From Remission to Relapse: the role of Inflammatory Cytokines in Course of Depression after Antidepressant Discontinuation

MRes Translational Neuroscience, 2022-2023

Module code : CLNE0034

Supervisor: Prof Quentin Huys

Candidate Number: CKTN5

Word Count: 14935

[NOTE TO STUDENTS: This can be up to 10% higher or lower than the set word count

UCL Queen Square Institute of Neurology – MSc/MRes Programmes

Statement of Originality

I, Kangxin Chen Huang confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Acknowledgements

Firslty, I would like to express my deepest gratitude to my supervisor Prof. Quentin Huys for providing me with this opportunity to work on this project in his lab, as well as for his mentorship and his guidance throughout the course of this project. I am profoundly thankful for the opportunity to learn under your direction. I would also like to express my thanks to Julia Siembiga, for the advice, time and moral support during the challenging period of this project. I would also like to thank the whole AIDA team, who I have not met but without their work this project would not have been possible. Finally, I would like to thank my family and close friends for their continuous encouragement, understanding, and unwavering belief in my abilities.

Statement of contribution:

Prof. Dr. med. Dirk Reinhold conducted the ELISA cytokines assays from the AIDA study.

Kangxin Chen Huang planned and performed the analyses and wrote the manuscript under supervision by Prof. Dr. Quentin J. M. Huys.

This work represents a collaborative effort among all authors, including the whole AIDA team from Zurich and Berlin.

ABSTRACT

Depression, a highly prevalent chronic disorder with an often relapsing and progressive course, is the leading disability worldwide (Lépine & Briley, 2011; Santomauro et al., 2021) . Emerging research highlights the potential role of the immune system in depression, evidenced by elevated levels of pro-inflammatory cytokines in depressed patients compared to never-depressed individuals (Köhler et al., 2017). Moreover, studies have suggested that antidepressant medications (ADM) may reduce the levels of pro-inflammatory cytokines (Liu et al., 2020). However, there is limited research on the effect of cytokine levels on ADM discontinuation and relapse rate.

To fill in this gap, this research aimed to explore the correlation between cytokine levels and relapse risk after antidepressant discontinuation. The present study uses data collected in the two-centre, longitudinal AIDA (Antidepressiva Absetzstudie) study. Plasma samples from remitted Major Depressive Disorder (MDD) patients and matched healthy controls (HCs) were analysed for levels of pro-inflammatory cytokines (TNF-α, IL6), anti-inflammatory cytokines (IL-10 and TGF-ß1) and the inflammation marker CRP assayed with ELISA. Regression models were employed to 1) to assess the predictive capability of cytokine levels in relapse risk after ADM discontinuation; 2) to explore the changes in cytokine levels during the disease course from remission to relapse. Results suggest that while cytokines may not robustly predict relapse, variations in TNF-α levels appear to be notably associated with both depression status and ADM discontinuation. This study highlights the potential utility of specific cytokine levels as biomarkers in the management and understanding of depression, paving the way for future research in personalised treatment strategies.

Tables of Content

List of Abbreviations

ADM	Antidepressant medication
ADS	Antidepressant discontinuation syndrome
AIDA	Antidepressiva Absetzstudie
AUC	Area Under the Curve
BMI	Body mass index
CNS	Central Nervous System
CRP	C-reactive protein
CI	confidence interval
DSM	Diagnostic and Statistical Manual of Mental Disorders
IFN	Interferons
IL	Interleukins
IQR	interquartile ranges
HCs	Healthy controls
HPA	Hypothalamic pituitary adrenal
MA	Main assessment
MAOIs	Monoamine oxidase inhibitors
MD	median differences
MDD	Major Depressive Disorder
NICE	The National Institute for Health and Care Excellence
NPV	Negative predictive value
offdrug	whether the patient discontinued (*offdrug* = 1) or not (*offdrug* = 0)
PPV	Positive predictive value
SE	Standard Error
SNRIs	Serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitors
T_H1	Type 1 T helper
T_H2	Type 2 T helper
TNF	Tumour necrosis factor
ß	Standardised coefficient
μ	Mean
n	sample size
ng	nanograms
pg	picograms
r_s	Spearman's rank correlation coefficient
z	z-value
1W2	Subject discontinued at MA1
12W	Subject discontinued at MA2

