howren, Lamkin, & Suls, 2009) among depressed individuals. Research has also identified an association between abnormal WBC counts and depressive symptoms (Maes et al., 1992; Schleifer et al., 2002).

1.2.5 Inflammation and Anxiety

Although less intensively studied, several lines of research suggest an association between anxiety-based disorders and inflammation. First, anxiety and depression are highly co-morbid, and the similar treatment effects of medication used for both disorders suggests the involvement of similar neurobiological substrates (Hou & Baldwin, 2012). Second, research shows a link between anxiety-based disorders and physical conditions such as metabolic syndrome (Carroll et al., 2009), coronary heart disease (Roest, Martens, De Jonge, & Denollet, 2010), and atherosclerosis (Seldenrijk et al., 2010), which are all associated with low grade systemic inflammation (Libby, 2012; Sharma, 2011; Willerson & Ridker, 2004). Finally, the experience of chronic stress or exposure to stressful and traumatic events has been linked to anxiety-based disorders (Hettema et al., 2005), and experimentally induced stress initiates an inflammatory reaction (Hamer, Gibson, Vuononvirta, Williams, & Steptoe, 2006). Although the underlying processes are not yet fully understood, it is theorised that stress and trauma lead to HPA axis reactivity, immune activation, and the release of cytokines (Haroon, Raison, & Miller, 2012). Continuous exposure to stress or stressful stimuli results in HPA and immune system dysregulation, facilitating increased immune activity in the periphery. Immune signalling from the periphery is transferred to the CNS via cytokines, which can influence processes of mood regulation such as anxiety, arousal, and alarm (Haroon et al., 2012). Furthermore, cytokine signals can affect neurotransmitter systems which control the release of serotonin and dopamine, creating or maintaining anxiety-based symptoms in these disorders (Michopoulous, Powers, Gillespie, Ressler, & Jovanovic, 2017).

1.3 Gaps in the Literature

An expanding body of research has compared circulating levels of inflammatory markers among individuals with anxiety-based disorders and healthy controls. However, findings are often inconsistent and based on adult populations. Studies investigating adults with PTSD have shown elevated levels of cytokines IL-1β, IL-6, IL-2, TNF-α (Guo et al., 2012; Hoge et al., 2009; Newton, Fernandez-Botran, Miller, & Burns, 2014; Oganesyan, Mkrtchyan, Sukiasyan, & Boyajyan, 2009), and CRP (Heath et al., 2013). However, other studies have failed to find significant differences in IL-6 levels (Song, Zhou, Guan, & Wang, 2007), CRP (Von Kanel et al., 2007), and have reported lower levels of IL-2 among adults with PTSD (Tucker et al., 2004). When comparing adult OCD patients with healthy controls, some studies have reported decreased TNF-α (Brambilla et al., 1997; Denys, Fluitman, Kavelaars, Heijnen, & Westenberg, 2004), and IL-6 levels (Fluitman et al., 2010) whereas others have reported increased TNF-α and IL-6 levels (Konuk et al., 2007). Similarly, while increased TNF-α levels have been reported in adults with panic disorder (Hoge et al., 2009), agoraphobia (Wagner et al., 2015) and GAD (Vieira et al., 2010), no differences in TNF-α levels have been described in adults with panic disorder (Brambilla, Bellodi, & Perna, 1999), and anxiety disorders in general (Vogelzangs, Beekman, De Jonge, & Pennix, 2013).

Research into anxiety-based disorders among children and adolescents is limited, yet characterised by similar inconsistencies. For example, Colak Sivri, Bilgic, and Kilinc (2018) found higher TNF-α and lower IL-12 levels among children and adolescents with OCD; whereas Gabbay et al. (2009) reported lower TNF-α and higher IL-12 levels. Furthermore, although heightened CRP levels have been reported in adolescents with GAD (Khandaker, Zammit, Lewis, & Jones, 2016), no differences have been reported in OCD (Simsek, Yuksel, Cim, & Kaya, 2016).

Due to these inconsistencies, the role of specific cytokines in anxiety-based disorders remains unknown. Furthermore, it is unclear whether the processes underlying anxiety and inflammation are similar or differ across the disorders. Inconsistencies in the literature may be explained by a failure to control for confounding variables. For example, medication, acute infection, chronic illness, mental health co-morbidities, and body mass index (BMI) can all influence inflammation (Michopoulous et al., 2017; Festa et al., 2001). A meta-analysis by Renna, O'Toole, Spaeth, Lekander, and Mennin (2018) was conducted to consolidate inconsistencies in the literature and investigate moderating factors. A significant overall difference in pro-inflammatory cytokines was observed between individuals with anxiety-

based disorders and healthy controls, with the cytokines IL-1β, IL-6, and TNF-α driving this effect (Renna et al., 2018). Type of diagnosis revealed a significant moderating effect, with only individuals with PTSD showing significant differences to healthy controls. No other significant moderators were identified (Renna et al., 2018). However, this meta-analysis only included adult populations. To date, no review has pooled together existing studies on inflammation and anxiety-based disorders among the population of children and adolescents.

There are several reasons why a review of this population is needed. First, cytokine production may differ between children and adults (Lilic, Cant, Abinun, Calvert, & Spickett, 1997), and age has been shown to affect the relationship between CRP levels and anxiety (Vogelzangs et al., 2013). Therefore, findings from adult studies may not be applicable to children. Second, the mechanisms which underlie the association between inflammation and anxiety may be more explicit among children and adolescents, as the confounding factor of medical co-morbidities is less prevalent when compared to adult populations (Mitchell & Goldstein, 2014). Finally, there are important clinical implications of research in this area. As childhood and adolescence represent a crucial risk phase for the development of anxiety-based disorders, prevention or treatment in this age group may lead to a reduction in the severity and duration of psychological illness (McGorry, Purcell, Goldstone, & Amminger, 2011). A greater understanding of the role of inflammation in the pathophysiology of specific anxiety-based disorders may lead to new approaches in the treatment of these conditions, such as anti-inflammatory medications (Kronfel & Remick, 2000). Implementation of these treatments in childhood and adolescence may reduce the burden of illness for both the individual and broader society.

1.4 Summary and Research Aims

Anxiety-based disorders are common, chronic conditions which have negative implications for individuals and the broader society. Immune activation and inflammation is increasingly being linked to psychological disorders. In particular, cytokine signalling is now known to travel from the periphery to the CNS, affecting behaviour and mood. Although a number of studies have investigated inflammation in anxiety-based disorders, research in this area is characterised by inconsistent results. Using meta-analytical techniques increases the sample size, statistical power, and accuracy of findings by combining effect sizes, consolidating the literature, and providing an overview of conflicting results (Borenstein, Hedges, Higgins, & Rothstein, 2009). Although a meta-analysis of anxiety-based disorders

and inflammation has been conducted with an adult population (Renna et al., 2018), to date, no studies have provided a comprehensive review among the population of children and adolescents. This represents an important knowledge gap as findings from adults may not be relevant for children and adolescents. Furthermore, anxiety-based disorders tend to develop in childhood and adolescence, and a better understanding of the specific inflammatory processes involved in these disorders may lead to the development of important therapeutic tools. This review aims to summarise and critically appraise the literature on inflammation and anxiety-based disorders in children and adolescents. The specific aims of this review are to:

1. Quantitatively pool the available data using meta-analytic techniques.
2. Examine the quality of studies and identify and investigate inconsistencies in the literature.
3. Explore potential sources of study heterogeneity.
4. Provide avenues for future research.

Chapter 2
Method

2.1 Literature Search

A comprehensive keyword-based search of five electronic databases (PsycINFO, PubMed, Embase, Scopus, and Web of Science) was conducted to source studies that examined inflammatory markers and anxiety-based disorders in children and adolescents. Database searches were undertaken for the period between database commencement and March 2019 using the search terms provided in Table 2. A senior research librarian helped identify and tailor search terms for specific databases. Search terms were kept broad to ensure all relevant studies were obtained. Email alerts were created to send notifications when any new publications met the search criteria. Additionally, reference lists of eligible studies and past meta-analyses (Gray & Bloch, 2012; Lamothe, Baleyte, Smith, Pelissolo, & Mallet 2018; Milkowska, Popko, Demkow, & Wolanczyk, 2017; Mitchell & Goldstein, 2014) were searched.

2.2 Eligibility Criteria

For inclusion in the present review, studies were required to meet all of the following criteria:

1. The sample comprised of children and adolescents, defined as 19 years of age or younger (World Health Organisation, 2019).
2. At least one blood-based marker of inflammation was measured; such as cytokines, CRP or WBC counts.
3. Inflammatory markers were compared between two independent groups: individuals meeting the diagnostic criteria for an anxiety-based disorder and healthy controls.
4. Quantitative data was provided from which an effect size could be calculated (e.g., group means and standard deviations (SD)).
5. The study was peer reviewed and published in English, with a full text available.

Studies were excluded if they met any of the following criteria:

1. The sample included individuals with anxiety based-disorders and other psychiatric/psychological disorders, for which data specific to anxiety-based disorders could not be extracted.
2. The sample included participants with severe co-morbid medical illness or acute infection, which can affect the inflammatory response (Pahwa & Jialal, 2019).

Table 2

Search Terms and Boolean Operators Used in Database Searches

AND ⇨

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
Inflammation	Anxiety disorders	Adolescent
OR	Anxiety based disorders	Child
⇩	Agoraphobia	Youth
Tumor necrosis factor	Posttraumatic stress	Paediatric
C-reactive protein	disorder	Teen
Interleukins	Generalized anxiety	
Chemokines	disorder	
White blood cells	Social phobia	
	Social anxiety disorder	
	Obsessive-compulsive	
	disorder	
	Phobic disorder	
	Panic Disorder	
	Separation anxiety	
	disorder	
	Selective mutism	

Note. Search terms included stated terms and derivatives. Both plural and singular terms were searched. See Appendix A for detailed search terms.

The initial literature search yielded 5,442 articles. After removal of duplicates, this number was reduced to 4,241. Study screening was conducted using the online software Covidence (Veritas Health Innovation, 2016). Titles and abstracts were screened against inclusion and exclusion criteria, from which 140 papers were retained. Full texts of the remaining articles were obtained and re-screened against eligibility criteria. Three authors (Copeland, Shanahan, Worthman, Angold, & Costello, 2012; Matz et al., 2012; Pervanidou et al., 2007) were contacted to provide additional information, as the available data was insufficient to calculate relevant effect sizes; however, no replies were received. These papers

were subsequently excluded from this review, resulting in a final sample of eight eligible studies. Each article was checked for independence of samples, no samples were found to overlap. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart was constructed to outline the screening process and reasons for exclusion (see Figure 2).