List of Figures and Tables

CHAPTER I - **Literature review**

1. On depression and depression relapse

Depression, also known as Major Depressive Disorder (MDD), is one of the most debilitating and deadly psychiatric condition. It is the leading cause of disability worldwide, affecting more than 300 million people worldwide (Friedrich, 2017). Depression is the most prevalent psychological disorder associated with suicide with more than 700 000 people die due to suicide every year, and more than half of the suicides are associated with depression (Brådvik, 2018). The economic cost to society of MDD is enormous as well, with an estimate of 236 billion dollars in 2018 in the US only, an increase of more than 35% since 2010 (Greenberg et al., 2021). The individual, social and economic burden that depression exerts makes it one of the most significant and pressing condition in the world.

One defining features of depression is its often remitting-relapsing nature, characterised by periods of well-being interrupted by potentially life-threatening episodes of anhedonia, helplessness, and low mood. Notably, in 2003, Jules Angst reported a life-long observational study whereby he followed the disease course of 186 depressed individuals (Angst et al., 2003). Angst found a positive linear relationship between age and cumulative time spent in illness, indicating a constant risk of recurrence over a depressed person's lifespan. This ceaseless proclivity for relapse, coupled with the disease's progressive nature, positions depression the leading cause of disability worldwide (Friedrich, 2017). Understanding the complexities of depression's relapse and recurrence is thus paramount for tackling depression.

While treatments including antidepressant medications and psychological therapies show a clinically significant response in around 7 out of 10 patients (A. John Rush et al., 2006), the risk of later recurrence and relapse following remission is high (DeRubeis et al., 2008). About 60% of individuals relapse after a first major depressive episode. The relapse risk increases with the number of depressive episodes, with 70% risk of recurrence for those with 2 episodes and 90% for those with three or more episodes (Monroe & Harkness, 2011; Mueller et al., 1999; Solomon et al., 2000). Each recurrence carries a 10-20% risk of developing unremitting and chronic depression, heightening the individual's risk of suicide (Lee et al., 2022). Therefore, preventing relapse is paramount following remission for managing the longer-term course of the illness. Yet, the transition between remission and relapse is under-researched and poorly understood.

1.1. Defining the course of depression pathology

Before delving into the underlying mechanisms of relapse, establishing a clear disease course is paramount for coherent research. Here, we define **remission** as the period of symptom improvement such that the individual is virtually asymptomatic; and **relapse** as the return of at least five out of nine symptoms following a minimum two-month interval during remission, as outlined by the (*Diagnostic and statistical manual of mental disorders (5th ed.)*, 2013).

The seminal report by The MacArthur Foundation Network on the Psychobiology of Depression in 1991 distinguishes remission from **recovery** - the resolution of the depressive episode following a sufficient period of sustained remission; and relapse from **recurrence** - the development of novel episode post-recovery) (Frank et al.,

1991). Whether these distinctions remain empirically valid or even necessary is a subject of further exploration (Burcusa & Iacono, 2007).

In this dissertation, we will explicitly reference remission and relapse due to the practical constraint of study duration. The distinction between remission and relapse will not be considered, and the terms may be used interchangeably. Nonetheless, readers should remain cognizant of the variations between these terms when interpreting the results and discussion.

1.2. Predictors/biomarkers of depression relapse after antidepressant discontinuation

Several strategies to prevent relapse episodes exist, with the most important one being continuation and maintenance treatment with antidepressants. Antidepressants are thought to correct the chemical imbalances of neurotransmitters in the brain (Kenis & Maes, 2002), which relieves the symptoms of depression and other related mood disorders. Some examples of antidepressants include selective serotonin reuptake inhibitors (SSRIs) like sertraline, serotonin and norepinephrine reuptake inhibitor (SNRIs) like venlafaxine, and older antidepressant medications like tricyclics and monoamine oxidase inhibitors (MAOIs). Antidepressants are mostly effective on moderate, severe and chronic depression, and less so in mild depression. Research has shown that continuation with antidepressants can prevent up to 70% of relapses, whereas discontinuation leads to relapse in approximately one in three patients (Geddes et al., 2003). Yet, there remains a risk of developing further depressive episodes during antidepressant treatment (A. John Rush et al., 2006). Additionally, the side effects that come with antidepressant use including weight gain and sexual

dysfunction may impact patients' adherence to treatment plan (Olfson et al., 2006), making the decision to continue treatment a complex one.

Therefore, continuation of antidepressant treatment does not uniformly benefit all patients, and individual trajectories following the initial responses to antidepressant medications can vary significantly. Markers that could distinguish these trajectories could guide clinical decision-making and determine which individuals can safely discontinue treatment compared to those at high risk of relapse after discontinuation. Current clinical guidelines on the length of maintenance treatment to prevent depression relapse considers clinical variables, including duration and number of prior episodes or chronicity (*NICE guideline [NG222]*, 2022). For individuals in their first depressive episode, the guidelines recommend at least 4-9 months of continuation after full or partial remission (Bauer et al., 2013), whereas those with recurrent episodes may be advised to continue treatment for two years or more. More recently, NICE guidelines have also incorporated residual symptoms and physical and psychological comorbidities as potential predictors of relapse risk (*NICE guideline [NG222]*, 2022)

However, the use of these factors as predictors of relapse after discontinuation remains somewhat contentious. The evidence supporting these predictors is primarily based on studies investigating the overall relapse risk in the natural course of depression, independent of medication discontinuation. Nonetheless, the relevance of these markers in predicting relapse, specifically after antidepressant discontinuation remains unclear, as meta-analyses have failed to find a significant effect of these markers on relapse risk after antidepressant discontinuation (Berwian et al., 2017; Gueorguieva et al., 2017).

Another study by (Berwian et al., 2022) investigated various demographic and clinical features but found little predictive power for relapse after antidepressant discontinuation. This reinforces the notion that the risk of relapse after antidepressant discontinuation differs from the overall relapse risk, as some patients may experience relapse regardless of whether they are prescribed medications. Consequently, our understanding of predictors of relapse after antidepressant discontinuation remains limited, and there is a pressing need for identifying more precise predictors of depression relapse after antidepressant discontinuation.

The complex nature of depression and its heterogeneous response to treatment underscores the importance of discovering reliable markers that can help differentiate individuals who can safely discontinue treatment from those at higher risk of relapse after discontinuation. Enhancing our understanding of these predictors will enable clinicians to make more informed decisions regarding the appropriate continuation or discontinuation of antidepressant treatment for individual patients. Ultimately, this research has the potential to lead to more personalized and effective treatment plans, thereby improving patient outcomes and preventing relapses in depression management.

Commented [RJ1]: To be included in discussion: On a positive note, a recent study by Berwian et al. (2019) revealed that patients in remission but still on medication exhibited heightened sensitivity to effort, which emerged as a predictive factor for the risk of relapse following antidepressant discontinuation. Such findings offer promising insights for identifying individual markers that can enhance the precision of clinical decisions related to continuation or discontinuation of antidepressant treatment.
Ultimately, identifying reliable predictors for relapse risk after antidepressant discontinuation has the potential to revolutionize clinical practice. By discerning the unique trajectories of individual patients, clinicians can tailor treatment plans more effectively, optimize outcomes, and prevent relapses more successfully. However, further research is needed to validate and expand upon these findings, with the ultimate goal of advancing personalized care for patients with depression.

2. On the role of inflammation in depression relapse

For the past 50 years, the “Monoamine-depletion hypothesis” has been the mainstay theory in depression research, proposing that an imbalance or depletion of the

monoamine neurotransmitters including serotonin, dopamine and noradrenaline explains the cause of depression (Delgado, 2000). This hypothesis is supported by the observed clinical effectiveness of first-generation antidepressants like tricyclics and monoamine oxidase inhibitors, as well as the second-generation antidepressant like selective serotonin reuptake inhibitors, targeting the enhancement and regulation of the monoamine system. Nevertheless, despite these advancements, our understanding of the precise biological mechanisms responsible for depression and the action of antidepressants remains incomplete. It is now recognized that depression is a complex and multifaceted disorder involving intricate interactions between various biological systems, and that the monoamine hypothesis alone cannot fully account for the complexity of depression (Massart et al., 2012).