2.3 Data Extraction, Organisation, and Preparation

In accordance with PRISMA guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009), key information was collected using a data extraction sheet (see Appendix B). This included information relating to study characteristics (e.g., study design and country), sample demographics (e.g., age and sex) and statistical data (e.g. means and SDs). The majority of studies ($N_{studies}$ = 6) reported means and SDs, however, data from two studies required re-calculation. Khandaker et al. (2016) reported baseline data in the form of medians and interquartile ranges. These statistics were converted to an estimated mean ($\bar{X}$) and standard deviation (SD) using the method outlined by Wan, Wang, Liu, and Tong (2014):

$$\overline{X} \approx \frac{q1 + m + q3}{3}$$

$$SD \approx \frac{q3 - q1}{2\Phi^{-1}\frac{0.75n - 0.125}{n + 0.25}}$$

Where m = median, $q1$ = lower quartile range, $q3$ = upper quartile range, Φ^{-1} = the inverse of the cumulative normal distribution, and n = sample size. This method has shown low bias and low imprecision in simulations and is preferred when data is skewed (Weir et al., 2018), as is the case for this data set. Rodriguez et al. (2017) reported standard error of the means (SEM), which were converted to SDs using the following formula as outlined by Higgins and Green (2011):

$$\text{SD} = \text{SEM x } \sqrt{n}$$

whereby SEM = standard error of the mean, and n = sample size.

Finally, authors of one study (Ozyurt & Binici, 2019) were contacted to provide further information concerning the log-transformation of their data; however, no reply was received. Subsequently, one marker of inflammation (neutrophil-lymphocyte ratio) was excluded from this review.

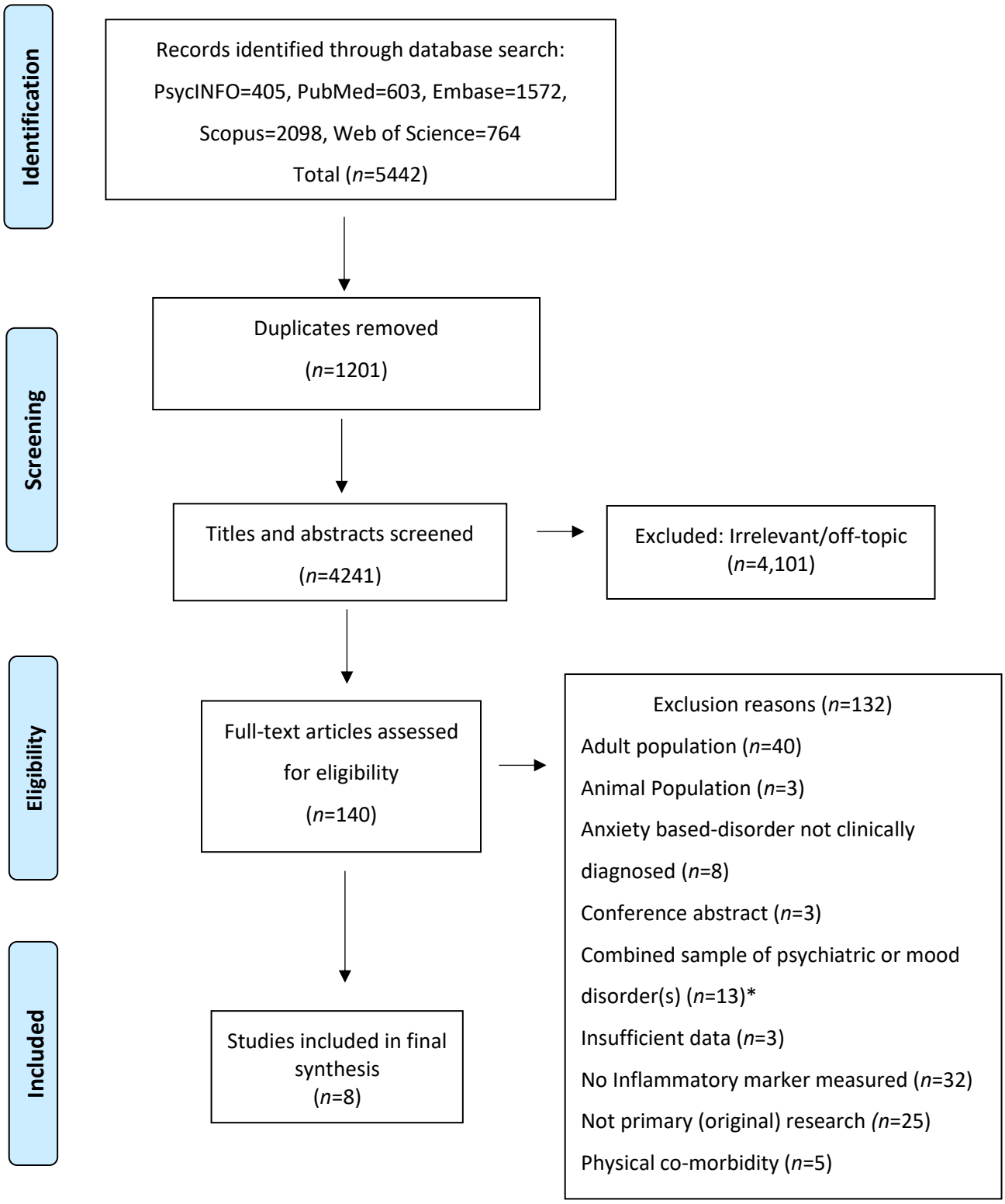

Figure 2: PRISMA flowchart of study selection process.

* The sample included individuals with anxiety based-disorders and other psychiatric/psychological disorders, for which data specific to anxiety-based disorders could not be extracted.

2.4 Quality Assessment

All included articles were evaluated for quality and risk of bias using the QualSyst tool (Kmet, Lee, & Cook, 2004). Studies were rated as either completely meeting criteria (score of 2), partially meeting criteria (score of 1) or not meeting the criteria (score of 0). Given the nature of the included studies, criteria 5-7 of the QualSyst tool (which relate to interventional designs) were not relevant. Consequently, studies were assessed on 11 criteria with a maximum score of 22. The total score and percentage of criteria met by each study was calculated. All studies were assessed for quality by the author and a second independent rater — an expert in this field (N.M.). Cohen's Kappa (κ) was calculated as a measure of inter-rater reliability, which takes into account the possibility of agreement occurring by chance (Cohen, 1960). Kappa values of 0—0.20 indicate slight agreement, 0.21—0.40 fair agreement, 0.41—0.60 moderate agreement, 0.61—0.80 substantial agreement, and 0.81—1 almost perfect agreement (Landis & Koch, 1977).

2.5 Statistical Analysis and Interpretation

Effect sizes were computed using means and SDs. Hedges' g—which provides the standardised difference between two groups (i.e., individuals with anxiety-based disorders vs healthy controls)—was the effect size estimate used in this study. The direction of effect size was standardised such that a negative Hedges' g suggested that levels of the inflammatory marker were lower in individuals with an anxiety-based disorder, whereas a positive Hedges' g suggested levels of the inflammatory marker were higher in individuals with an anxiety-based disorder, when compared with healthy controls.

Hedges' g was calculated for each individual measure of inflammation utilised by a study. Where two or more studies investigated the same inflammatory marker, the means and SDs were pooled to provide an overall effect size. Data was analysed using the software package Review Manager (RevMan) 5. 3 (The Nordic Cochrane Centre, 2014). A random-effects model of meta-analysis was used, which allows the true effect size to vary due to random error, or true variation in effect size between studies (Borenstein et al., 2009). This was considered important as the included studies were not identical in design. To account for variance in sample size, RevMan automatically assigns weighting to studies using inverse variance; where greater weighting is given to larger sample sizes (Higgins & Green, 2011). Effect sizes were interpreted using the boundaries proposed by Cohen (1988) whereby 0.2, 0.5, and 0.8 are considered small, medium, and large, respectively. As an estimate of

statistical significance, 95% confidence intervals (CIs) were calculated for individual and pooled effect sizes. A CI is deemed statistically significant when it does not include zero as a value (Thompson, 2007).

As unpublished and non-peer reviewed articles were excluded from this study, Orwin's (1983) Fail-safe N statistic (N_{fs}) was calculated to assess for publication bias, i.e., the tendency for journals to favour statistically significant results. The N_{fs} provides the number of unpublished studies required to reduce a finding to a small effect, and is outlined below:

$$N_{fs} = \frac{N(d - d_c)}{d_c}$$

Where N = the number of studies in the meta-analysis, d = the average effect size for the pooled studies, and d_c = the criterion value selected that *d* would equal when a knowable number of hypothetical studies (i.e., N*fs*) are added to the meta-analysis. The value for d_c was set at 0.2 (a small effect). The higher the N_{fs}, the more likely an effect size can be considered robust. For the purpose of this meta-analysis, findings were considered robust to publication bias when the N_{fs} value was greater than the number of studies contributing to the effect size being measured (i.e., $N_{fs} > N_{studies}$).

Heterogeneity between studies was assessed using Cochran's Q (also referred to as chi-squared, X^2) and the I^2 index. Cochran's Q calculates the weighted sum of squared differences between individual study effects and pooled effects across studies. When significant p-values (≤.05) for Q are observed, you can reject the null hypothesis that all studies share a common effect size (Borenstein et al., 2009). The I^2 value provides the percentage of variation across studies resulting from heterogeneity and not chance. An I^2 value of 0% indicates no heterogeneity, whereas values of 25%, 50%, and 75% represent low, moderate, and high heterogeneity, respectively (Higgins, Thompson, Deeks, & Altman, 2003).

A combination of these statistics was used to assess whether inflammatory markers differed between individuals with anxiety-based disorders and healthy controls. Specifically, group differences were considered meaningful if the weighted effect size was: (1) small (Hedge's g ≥ 0.2) to large (Hedge's g ≥ 0.80), (2) statistically significant (i.e., 95% CI≠ 0; p <.05), and (3) the N_{fs} score indicated that the finding was unlikely to be influenced by publication bias ($N_{fs} > N_{studies}$). Heterogeneity statistics were also considered when interpreting results.