More recently, researchers have suggested that inflammation may be involved in the onset and maintenance of MDD in interplay with the monoamine-depletion hypothesis. Depression relapse may be associated with immune dysregulation and chronic inflammation. The cytokine theory of depression has drawn significant attention within the scientific community: elevated levels of pro-inflammatory cytokines have been found in the blood of patients with MDD in several studies. In the next section, we delve deeper into the role of inflammation in depression and depression relapse, with a particular focus on the contribution of cytokines.

2.1. Cytokines

Cytokines are small proteins that act as key signalling molecules in initiating and regulating the inflammatory response They are produced by various cells in the immune system, including macrophages, lymphocytes and mast cells. This group of messenger molecules is broadly categorised into chemokines, interferons (IFN),

interleukins (IL), lymphokines and tumour necrosis factors (TNF). Notably, the production of cytokines is not limited to immune cells alone, other cells including the endothelial cells, fibroblasts and epithelial cells of the body's periphery, as well as the neurones, astrocytes and microglia of the brain, also release cytokines.

Most cytokines primarily act in their local micro-environment, but certain cytokines, such as those involved in the acute phase response such as TNF-α and IL6, can be released into the bloodstream, to act on distant organs in a hormone-like manner. Once in the blood, these peripherally produced cytokines can access the brain through three different pathways (H. Himmerich et al., 2019): the humoral pathway, where cytokines cross through leaky sections of the blood-brain barrier (Banks et al., 1995); the neural pathway, which involves the stimulation of primary afferent nerve fibres in the vagus nerve by the cytokines (Johnston & Webster, 2009); and the cellular pathway, where peripheral cytokines stimulate cerebral microglia, possibly through cerebral endothelial cells, leading to the production and subsequent recruitment of monocytes to the meninges and brain parenchyma (D'Mello et al., 2009). Once in the brain, these cytokines can impact neural activity and neurotransmitter systems, potentially contributing to changes in mood and cognition. It is the discovery of these immune-to-brain communication pathway that revolutionised the understating of our immune system, as it was previously believed that the immune system was confined to the periphery. The involvement of inflammation in depression and other neurological conditions is increasingly recognised, demonstrating the complex and bidirectional relationship between the immune system and the Central Nervous System (CNS).

2.2. Cytokine theory of depression

The first hint to a potential relationship between the peripheral inflammation and major depression comes from early studies in the 1990s. Researchers conducted investigations into the immune cell profile of patients with depression and observed alterations in their total count of immune cells (Maes et al., 1992). Subsequent studies further supported these findings and reported increased levels of both central and peripheral pro-inflammatory cytokines, including IL-1, IL6, TNF-α, and the acute phase protein CRP, in depressed patients even in the absence of somatic comorbidity (Maes, 1999).

Since then, these findings have been substantiated by multiple meta-analyses (Dowlati et al., 2010; Goldsmith et al., 2016; Haapakoski et al., 2015; Howren et al., 2009; Köhler et al., 2017; Liu et al., 2012; Osimo et al., 2020), which consistently reported elevated levels of pro-inflammatory cytokines in individuals with depression compared to non-depressed individuals. Notably, administration of pro-inflammatory cytokine IFN-α in immunotherapy induced depressive symptoms in cancer patients, and discontinuation of treatment alleviated these depressive symptoms almost immediately (Lotrich, 2009). From these observations , the “theory of cytokine-induced depression” was proposed (Maes, 1999), suggesting that inflammatory factors, in particular cytokines, play a crucial role in the development of depressive symptoms.

Further evidence to support the cytokine theory of depression comes from meta-analyses of randomized controlled studies investigating the effects of pro-inflammatory cytokine inhibitors. These studies found that anti-inflammatory drugs treatments improved depressive symptoms (Kappelmann et al., 2018; Köhler et al., 2014), supporting the cytokine theory of depression. Moreover, a recent meta-analysis

of 45 treatment studies showed that antidepressant treatment significantly decreased peripheral levels of pro-inflammatory cytokines IL6 and TNF-α, suggesting that antidepressants may have an anti-inflammatory property (Köhler et al., 2018; Liu et al., 2020).