Chapter 3

Results

3.1 Study Characteristics

This review included eight independent studies, published in peer-reviewed journals between 2009 and 2019 (see Table 3). The total pooled sample comprised of 347 individuals with anxiety-based disorders and 3,688 controls, totalling 4,035 individuals. The majority of studies were conducted in Turkey (*Nstudies* = 5), with single studies originating from England, Spain, and America. The primary study design was cross-sectional (*Nstudies* = 7), with one prospective study included (Khandaker et al., 2016). With the exception of Khandaker et al. (2016), samples sizes tended to be small, with a median of 81 (range = 33—3,392). The most common anxiety-based disorder investigated was OCD (*Nstudies* = 6), with single studies investigating PTSD (Ayadin et al., 2016) and GAD (Khandaker et al., 2016). Ozyurt and Binici (2019)—who recruited individuals with a primary diagnosis of OCD—also included individuals with co-morbid GAD, social anxiety disorder, and specific phobia. No studies investigating other anxiety-based disorders were found.

A total of 25 inflammatory markers were measured across the studies. Four studies measured TNF-α, three measured IL-1β, IL-6, neutrophils, lymphocytes, and WBCs, and two measured CRP, IL-12, IL-8, and IL-17A. Furthermore, individual studies measured monocytes, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD3^+$ T lymphocytes, $CD3^+$ HLA-DR (human leukocyte antigen-DR isotype) lymphocytes, $CD16^+$ 56^+ natural killer (NK) cells, TGF-β, sTNFR1, sTNFR2, CCL3, GM-CSF, IL-10, IL-4, IL-2, and NLR. All inflammatory markers were measured in blood. Most studies used blood serum samples (*Nstudies* = 4), one used blood plasma (Gabbay et al., 2009), and three did not report this information. Time of blood collection varied across the studies, but usually occurred before 12pm (*Nstudies* = 6). Three studies reported participant fasting, which ranged from 6-12 hours.

Anxiety-based disorders were primarily diagnosed using the Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime (K-SADS-PL) interview (*Nstudies* = 7), however, Khandaker et al. (2016) used the child version of the Development and Well Being Assessment (DAWBA). Participants with anxiety-based disorders were recruited from outpatient clinics or hospital settings in the majority of studies (*Nstudies* = 7). Controls were also primarily recruited from hospital settings (*Nstudies* = 5), however, one study recruited from schools (Rodriquez et al., 2017), and one used family members of staff (Gabbay et al., 2009). Khandaker et al. (2016) recruited all participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort.

Table 3.

Study Characteristics

Lead Author /year	Country	Study Design	Sample Size		Primary Diagnosis	Diagnostic Tool	Inflammatory Marker(s)	Sample Type	Time of Sample /fasting
			ABD	Control					
1.Ayadin (2016)	Turkey	Cross-sectional	10	33	PTSD	K-SADS-PL	Neutrophils, Lymphocytes, WBC, $CD3^+$ T, $CD3^+$ HLA-DR, $CD4^+$T, $CD8^+$, $CD16^+$ 56^+, monocytes	NR	8-10.30 am fasting NR
2. Colak Sivri (2018)	Turkey	Cross-Sectional	44	40	OCD	K-SADS-PL	TNF-α, TGF-β, IL-1β, IL-12, IL-17A, CRP, IL-8, sTNFR1, sTNFR2, CCL3	Serum	NR
3. Gabbay (2009)	America	Cross-sectional	16	17	OCD	K-SADS-PL	TNF-α, IL-1β, IL-12, IL-6	Plasma	8-9 am overnight fasting
4.Khandaker (2016)	England	Cohort, prospective	26	3366	GAD	DAWBA	CRP	Serum	Any time of day 6 hours fasting
5. Ozyurt (2019)	Turkey	Cross-sectional	60	128	OCD	K-SADS-PL	Neutrophils, Lymphocytes, WBC	NR	8.30-10.30 am 12 hours fasting
6. Rodriguez (2017)	Spain	Cross - sectional	102	47	OCD	K-SADS-PL	IL-1β, IL-6, TNF-α, IL-8, GM-CSF	Serum	Before 12pm fasting NR
7. Simsek (2016)	Turkey	Cross-sectional	34	34	OCD	K-SADS-PL	IL-17A, TNF-α, IL-10, IL-6, IL-4, IL-2	Serum	10-12 am fasting NR
8. Ucar (2019)	Turkey	Cross-sectional	31	47	OCD	K-SADS-PL	Neutrophils, Lymphocytes, NLR	NR	Early morning 12 hours fasting

Notes. ABD: anxiety-based disorder; PTSD: posttraumatic stress disorder; OCD: obsessive-compulsive disorder; GAD: generalised anxiety disorder; K-SADS-PL: Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime; DAWBA: Development and Well Being Assessment; NR: not reported; TNF: tumour necrosis factor; TGF: tumour growth factor; WBC: white blood cells; IL: interleukin; NLR: neutrophil-lymphocyte ratio; CRP: C-reactive protein. CD: cluster of differentiation; HLA-DR; human leukocyte antigen-DR isotype; sTNFRI: soluble tumour necrosis factor receptor; CC: chemokine; GM-CSF: granulocyte macrophage colony stimulating factor; NLR: neutrophil-lymphocyte ratio.

3.2 Participant Characteristics

Participant sociodemographic details are provided in Table 4. Age was well matched across individuals with anxiety-based disorders (M = 14.37, SD = 1.79) and controls (M = 14.76, SD = 1.74). The exact age range is unknown, as this information was not provided by the majority of studies. Samples were comprised of marginally more females for participants with anxiety-based disorders (56.20%) and controls (53.09%). BMI was reported by four studies, with a mean of 21.03 (SD = 3.51) for individuals with anxiety-based disorders and 20.69 (SD = 3.37) for controls. Five studies excluded individuals who were taking psychotropic or anti-inflammatory medication, two studies did not (Gabbay et al., 2009; Rodriguez et al., 2017) and one study did not report this information (Khandaker et al., 2016). Among individuals with an anxiety-based disorder, the percentage of participants taking medication was 27.95%. No controls were taking psychotropic or anti-inflammatory medication. Individuals with major physical co-morbidities were excluded from the majority of studies ($N_{studies}$ = 7), however, Khandaker et al. (2016) did not report exclusion criteria in relation to physical health. Individuals with mental health co-morbidities were excluded from three studies. Among the remaining studies, two included participants with co-morbid major depressive disorder (MDD) (Ayadin et al., 2016; Khandaker et al., 2016), tic disorder, and attention deficit hyperactivity disorder (ADHD)(Gabbay et al., 2009; Rodriguez et al., 2017). Additionally, one study included individuals with co-morbid paediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS)(Gabbay et al., 2009). Rodriguez et al. (2017) included participants with co-morbid anxiety or mood disorders but did not outline their specific diagnoses. Among individuals with anxiety-based disorders, 22.19% had at least one co-morbid psychological disorder (excluding other anxiety-based disorders). No controls had mental health disorders.

Table 4.

Participant Characteristics

Lead Author	Age in years (*M*, *SD*)		Female (N, %)		BMI (*M*, *SD*)		Medicated (% of participants*)	Physical Health Co-morbidities	Mental Health Co-morbidities (% of participants*)
	Anxiety-Based Disorder	Control	Anxiety-Based disorder	Control	Anxiety-Based Disorder	Control			
1. Ayadin	15.20 (0.90)	15.30(1.30)	27 (81%)	6 (60%)	22.40 (3.80)	21.70 (3.80)	EXCLUDED	EXCLUDED	MDD (84%)
2.Colak Sivri	14.50 (2.70)	14 (3)	22 (50%)	17 (42.50%)	NR	NR	EXCLUDED	EXCLUDED	EXCLUDED
3. Gabbay	12.60 (3.35)	15.10(3.30)	3(17.60%)	9 (56.25%)	NR	NR	88%	EXCLUDED	Tic disorder (100%) ADHD(65%) PANDAS (6%)
4. Khandaker	15.56 (0.24)	15.53(0.31)	23 (90%)	1760 (52.30%)	22.53 (3.52)	21.40 (3.63)	NR	NR	MDD (30.77%)
5. Ozyurt	14.61(1.81)	14.37 (1.80)	43 (72%)	101 (79%)	NR	NR	EXCLUDED	EXCLUDED	EXCLUDED
6. Rodriguez	14.78 (0.26)	16.29 (0.26)	50 (49%)	33 (71%)	NR	NR	80.40%	EXCLUDED	ADHD or Tic disorder (21.60%) Mood disorder (45.10%)
7. Simsek	12.80 (2.70)	12.80 (2.60)	11 (31.40%)	17 (50%)	19.70 (4.10)	19.70 (2.80)	EXCLUDED	EXCLUDED	Tic disorder (11.80%)
8. Ucar	14.90 (2.38)	14.65 (1.37)	16 (51.60%)	15 (31.90%)	19.50 (2.62)	19.97 (3.25)	EXCLUDED	EXCLUDED	EXCLUDED
Combined	14.37 (1.79)	14.76 (1.74)	195 (56.20%)	1958 (53.09%)	21.03 (3.51)	20.69 (3.37)	27.95%	—	22.19%

Notes. *M*: mean; *SD:* standard deviation; N: number of participants; BMI: body mass index; medicated: refers to psychotropic or inflammatory medication; physical co-morbidities: including acute illness or disease known to cause inflammation; mental health co-morbidities: excluding other anxiety-based disorders; NR: not reported; MDD: major depressive disorder; PANDAS: paediatric autoimmune neuropsychiatric disorder associated with streptococcal infections ; ADHD; attention deficit hyperactivity disorder. * % refers only to individuals with anxiety-based disorders, no controls were taking medication or reported having mental health disorders.