While the cytokine theory of depression implicates pro-inflammatory cytokines in the pathogenesis of depressive symptoms, the role of anti-inflammatory cytokines, including IL-10 and TGF-ß, remains controversial. Animal studies using knock-out mice lacking IL-10 expression (IL10-KO) have shown that these mice exhibit depressive-like behaviours, which can be reverted by administration of IL10 (Mesquita et al., 2008). This observation aligns with the cytokine theory of depression, positing that increased inflammation contributes to the development of depressive-like symptoms. However, meta-analyses of human studies have yielded mixed results, reporting both elevated (Köhler et al., 2018) and unaltered levels (Dowlati et al., 2010; Hiles et al., 2012; Lee & Kim, 2006) of anti-inflammatory cytokines IL-10 and TGF-ß in depressed patients compared to healthy individuals. Given the complexity and variability in cytokine involvement, understanding the current evidence supporting each cytokine's role in depression is crucial for formulating hypotheses needed in this study.

2.3. A comprehensive review on the current evidence on the cytokines TNF-α, IL6, IL10, TGF-ß1, CRP in depression and antidepressant treatment

Cytokines are often divided into four different categories in the psychoimmunological literature based on their functions and immune responses: 1) T_H1 cytokines, including IL-2 and IFN-γ, are responsible for inducing cytotoxic cell contacts. 2) T_H2 cytokines,

such as IL-4 and IFN-5, primarily facilitate the production of antibodies. 3) Proinflammatory cytokines, including IL6, IFN-α and TNF-α promote inflammation and activate immune responses against infections or tissue damage and 4) Anti-inflammatory cytokines, such as IL-10 and TGF-ß1, are influenced by regulatory T cells and serve to counteract the pro-inflammatory responses, promoting resolution of inflammatory processes (Roohi et al., 2021).

Many cytokines have been suggested to play a role in depression, including IL-1β, IL-2, IL-4, IL-5, IL6, IL-8, IL-10, IL-12, CRP, TNF-α, IFN-γ, etc (Liu et al., 2020). This study focuses on the role of - the pro-inflammatory cytokines TNF-α, IL6 and the acute phase protein CRP, as well as the anti-inflammatory cytokines IL-10 and TGF-ß1 – in depression relapse after antidepressant discontinuation; as these are the most explored cytokines in the literature.

2.3.1. TNF-α

TNF-α is commonly known to be the master regulator of other inflammatory cytokines and the major cytokine in the pathogenesis of chronic inflammatory disease (Berthold-Losleben & Himmerich, 2008). Multiple metanalyses have consistently reported elevated levels of TNF-α in depressed patients compared to healthy controls (Dowlati et al., 2010; Köhler et al., 2017; Min et al., 2023; Osimo et al., 2020), thus there is strong evidence to support a role of TNF-α in depression.

The evidence on the relationship of TNF-α levels and antidepressant treatment is less consistent. Studies have reported varying outcomes, with some indicating an increase in TNF-α levels after effective ADM treatment (Eller et al., 2008), while others have shown a decrease (Narita et al., 2006; Yao et al., 2020) or no change (Hannestad et al., 2011). Several studies have showed that different types of antidepressants may

have different effects on TNF-α levels. For instance, an open study by (Pérez-Sánchez et al., 2018) found that TNF-α levels decreased after SSRI fluoxetine treatment in patients. Conversely, (Kraus et al., 2002) reported that atypical tetracyclic antidepressant mirtazapine induced a significant increase in TNF-α plasma levels, while the SNRI venlafaxine did not alter TNF-α plasma levels. Therefore, different antidepressants may exert different effects on cytokine levels, which may explain the disparate findings in the literature.

Higher baseline TNF-α levels have been associated with poorer response to antidepressant treatment. A study by (Xu et al., 2019) reported significantly lower baseline serum TNF-α levels in patients responsive to antidepressant treatment compared to those who are insensitive to treatment. Similarly, increased circulating levels of baseline TNF-α are associated with a reduced efficacy of antidepressant treatment (Eller et al., 2008; O'Brien et al., 2008; Yao et al., 2020). These findings suggest that TNF-α may hold predictive value for the antidepressant treatment response in patients with MDD.

2.3.2. IL6

IL6 is a multifunctional cytokine with pleiotropic functions, displaying both pro- and anti-inflammatory properties (Roohi et al., 2021). Its involvement in regulating immune responses and neural processes makes it a key focus in depression research. IL6 elevation may be considered a biological marker of depression (Mössner et al., 2007).

Multiple meta-analyses consistently reported elevated IL6 levels in individuals with MDD compared to healthy controls (Dowlati et al., 2010; Haapakoski et al., 2015; Hiles et al., 2012; Howren et al., 2009; Köhler et al., 2017; Liu et al., 2012). Notably, a longitudinal study reported that children with higher circulating levels of IL6 at age 9

had 10% greater risk of developing MDD by age 18, suggesting that higher circulating IL6 levels in childhood may predict an increased risk of developing MDD in later years (Khandaker et al., 2014).

ADM treatment has been associated with a decrease and normalization of IL6 levels, as substantiated by various meta-analyses (Hannestad et al., 2011; Hiles et al., 2012; Köhler et al., 2018; Więdłocha et al., 2018). However, research suggests that distinct antidepressant types carry varying effects on IL6 levels. (Carboni et al., 2019) undertook a longitudinal study comparing plasma IL6 levels in MDD patients treated either with the SSRI paroxetine or SNRI venlafaxine, and matched controls. Changes in IL6 levels were measured as the difference between baseline values and week 10 treatment. In the paroxetine-treated group, IL6 levels correlated positively with HAM-D (Hamilton Rating Scale for Depression) scores after 10 weeks, implying that higher IL6 levels were associated with a greater reduction in depressive symptoms. Conversely, in the venlafaxine group, no significant correlation between IL6 levels and HAM-D scores were observed.

Even the effect of SSRIs on IL6 levels appears to vary across studies. A study by (Yoshimura et al., 2013) reported substantial reductions in IL6 levels after 8 weeks of SSRI treatment in responders but not in non-responders. In contrast, (Manoharan et al., 2016) reported no statistically significant variation in IL6 levels changes after 6 weeks of fluoxetine treatment between responder and non-responder groups. These differences could potentially be attributed to variations in timing of the second measurement of the IL6-level (Mosiołek et al., 2021),

2.3.3. IL-10

IL-10 is a multifaceted cytokine involved in maintaining immune homeostasis with anti-inflammatory properties. There are many ways IL-10 minimise inflammation, including reducing pro-inflammatory cytokine levels such as TNF-α (Mosiołek et al., 2021). In the context of the cytokine theory of depression, it is thought that individuals with elevated inflammation due to depression should exhibit decreased anti-inflammatory IL-10 levels. Animal studies corroborate this hypothesis; for instance, depression-like symptoms have been observed in IL-10 knockout mice (Worthen et al., 2020).

However, the overall clinical data on IL-10 levels in MDD patients are inconsistent, with many studies reporting no significant difference in IL-10 levels between MDD patients and healthy individuals, both at baseline and after treatment (Fornaro et al., 2020). While such discrepancies may be attributed to variations in study populations, sample sizes, and methodologies; the role of IL-10 in depression remains inconclusive.

The effects of ADM treatment on IL-10 levels have garnered significant interest. Early studies suggested that ADMs might increase IL-10 expression, aligning the potential anti-inflammatory effect of these medications (Kubera et al., 2001; Maes, 1999). However, more recent meta-analyses indicate that ADM treatment tends to moderately reduce IL-10 levels (Köhler et al., 2018; Więdłocha et al., 2018). Notably, (Carboni et al., 2019) revealed that high baseline IL-10 levels differ between responders and non-responders to antidepressants. Consequently, although the impact of antidepressants on IL-10 levels remains inconclusive, a correlation is apparent.