3.3 Quality Assessments of Included Studies

Substantial agreement was observed between the two independent raters in regard to study quality ($\kappa = 0.62$, 95% CI [0.43 to 0.81], $p < .001$). Reporting quality across the studies was adequate, with an average raw score of 18.88 out of a possible 22 (*SD* = 1.17, range = 17—20) and percentage score of 85.81% (range: 77.27—90.90%). Therefore, all studies met the inclusion threshold proposed by Kmet et al. (2004) of 75% of items (see Figure 3 and Appendix C). Specifically, all studies clearly articulated their objectives and used appropriate study designs (criterion 1 and criterion 2: 100% fulfilled). Reporting of the recruitment method often lacked sufficient detail, particularly in regard to control participants (criterion 3: 62.50% fulfilled). Subject characteristics (e.g., gender and age) and outcomes were well reported (criterion 4 and 5: 87.5% fulfilled). Sample sizes tended to be inadequate in size and were underpowered for both Mann-Whitney tests (used for non-parametric data) and independent sample t-tests (used for parametric data: criterion 6: 37.5% fulfilled). More specifically, when adopting power = 0.80 and a significance level of $\alpha = 0.05$, seven studies met the minimum sample size required to reliably detect a large effect, three met the minimum sample size to reliably detect a medium effect, and none met the sample size required to reliably detect a small effect (Cohen, 1988).

Analytic procedures were only partially reported; in particular, the median, inter-quartile range (IQR) and log-transformation of non-parametric data was not always provided or described in adequate detail (criterion 7: 37.5% fulfilled). All studies reported some measure of variance (criterion 8: 100% fulfilled). Although most studies adopted appropriate exclusion criteria (e.g., acute illness), control for confounding variables was not always undertaken or clearly reported, particularly in regard to BMI, mental health co-morbidities, and medication use (criterion 9: 50% fulfilled). Most studies reported results in sufficient detail (criterion 10: 87.5% fulfilled), and conclusions generally followed from results (criterion 11: 62.5% fulfilled).

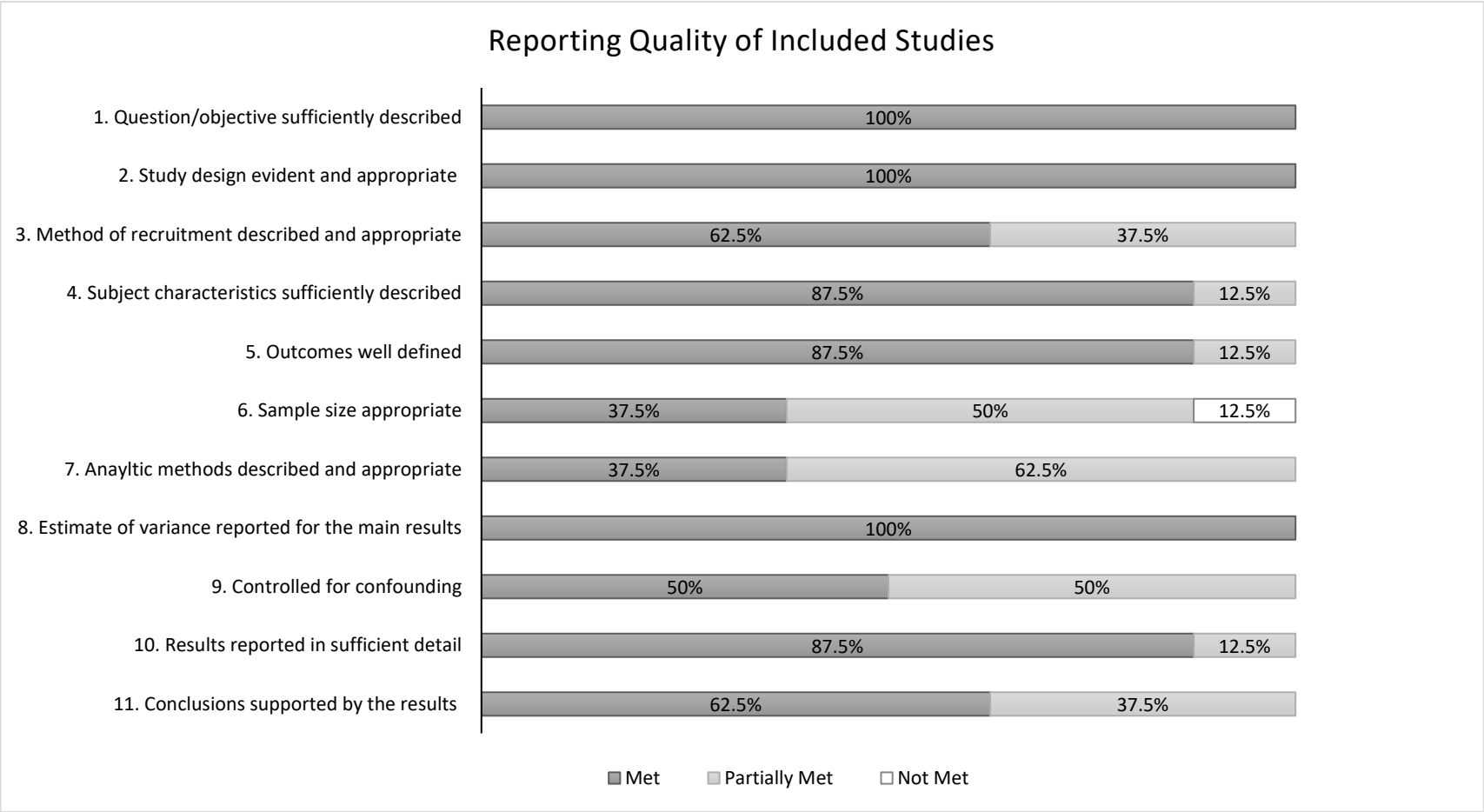

Figure 3. Percentage of included studies which met the reporting quality criteria based on the Qualsyst tool (Kmet et al., 2004

3.4 Meta-analyses

3.4.1 Cytokines

Four studies compared TNF-α levels among children and adolescents with anxiety-based disorders and healthy controls, the results of which are displayed in Figure 4. The overall between-group difference was small and non-significant (Hedges' g = 0.35, 95% CI [-0.18, 0.89], p = .19, $N_{fs} < N_{studies}$). However, substantial heterogeneity between studies was reported (Table 5 displays heterogeneity statistics for all meta-analyses). Specifically, large and significant group differences were reported by Colak Sivri et al. (2018) and Simsek et al. (2016), who found TNF-α to be increased among participants with anxiety-based disorders. Conversely, small and non-significant group differences were reported by Gabbay et al. (2009) and Rodriguez et al. (2017). Studies that reported increased TNF-α levels excluded individuals who were taking medication, which may explain the observed heterogeneity. The fail-safe N of three indicates that this finding may be influenced by publication bias.

Data from three studies investigating IL-1β levels in individuals with anxiety-based disorders and healthy controls is displayed in Figure 5. Overall, no significant between-group difference was observed (Hedges' g = - 0.04, 95% CI [-0.41, 0.34], p = .85, $N_{fs} < N_{studies}$), and heterogeneity was moderate.

Figure 6 displays results from three studies which investigated IL-6 levels in individuals with anxiety-based disorders and healthy controls. A small, negative effect size was observed, indicating mean IL-6 levels were lower among individuals with anxiety-based disorders; however, this difference was not statistically significant (Hedges' g = -0.11, 95% CI [-0.44, 0.21], p = .49, $N_{fs} < N_{studies}$). Low heterogeneity was observed between studies.

Figure 7 displays the meta-analysis of two studies investigating IL-17A levels. A small but non-significant between-group difference was observed (Hedges' g = 0.16, 95% CI [-0.71,1.03], p = .72, $N_{fs} < N_{studies}$), and high heterogeneity was reported. Specifically, although Simsek et al. (2016) found significantly higher levels of IL-17A among individuals with anxiety-based disorders, Colak Sivri et al. (2018) found lower but not statistically significant levels of IL-17A.

The meta-analysis of two studies comparing IL-8 levels (Figure 8) found no overall between-group difference between individuals with anxiety-based disorders and controls (Hedges' g = 0.02, 95% CI [-0.54, 0.57], p = .96, $N_{fs} < N_{studies}$). High heterogeneity was reported.

Figure 9 depicts results from two studies comparing IL-12 levels. No significant difference was observed between individuals with anxiety-based disorders and controls (Hedges' g = 0.10, 95% CI [-0.73, 0.93], p = .82, $N_{fs} < N_{studies}$), and heterogeneity was high.

Lead Author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	0.87	0.42	1.32			
Gabby	1	33	-0.37	-1.05	0.32			
Rodriguez	1	142	0.04	-0.03	0.40			
Simsek	1	68	0.76	0.27	1.26			
Overall	4	327	0.35	-0.18	0.89	.19	3	

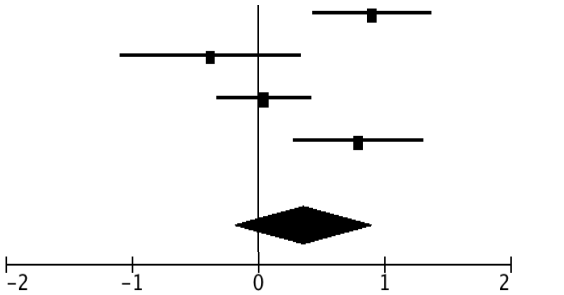

Figure 4. Random effects meta-analysis of TNF-α levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI = lower and upper limit of the 95% confidence interval.

Lead Author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	-0.37	-0.80	0.07			
Rodriguez	1	143	0.03	-0.32	0.39			
Gabbay	1	33	0.39	-0.30	1.08			
Overall	3	260	-0.04	-0.41	0.34	.85	0	

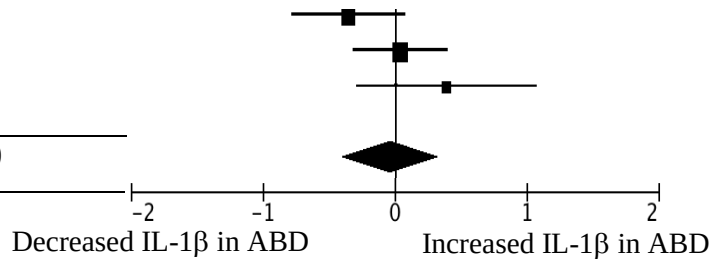

Figure 5. Random effects meta-analysis of IL-1β levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of 95% confidence interval.

Lead Author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Gabbay	1	33	-0.19	-0.87	0.50			
Rodriguez	1	118	-0.31	-0.69	0.08			
Simsek	1	68	0.19	-0.28	0.67			
Overall	3	219	-0.11	-0.44	0.21	.49	0	

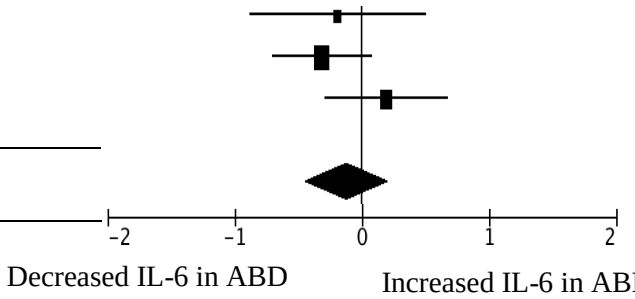

Figure 6. Random effects meta-analysis of IL-6 levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead Author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	-0.28	-0.71	0.15			
Simsek	1	68	0.61	0.13	1.10			
Overall	2	152	0.16	-0.71	1.03	.72	0	

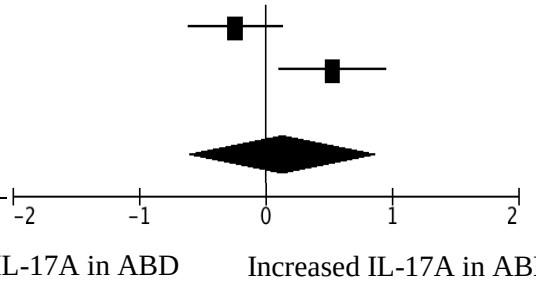

Figure 7. Random effects meta-analysis of IL-17A levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	0.31	-0.12	0.74			
Rodriguez	1	119	-0.26	-0.64	0.12			
Overall	2	203	0.02	-0.54	0.57	.96	0	

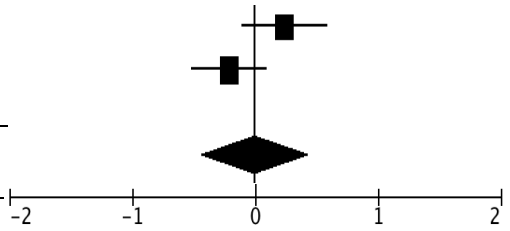

Figure 8. Random effects meta-analysis of IL-8 levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead author	*Nstudies*	*Nparticipants*	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	-0.28	-0.71	0.15			
Gabbay	1	33	0.57	0.13	1.27			
Overall	2	117	0.10	-0.73	0.93	.82	0	

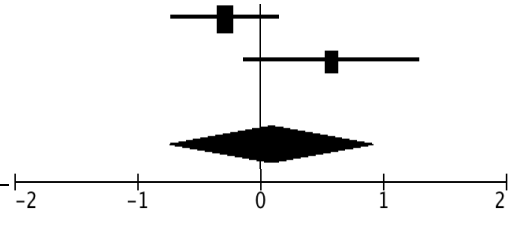

Figure 9. Random effects meta-analysis of IL-12 levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

3.4.2. C-Reactive Protein

Figure 10 displays pooled data from two studies investigating CRP levels. A moderate but non-significant between-group difference was found (Hedges' g = 0.63, 95% CI [-0.94, 2.21], p = .47, $N_{fs} > N_{studies}$). Heterogeneity between studies was high; more specifically, Khandaker et al. (2016) reported a large and significant between-group difference, with higher CRP levels observed among individuals with GAD. Conversely, Colak Sivri et al. (2018) found individuals with OCD had lower CRP levels, however this difference was not significant. The observed heterogeneity may reflect the difference in primary mental health diagnosis (OCD vs GAD). The fail-safe N was acceptable, however, should be interpreted with caution as the meta-analysis was based on only two studies.

3.4.3 White Blood Cells

Three studies compared total WBC counts between individuals with anxiety-based disorders and healthy controls (see Figure 11). A small, non-significant between-group difference was observed (Hedges' g = 0.04, 95% CI [-0.76, 0.83], p = .93, $N_{fs} < N_{studies}$). Heterogeneity between studies was high; although Ozyurt and Binici (2019) found individuals with OCD had significantly higher WBC counts, Ayadin et al. (2016) and Ucar, Murat, and Eray (2019) found individuals with PTSD and OCD, respectively, had lower but not statistically significant WBC counts.

Three studies provided data on neutrophil levels, displayed in Figure 12. A small, non-significant between-group difference was observed (Hedges' g = 0.13, 95% CI [-0.64, 0.89], p =.74, $N_{fs} < N_{studies}$), with large heterogeneity. Specifically, Ozyurt and Binici (2019) found individuals with OCD had significantly higher neutrophil levels, whereas Ucar et al. (2019) found no difference between groups, and Ayadin et al. (2016) reported lower, but not statistically significant levels of neutrophils among individuals with PTSD.

Figure 13 depicts results of three studies investigating lymphocyte levels. A small negative effect was observed, indicating lymphocytes were decreased in individuals with anxiety-based disorders when compared with controls, however, this was not statistically significant (Hedges' g = - 0.20, 95% CI [-0.54, 0.14], p = .25, $N_{fs} < N_{studies}$). Heterogeneity was moderate: although Ucar et al. (2019) found individuals with OCD had significantly lower levels of lymphocytes, Ozyurt et al. (2019) and Ayadin et al. (2016) did not find a statistically significant between-group difference.

Lead author	*Nstudies*	*Nparticipants*	Hedges' *g*	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Colak Sivri	1	84	-0.17	-0.60	0.26			
Khandaker	1	3356	1.44	1.05	1.83			
Overall	2	3440	0.63	-0.94	2.21	.43	4	

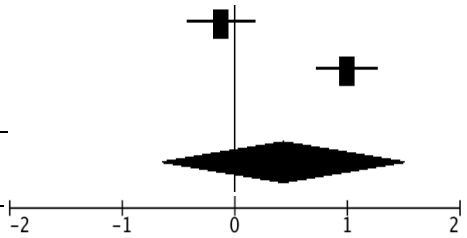

Figure 10. Random effects meta-analysis of CRP levels in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead author	*Nstudies*	*Nparticipants*	Hedges' *g*	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Ayadin	1	43	-0.54	-1.25	0.18			
Ozyurt	1	188	0.73	0.42	1.05			
Ucar	1	78	-0.21	-0.67	0.24			
Overall	3	309	0.04	-0.76	0.83	.93	0	

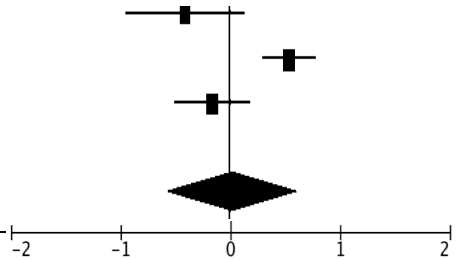

Figure 11. Random effects meta-analysis of total WBC count in individuals with anxiety-based disorders (ABD) compared with controls. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead author	*N*studies	*N*participants	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Ayadin	1	43	-0.59	-1.31	0.13			
Ozyurt	1	188	0.79	0.47	1.11			
Ucar	1	78	0.02	-0.43	0.48			
Overall	3	309	0.13	-0.64	0.89	.74	0	

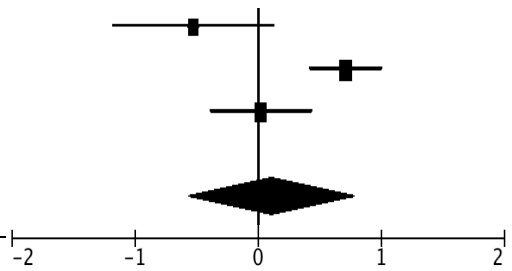

Figure 12. Random effects meta-analysis of neutrophil levels in individuals with anxiety-based disorders (ABD) compared with controls. *N*studies = number of studies providing data; *N*participants = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Lead author	*N*studies	*N*participants	Hedges' g	95% CI Lower	95% CI Upper	*p*	Fail-safe N	Hedges' g and 95% CI
Ayadin	1	43	-0.18	-0.89	0.53			
Ozyurt	1	188	0	-0.31	-0.31			
Ucar	1	78	-0.52	-0.98	-0.06			
Overall	3	309	-0.20	-0.54	0.14	.25	0	

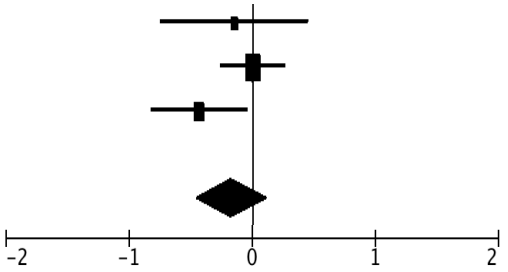

Figure 13. Random effects meta-analysis of lymphocyte levels in individuals with anxiety- based disorders (ABD) compared with controls. *N*studies = number of studies providing data; *N*participants = number of participants providing data; 95% CI: lower and upper limit of the 95% confidence interval.

Table 5.

Heterogeneity Statistics for Meta-Analyses

Inflammatory Marker	*Nstudies*	*Nparticipants*	Q	*p*	I^2
TNF-α	4	327	14.79	<.001	80%
IL-1β	3	260	3.83	.15	48%
Il-17A	2	152	7.24	.007	86%
Il-8	2	203	3.74	.05	73%
IL-12	2	117	4.15	.04	76%
IL-6	3	219	2.61	.27	23%
CRP	2	3440	29.85	<.001	97%
Lymphocytes	3	124	3.35	.19	40%
Neutrophils	3	124	15.56	<.001	87%
WBC count	3	124	17.21	<.001	88%

Notes. *Nstudies* = number of studies providing data; *Nparticipants* = number of participants providing data; Q = Cochran's Q (chi-squared); *p* = associated *p*-value, a significant *p* value (≤.05) indicates homogeneity; I^2 = measure of study inconsistency, expressed as a percentage; TNF: tumour necrosis factor; IL: interleukin; CRP: C-reactive protein; WBC: white blood cell.

3.5 Findings from Individual Studies

A further 15 inflammatory markers were measured by individual studies included in this review. Hedges' g and 95% confidence intervals for these markers are displayed in Figure 14. A significant, moderate Hedges' g was observed for IL-2 levels (Hedges' g = 0.57, 95% CI [0.08,1.05]), indicating that individuals with OCD showed significantly higher IL-2 levels when compared with healthy controls (Simsek et al., 2016). No other inflammatory markers differed significantly between groups.

Lead Author	Diagnosis	Inflammatory Marker	*N*participants	Hedges' g	95% CI Lower	95% CI Upper	Hedges' g and 95% CI
Ayadin	PTSD	$CD3^+$ T lymphocytes	43	-0.21	-0.91	-0.50	
		$CD3^+$ HLA-DR lymphocytes	43	-0.48	-1.20	0.23	
		$CD4^+$ T lymphocytes	43	-0.23	-0.94	0.47	
		$CD8^+$ lymphocytes	43	-0.18	-0.89	0.53	
		$CD16^+ 56^+$ NK cells	43	-0.19	-0.90	0.52	
		monocytes	43	-0.39	-1.10	0.32	
Colak Sivri	OCD	TGF-β	84	-0.26	-0.69	0.17	
		sTNFR1	84	-0.28	-0.71	0.15	
		sTNFR2	84	-0.11	-0.54	0.32	
		CCL3	84	0.20	-0.23	0.63	
Rodriguez	OCD	GM-CSF	149	0.09	-0.27	0.44	
Simsek	OCD	IL-2	68	0.57	0.08	1.05	
		IL-4	68	0.48	0	0.96	
	OCD	IL-10	69	-0.04	-0.52	0.43	
Ucar	OCD	NLR	78	0.06	-0.39	0.51	

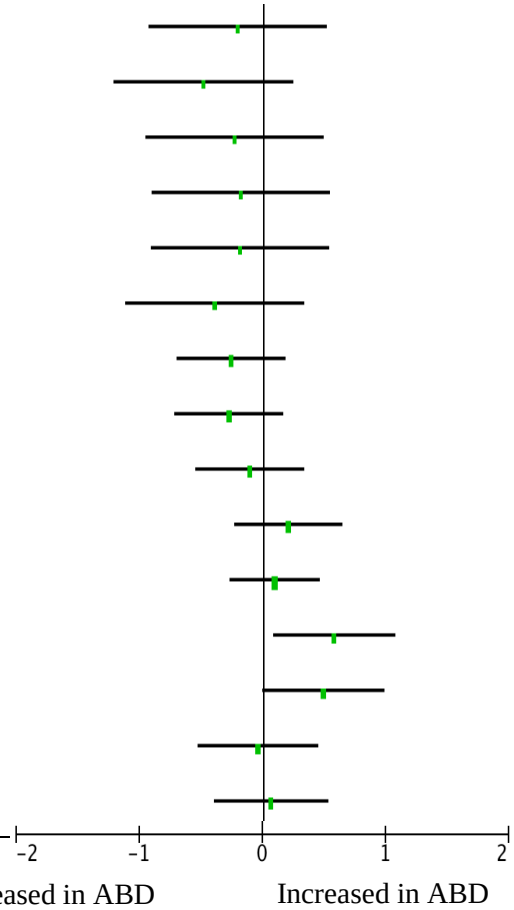

Figure 14. Hedges' g and 95% CIs comparing individuals with anxiety-based disorders (ABD) and controls for inflammatory markers investigated in individual studies. CD: cluster of differentiation; HLA-DR; human leukocyte antigen-DR isotype; NK: natural killer; TGF: transforming growth factor; sTNFR: soluble tumour necrosis factor receptor; CC: chemokine; GM-CSF: granulocyte macrophage colony stimulating factor; IL: interleukin; NLR: neutrophil-lymphocyte ratio.

Chapter 4
Discussion

4.1 Key Findings

This systematic review and meta-analysis examined data from eight studies (*Nparticipants* = 4035) to investigate the role of inflammation in anxiety-based disorders among children and adolescents. Meta-analyses of six cytokines, CRP, total WBC count, lymphocytes, and neutrophils were conducted, as well as quality assessments for all included studies. No significant differences were observed between children and adolescents with anxiety-based disorders and healthy controls for any inflammatory marker. However, these findings were based on a small number of studies with substantial heterogeneity. Furthermore, limitations in study quality were observed. Consequently, it is premature to conclude whether inflammation plays a role in anxiety-based disorders among this population. This chapter will critically discuss these findings with reference to their implications in clinical and research settings.

4.1.1 Cytokines

Results from meta-analyses of six cytokines—TNF-α, IL-1β, IL-6, IL-17A, IL-8, and 1L-12—showed no differences between children and adolescents with anxiety-based disorders and healthy controls. All studies included in these meta-analyses investigated individuals with a primary diagnosis of OCD. As Renna et al. (2018) found diagnosis (i.e., PTSD, OCD, or other anxiety disorder) to have a moderating effect on inflammation, the findings from these meta-analyses may not be applicable to other anxiety-based disorders.

Although the meta-analysis of TNF-α levels was not significant, studies by Colak Sivri et al. (2018) and Simsek et al. (2016) did report significantly higher TNF-α levels among children and adolescents with OCD. The studies which did not find group differences in TNF-α levels (Gabbay et al., 2009; Rodriguez et al., 2017) included participants who were taking psychotropic medication, which may explain the observed heterogeneity. In the broader literature, differences in TNF-α levels have also been reported between medicated and non-medicated adults with PTSD (Passos et al., 2015). Psychotropic medication may modulate immune activation; for example, the tricyclic antidepressant clomipramine and selective serotonin reuptake inhibitor (SSRI) fluoxetine have been associated with decreased TNF-α levels (Baumeister, Ciufolini, & Mondelli, 2016), and these treatments are commonly used in OCD (Simeon, Thatte, & Wiggins, 1990) and other anxiety-based disorders (Allen,

Leonard, & Swedo, 1995). Although this review provides preliminary evidence that TNF-α levels may be increased in non-medicated children and adolescents with OCD, further research is needed to replicate this finding and delineate the effect of psychotropic medication on cytokines and inflammation.

Increased IL-17A and IL-2 levels were observed by one study on non-medicated children and adolescents with OCD (Simsek et al., 2016). However, these findings should be interpreted with caution as they are based on a single study. Findings of this review differ from a meta-analysis by Gray and Bloch (2012) which found reduced IL-1β levels among adults and children with OCD, and reduced IL-6 levels among medicated children with OCD. Gray and Bloch (2012) included studies with adult populations, and combined studies investigating OCD and tic disorder, which may explain the observed discrepancies. Differences were also observed between research investigating inflammation in other psychological disorders, where meta-analyses have reported increased IL-6, IL-12, and TNF-α levels among adults with depression (Dowlati et al., 2010; Howren et al., 2009; Kohler et al., 2017), and IL-1β, TNF-α (Renna et al., 2018) and IL-6 (Passos et al., 2015) levels among adults with PTSD. It is unclear whether these discrepancies reflect differences in the biological mechanisms—in relation to immune and inflammatory responses—between the different study populations (adults vs children/adolescents) or diagnoses. Further research which encompasses a range of anxiety-based disorders may help disentangle these relationships.

4.1.2 C-Reactive Protein

The meta-analysis of CRP levels reported no significant difference between children and adolescents with anxiety-based disorders and healthy controls. However, this finding should be interpreted with caution as it is based of two studies, with large heterogeneity. Khandaker et al. (2016) found participants with GAD had significantly higher levels of CRP compared to those without GAD, which remained after adjusting for a range of confounders, including: age, sex, BMI, and ethnicity. Conversely, Colak Sivri et al. (2018) found lower, but not statistically significant levels of CRP among participants with OCD. This heterogeneity may be explained by the difference in primary diagnosis of participants (GAD vs OCD). GAD and OCD are known to have different clinical features in relation to symptomology (Brown, Moras, Zinbarg, & Barlow, 1993), and the results of this review

provide preliminary evidence that biological differences may also underlie these conditions, in terms of their association with inflammation and immune dysregulation.

Previous research by Renna et al. (2018) found no evidence for dysregulated CRP levels among adults with a range of anxiety-based disorders; however, a meta-analysis of CRP and GAD using primarily adult studies found evidence for increased levels of CRP in GAD (Costello, Gould, Abrol, & Howard, 2019). A longitudinal study by Copeland et al. (2012)—which could not be included in this meta-analysis due to missing baseline data—reported increased CRP levels in children and adolescents with GAD through both bivariate and cross-sectional associations. However, these associations were attenuated in models which adjusted for health-related covariates, providing evidence that BMI and medication use mediated the CRP-GAD relationship. Khandaker et al. (2016) did not adjust for medication use, which may explain the discrepancy in their findings. Future research is needed to replicate the findings of Khandaker et al. (2016) in relation to GAD and CRP levels, considering medication use as a possible confounder. This review highlights the lack of research investigating CRP and other anxiety-based disorders among children and adolescents, indicating a need for future research.

4.1.3 White Blood Cells

No significant differences were observed for meta-analyses investigating white blood cell count parameters—total WBC, neutrophils, and lymphocytes—between children and adolescents with anxiety-based disorders and healthy controls. Ozyurt and Binici (2019) reported significantly higher total WBC and neutrophil levels among individuals with OCD. Contrastingly, Ucar et al. (2019) and Ayadin et al. (2016) found no significant differences in total WBC and neutrophil levels. Ucar et al. (2019), however, reported significantly lower lymphocyte levels among individuals with OCD, whereas Ayadin et al. (2016) and Ozyurt and Binici (2019) did not find any significant differences. Ozyurt and Binici (2019) included a sample of participants with co-morbid anxiety disorders, specifically: GAD, social anxiety disorder, and specific phobia. Subgroup analyses found inflammation was higher among these individuals (Ozyurt & Binici, 2019. It may be that symptom severity is higher among individuals with co-morbidities, leading to an increase in inflammation.

Few studies in the literature have investigated WBC parameters in relation to mental health disorders. However, higher WBC counts are associated with increased symptom severity in adult men with depression and anxiety (Shafiee et al., 2017) and correlate with mood symptom severity in bipolar (Kohler et al., 2017). Furthermore, Maes et al. (1992)

found WBC, neutrophil, and monocyte levels were disturbed in adults with depression, noting that severity of illness was associated with higher levels of WBC parameters. Future research is needed to investigate WBC parameters in a range of anxiety-based disorders, taking into account symptom severity.

4.1.4 Quality Findings

Findings from this review should be interpreted with reference to study quality. Quality assessments identified a number of methodological limitations in the included studies, which may help inform future research. The primary limitation was small sample sizes, resulting in underpowered studies. Low statistical power leads to an increase in Type II errors —the failure to reject a false null hypothesis—reducing the probability of finding a difference between groups, where a difference exists (Christley, 2010). No studies were sufficiently powered to reliably detect a small effect, and only three studies were sufficiently powered to reliably detect a moderate effect. This is problematic as previous research on inflammation in adults with depression (Howren et al., 2009) and anxiety-based disorders (Renna et al., 2018) indicates that effect sizes are small to moderate. Low statistical power may also lead to an increased likelihood that a statistically significant finding is a Type I error; the rejection of a true null hypothesis. Due to the file-draw problem—the tendency for journals to favour significant findings—the use of underpowered studies contributes to inconsistent and misleading results in the literature (Christley, 2010).

Limitations were also identified in relation to recruitment method and reporting. The vast majority of studies recruited participants from hospital settings (e.g., outpatients of psychiatric wards). Although recruiting from hospital settings has clear advantages in relation to access to participants and ease in acquiring biological data, these samples may be bias in regard to the severity and acuity of clinical presentation (Carr et al., 2002). Consequently, findings based on these samples may not be generalisable to other sample sources, or the broader population of individuals with anxiety-based disorders. Controls were also primarily recruited from hospital settings; however, their reason for admittance was not reported. This is important as many factors can influence inflammation, one of which is acute and chronic stress (Maydych, 2019). Even in the absence of acute illness, admittance to hospital may be associated with acute stress. Gabbay et al. (2009) recruited controls from family members of staff. Similarly, it is questionable as to whether these controls are representative of the healthy population. Future research would benefit from large community-based samples which better represent the population of interest.

In relation to analytic procedures, the reporting of statistics and data analysis was not always provided in adequate detail. Complications can arise when conducting meta-analyses which incorporate skewed data, as raw data is often reported in different forms (e.g., means and SDs vs medians and IQRs), or is log transformed (Higgins, White, & Anzures-Cabrera, 2008). Standardised reporting procedures for non-parametric data should be adhered to (Whitley & Ball, 2002), and where data has been transformed, summary statistics of the raw data should be provided, along with sufficient description of the transformation which was applied, and reason for the transformation (Manikandan, 2010). This allows reviewers to extract the appropriate data.

A number of potential confounding variables were not controlled for by studies, such as BMI, medication use, and co-morbid mental health disorders. A higher BMI has been linked to increased inflammation (Kitahara, Trabert, Katki, 2014; Festa et al., 2001); however, only four studies measured and reported participant BMI. Psychotropic medications have also been shown to modulate immune activation and the inflammatory response (Baumeister et al., 2016). Samples of two studies (Gabbay et al., 2009; Rodriguez et al., 2017) included a large number of participants taking medication (88% and 80.40%, respectively). In addition to the potential biological effects of medication on inflammation, individuals who are taking medication may differ from those who are unmedicated in relation to symptom severity. Samples also comprised of individuals with mental health co-morbidities. Two studies (Ayadin et al., 2016; Khandaker et al., 2016) included individuals with co-morbid depression, which has been associated with increased levels of IL-6, IL-1, TNF-α, and CRP in adults (Dowlati et al., 2010; Howren et al., 2009; Kohler et al). Two studies included participants with ADHD and tic disorder (Gabbay et al., 2009; Rodriguez et al., 2017) which have also been associated with inflammation and immune dysregulation in adults (Anand, Colpo, Zeni, Zeni, & Teixeira, 2017; Krause & Muller, 2012). As medication use (Hollingworth, Burgess & Whiteford, 2010), and co-morbidities (Jacobi et al. 2004) are common among individuals with mental health disorders, excluding these participants may result in samples which are unrepresentative of the population of interest. Alternatively, recruiting samples which are sufficiently sized to measure and control for the effect of these variables may increase study quality.

4.1.5 Study Heterogeneity

High and statistically significant heterogeneity was reported among all but one meta-analysis (IL-6). High heterogeneity is characteristic of research in this area and has been observed by meta-analyses examining inflammation in adults with anxiety-based disorders (Renna et al., 2018), and depression (Kohler et al., 2017; Dowlati et al., 2010; Howren et al., 2009). Due to the limited number of studies in this review, potential sources of heterogeneity could not be statistically investigated through moderator analyses. However, the observed heterogeneity may reflect differences in participant characteristics (e.g., BMI, medication use, and co-morbid mental health conditions) and in study characteristics (e.g., blood sampling procedures).

Variations in blood sampling procedures—the time of blood sample and participant fasting—were observed between studies. Although most studies sampled blood in the morning, this varied by up to 6 hours, and Khandaker et al. (2016) sampled blood at any time of the day. This is important as many markers of inflammation follow diurnal rhythms which peak in early morning (Petrovsky, McNair, & Harrison, 1998). Furthermore, participant fasting ranged from 6-12 hours. In response to issues of study reproducibility, validity, and reliability in research involving blood-based biomarkers, Andreazza et al. (2019) have provided guidelines for the standardised collection of blood samples. These guidelines include the reporting of participant age, gender, race, ethnicity, BMI, time of withdrawal, menstrual cycle/ contraceptive use, fasting status, diet, smoking status, and use of alcohol or other drug use. Participant medication use, co-morbidities, family history of neuropsychiatric disorder, and symptom severity should also be measured. Furthermore, a fasting time of 9-10 hours has been suggested. Andreazza et al. (2019) also provide detailed recommendations specific to blood collection procedures (e.g., storage, sample shipment, and data management). Implementation of these guidelines should minimise laboratory-testing errors and improve reproducibility of research in this area.

Another potential source of study heterogeneity is the dichotomous categorisation of participants (i.e., anxiety-based disorder vs healthy controls). This may lead to individuals with severe symptomology being grouped with those who only just meet the diagnostic criteria for an anxiety-based disorder. Categorising individuals in this manner leads to a loss of information concerning symptom severity. As few included studies measured symptom severity, only group differences could be examined. Conway et al. (2019) have suggested that research in mental health may benefit from adopting a hierarchical approach to mental illness

which focuses on psychological symptoms rather than categorical diagnoses. Future research may benefit from the addition of symptom severity measures.

4.2 Clinical Implications and Future Research

Based on the results of this review, it cannot be concluded whether inflammation plays a role in anxiety-based disorders among children and adolescents. However, there remains important, potential clinical implications of research in this area; primarily, in relation to therapeutic interventions. Among adults, anti-inflammatory drugs—in combination with antipsychotics and antidepressants—have shown a beneficial effect on symptoms of patients with schizophrenia (Cho et al., 2019) and depressive symptoms in MDD (Faridhosseini, Sadeghi, Farid, & Pourgholami, 2014). If inflammation does play a role in the aetiology or severity of anxiety-based disorders, similar treatment options may prove beneficial. Examining the link between inflammation and anxiety-based disorders in children and adolescents may lead to early treatment options for these individuals, reducing the severity and duration of these disorders. Furthermore, as anxiety is linked with inflammatory conditions such as metabolic syndrome (Carroll et al., 2009), coronary heart disease (Roest et al., 2010), and atherosclerosis (Seldenrijk et al., 2010), early treatment may decrease the incidence of these co-morbidities and improve the overall health of these individuals.

In light of these implications, this review highlights the need for further, high quality research in this area. To ensure progress is made in this emerging field, the following methodological recommendations should be adhered to. First, studies should aim to recruit large sample sizes, which are sufficiently powered to detect small to moderate effects. Recruitment and retention of participants in clinical trials is often the most labour-intensive and difficult aspect of research (Furimsky, Cheung, Dewa, & Zipursky, 2008). Gaining consent for blood collection is particularly problematic among a population characterised by anxiety. Furthermore, additional barriers exist in conducting clinical studies of children and adolescents, in regard to family engagement. Furimsky et al. (2008) provide a review of barriers and potential solutions for conducting research in this area, in which they recommend collaboration between research and treatment teams, incentives, and flexibility in scheduling visits. The Australian Department of Health (2016) also offer suggestions for the recruitment and retention of clinical participants, including: raising awareness among health care professionals of clinical research before research commences, dedicating research staff to recruitment procedures, improving training of research staff to ensure communication and

trust with participants, and emphasising the benefits of research to potential clinical participants, in relation to therapeutic treatments.

Second, researchers should adhere to the basic guidelines provided by Andreazza et al. (2019) in relation to blood collection procedures, ensuring their methodology is reported in sufficient detail. In particular, it is recommended that researchers include the measurement and analysis of symptom severity, BMI, specific medication use, and co-morbidities. Finally, studies should aim to report statistical procedures with sufficient clarity, to enhance replicability and ensure their inclusion in relevant reviews.

4.3 Study Limitations and Strengths

A number of methodological limitations should be considered when interpreting findings from this review. First, despite the use of intensive search strategies for major databases, this study did not search grey literature, leaving the possibility that unpublished studies were missed. Furthermore, the eligibility criteria required that participants meet the diagnostic criteria for an anxiety-based disorder. This did not allow the investigation into trait anxiety or anxiety symptoms. Future research may benefit from investigating the relationship between inflammation and anxiety symptomology.

Second, although meta-analytic techniques increase statistical power and precision, Borenstein et al. (2009) suggest that for sufficient power, meta-analyses include at least four studies, particularly when adopting a random-effects model (Jackson & Turner, 2017). Ideally meta-analyses would include a larger number of studies, however, the statistical combination of only two studies is observed in the literature (Friede, Rover, Wandel, & Neuenschwander, 2017). Despite the limited number of studies, the included meta-analyses still provide a valuable overview of the current research in this area. The lack of studies included in this review should be viewed as evidence that this area is under researched.

Finally, as data from the majority of studies was skewed, Hedges' g—calculated using means and SDs—may not accurately reflect the true distribution of the data. Making inferences based on the means of skewed data is valid when sample sizes are large, according to central limit theorem. However, using raw outcome measurements is problematic with small samples, as the mean and SD may be influenced by outliers and extreme values (Higgins et al., 2008). However, this remains the most effective way of combining studies based on the available data, and is the current standard practice observed by other meta-analyses in this area (e.g., Renna et al., 2018).

Despite these limitations, the current review also contains strengths. Primarily, it represents the first comprehensive review of inflammation and anxiety-based disorders among the population of children and adolescents. Furthermore, the quality assessments have highlighted important limitations in current methodology, and avenues through which future research can improve.

4.4 Conclusions

This systematic review and meta-analysis provides an overview of the literature on inflammation and anxiety-based disorders in children and adolescents. Although no significant effects were observed in relation to any inflammatory marker, the small number of included studies and limitations in study quality render these findings provisional. Research in this area has the potential for important clinical implications in relation to therapeutic interventions. This review highlights the need for further, high quality research, which encompasses a broad range of anxiety-based disorders. By adhering to the recommendations provided by this study, issues of heterogeneity and reproducibility may be minimised, leading to a greater understanding of the potential relationship between anxiety and inflammation among this population.

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Appendices

Appendix A

Search Strategies by Database

PsycINFO Logic Grid

AND →

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
Inflammation.sh OR	Anxiety Disorders.sh OR	Childhood birth 12 yrs.ag
Inflamm*.tw OR	Anxiety disorder*.tw OR	OR
Exp neuroimmunology OR	Anxiety based disorder*.tw	Adolescence 13 17 yrs.ag
Neuroimmun*.tw OR	OR	OR
Psychoimmun*.tw OR	exp Posttraumatic stress	Adolescen*.tw OR
Immun*.tw OR	disorder OR	Child*.tw OR
Exp Cytokines OR	Posttraumatic stress	Youth.tw OR
Cytokine*.tw OR	disorder*.tw OR	Paediatric.tw OR
Tumor necrosis factor*.tw	Post traumatic stress	Pediatric.tw OR
OR	disorder*.tw OR	Teen*.tw
Tumour necrosis factor*.tw	PTSD.tw OR	
OR	Generalized anxiety	
TNF*.tw OR	disorder.sh OR	
C-reactive protein.tw OR	Generalized anxiety	
CRP.tw OR	disorder*.tw OR	
Interleukin*.tw OR	Generalised anxiety	
Chemokine*.tw OR	disorder*.tw OR	
White blood cell*.tw	Social Phobia.sh OR	
	Social phobia*.tw OR	
	Social anxiety disorder*.tw	
	OR	
	obsessive compulsive	
	disorder.sh OR	
	obsessive compulsive	
	disorder*.tw OR	
	OCD.tw OR	
	Agoraphobi*.tw OR	
	Phobi*.tw OR	
	Panic disorder.sh OR	
	Panic disorder*.tw OR	
	Separation anxiety	
	disorder.sh OR	
	Separation anxiety	
	disorder*.tw OR	
	Selective mutism.tw	

PubMed Logic Grid

AND →

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
"Inflammation"[mh:noexp]OR Inflamm*[tiab] OR Immun*[tiab] OR Psychoimmun*[tiab] OR Neuroimmun*[tiab] OR "Cytokines"[mh] OR Cytokine*[tiab] OR Interleukin*[tiab] OR Tumor necrosis factor*[tiab] OR Tumour necrosis factor*[tiab] OR TNF*[tiab] OR "C-reactive protein" [mh] OR C-reactive protein[tiab] OR CRP[tiab] OR Chemokine*[tiab] OR White blood cell*[tiab]	"Anxiety Disorders"[mh] OR Anxiety disorder*[tiab] OR Anxiety based disorder*[tiab] OR "stress disorders, post- traumatic"[mh] OR Post-traumatic stress disorder*[tiab] OR Posttraumatic stress disorder*[tiab] OR PTSD[tiab] OR Generalised anxiety disorder*[tiab] OR Generalized anxiety disorder*[tiab] OR GAD[tiab] OR "Phobia, social"[mh] OR Social phobia*[tiab] OR social anxiety disorder*[tiab] OR "obsessive-compulsive disorder"[mh] OR obsessive-compulsive disorder*[tiab] OR OCD[tiab] OR "phobic disorders"[mh] OR Agoraphobi*[tiab] OR phobi*[tiab] OR "Panic disorder"[mh] OR Panic disorder*[tiab] OR "Anxiety, Separation"[mh] OR Separation anxiety disorder*[tiab]OR Selective mutism[tiab]	"adolescent" [mh] OR adolescen*[tiab] OR "Child"[mh] OR Child*[tiab] OR Youth[tiab] OR Paediatric[tiab] OR Pediatric[tiab] OR Teen*[tiab]

Embase Logic Grid

AND →

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
Inflammation/de OR	'Anxiety Disorder'/exp	Adolescent/de OR
Inflamm*:ti,ab OR	OR	adolescen*:ti,ab OR
Immunology/de OR	'anxiety disorder*':ti,ab	Child/de OR
Neuroimmun*:ti,ab OR	OR	Child*:ti,ab OR
Psychoimmun*:ti,ab OR	'anxiety based	Youth:ti,ab OR
Immun*:ti,ab OR	disorder*':ti,ab OR	Paediatric:ti,ab OR
Cytokine/de OR	'Posttraumatic stress	Pediatric:ti,ab OR
Cytokine*:ti,ab OR	disorder'/de OR	Teen*:ti,ab
'Tumor necrosis factor'/de	'posttraumatic stress	
OR	disorder*':ti,ab OR	
'Tumor necrosis	'Post-traumatic stress	
factor*':ti,ab OR	disorder*':ti,ab OR	
'tumour necrosis	PTSD:ti,ab OR	
factor*':ti,ab OR	'Generalized anxiety	
TNF*:ti,ab OR	disorder'/de OR	
'C reactive protein'/de OR	'Generalized anxiety	
'C reactive protein':ti,ab OR	disorder*':ti,ab OR	
CRP:ti,ab OR	'Generalised anxiety	
Interleukin*:ti,ab OR	disorder*':ti,ab OR	
Chemokine/de OR	'social phobia'/de OR	
Chemokine*:ti,ab OR	'social phobia*':ti,ab OR	
'White blood cell*'/de OR	'Social anxiety	
'White blood cell*:ti,ab	disorder*':ti,ab OR	
	'obsessive compulsive	
	disorder'/de	
	OR	
	'obsessive compulsive	
	disorder*':ti,ab OR	
	OCD:ti,ab OR	
	Agoraphobi*:ti,ab OR	
	Phobia/exp OR	
	Phobi*;ti,ab OR	
	'Panic disorder'/de OR	
	'Panic disorder*':ti,ab OR	
	'Separation anxiety	
	disorder'/de OR	
	'Separation anxiety	
	disorder*':ti,ab OR	
	'Selective mutism:ti,ab'	

Scopus Logic Grid

AND →

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
TITLE-ABS-KEY(Inflamm* OR Immun* OR Psychoimmun* OR Neruoimmun* OR Cytokine* OR "Tumor necrosis factor*" OR "Tumour necrosis factor*" OR TNF* OR "C-reactive protein" OR CRP OR Interleukin* OR Chemokine* OR "White blood cell*")	TITLE-ABS-KEY("Anxiety Disorder*" OR "Anxiety based disorder*" OR "posttraumatic stress disorder*" OR "Post traumatic stress disorder*" OR PTSD OR "Generalised anxiety disorder*" OR "Generalized anxiety disorder*" OR "Social phobia" OR "Social anxiety disorder*" OR "obsessive compulsive disorder*" OR OCD OR Agoraphobi* OR Phobi* OR "Panic disorder*" OR "separation anxiety disorder*" OR Selective mutism")	TITLE-ABS-KEY(Adolescen* OR Child* OR Youth OR Paediatric OR Pediatric OR Teen*)

Web of Science Logic Grid

AND →

Inflammatory Markers	Anxiety-Based Disorders	Children and Adolescents
TS=(Inflamm* OR Immun* OR Psychoimmun* OR Neruoimmun* OR Cytokine* OR "Tumor necrosis factor*" OR "Tumour necrosis factor*" OR TNF* OR "C-reactive protein" OR CRP OR Interleukin* OR Chemokine* OR "White blood cell*")	TS=("Anxiety Disorder*" OR "Anxiety based disorder*" OR "posttraumatic stress disorder*" OR "Post traumatic stress disorder*" OR PTSD OR "Generalised anxiety disorder*" OR "Generalized anxiety disorder*" OR "Social phobia" OR "Social anxiety disorder*" OR "obsessive compulsive disorder*" OR OCD OR Agoraphobi* OR Phobi* OR "Panic disorder*" OR "Separation anxiety disorder*" OR "Selective mutism")	TS=(Adolescen* OR Child* OR Youth OR Paediatric OR Pediatric OR Teen*)

Appendix B

Data Extraction Sheet Example

1. Study Information

Lead Author/Year:

Country:

Study Design:

Anxiety-Based Disorder investigated(s):

Diagnostic Tool:

Inflammatory marker measured:

Sample Type:

Time of Sample/ Fasting:

Recruitment Source:

Total Sample (N):

Anxiety- Based Disorder Sample (N):

Control Sample (N):

Outcome:

Additional Comments:

2. Participant Information

	Total Sample	Anxiety-Based Disorder	Controls
Nparticipants			
Age (M/SD)			
Gender (N/%)			
Male			
Female			
BMI (M/SD)			
Medicated			
Co-morbidities			
Physical health			
Mental health			

3. Statistical Information

Inflammatory Marker	Statistic Used	Anxiety-Based Disorder Data	Control Data	Effect size	P value

Appendix C

Quality Assessment Criteria

Lead Author	Question/ objective sufficiently described	Study design evident and appropriate?	Method of recruitment described and appropriate?	Subject characteristics sufficiently described?	Outcomes well described?	Sample size appropriate?	Analytic methods described and appropriate?	Some estimate of variance is reported for main results?	Controlled for confounding?	Results reported in sufficient detail?	Conclusions supported by the results?	Total Score (0-22)	Percentage (0-100%)
Ayadin	2	2	1	2	2	0	1	2	2	2	1	17	77%
Colak Sivri	2	2	1	2	2	1	1	2	2	2	2	19	86%
Gabbay	2	2	2	2	2	1	2	2	1	1	1	18	82%
Khandaker	2	2	2	1	2	2	2	2	1	2	2	20	91%
Ozyurt	2	2	2	2	1	2	1	2	2	2	2	20	91%
Rodriguez	2	2	2	2	2	2	1	2	1	2	2	20	91%
Simsek	2	2	1	2	2	1	2	2	1	2	2	19	86%
Ucar	2	2	2	2	2	1	1	2	2	2	1	19	86%

Notes. 2 = criteria fully met, 1 = criteria partially met, 0 = criteria not met.