2.3.4. TGF-ß1

TGF-ß1 is a pivotal cytokine for immune regulation and tissue repair with anti-inflammatory properties, with potential relevance to depression-associated inflammation. However, most meta-analyses and clinical studies have failed to find a significant difference in TGF-ß1 level between depressed and non-depressed individuals (Köhler et al., 2017; Lee & Kim, 2006; Pallavi et al., 2015).

Conversely, several studies have reported an increase in TGF-ß1 levels following antidepressant treatment (Myint et al., 2005). SSRIs like fluoxetine and sertraline elevate circulating TGF-ß1(Lee & Kim, 2006; Sutcigil et al., 2007). Similarly, medicated patients have higher TGF-ß1 levels compared to non-medicated patients and matched controls (Pallavi et al., 2015).

The TGF-ß1 and depression relationship remains intricate. While depressed and non-depressed individuals show no significant TGF-ß1 level differences, ADM treatment increase TGF-ß1 level, implying that antidepressants might modulate its expression. TGF-ß1's role in depression pathophysiology and response to ADMs warrants further study. It is possible that TGF-ß1, alongside other inflammatory cytokines, contributes to antidepressants' mechanisms and immune regulation in depression.

2.3.5. CRP

CRP is an acute-phase reactant protein produced by the liver in response to inflammation. Its ease of measurement through a blood sample in its high-sensitivity form, has made it a valuable biomarker widely used in clinical practice. Several meta-analyses (Osimo et al., 2019; Osimo et al., 2020) have shown a positive association between elevated CRP levels and depression, particularly with the severity of

depressive symptoms (Valkanova et al., 2013), thus suggesting a potential role of CRP in the pathophysiology of depression. Moreover, additional investigations have explored the predictive value of CRP in depression. For instance, (Foley et al., 2021) reported that elevated CRP levels were associated with an increased risk of late-life depression symptoms. Similarly, (Copeland et al., 2012) found that multiple occurrences of depressive symptoms were linked to increasing CRP levels.

Researchers have suggested that antidepressant treatment can lead to a decrease in CRP levels, aligning with the inflammatory theory of depression. However, the overall evidence remains inconclusive, as a recent meta-analysis conducted by (Więdłocha et al., 2018) found no significant effect on CRP from antidepressant treatment. Nevertheless, a consistent pattern observed in many studies is that elevated CRP relates to treatment-resistant depression. Particularly, lower CRP predicts more favourable SSRI responses (Orsolini et al., 2022). This implies that CRP could potentially serve as a predictor of antidepressant effectiveness. About one-third of depressed patients exhibit a low-grade inflammatory state, possibly explaining unresponsiveness to standard antidepressants in around 30% of cases (Wysokiński et al., 2016). This chronic inflammation suggests a distinct subgroup within MDD, potentially making CRP a valuable biomarker for this subgroup.

2.4. Cytokines in depression relapse

Given the significant link between inflammation and depression, cytokines could also play a role in the intricate interplay between depression and relapse after antidepressant discontinuation. Little research has been conducted on the relationship between cytokine levels after ADM discontinuation and relapse rate. However, there is some experimental evidences that suggests the contribution of pro-inflammatory

cytokines, especially TNF-α, IL-1ß, IL6 and the acute phase reactant CRP to depression relapse (Liu et al., 2019).

Firstly, baseline levels of TNF-α in patients receiving antidepressants are significantly higher for non-remitted and 24-month relapse patients (Choi et al., 2021). Similarly, a study by (Belzeaux et al., 2012) investigated the peripheral blood transcriptional profiled of patients with major depressive episode and found that the expression of the cytokines IL-1ß and TNF genes before antidepressant treatment was higher in antidepressant non-responders compared to responders. These findings support that pro-inflammatory cytokines may have potential predictive value in MDD for treatment response (Janssen et al., 2010). Other studies also have found elevated levels of baseline CRP and IL6 before antidepressant treatment in recurrent MDD patients compared to healthy controls (Goldsmith et al., 2016; Topic et al., 2013), implying inflammation's partial involvement in the recurrence of depressive episodes. Furthermore, (Copeland et al., 2012) found a positive correlation between increased CRP levels and cumulative episodes of depression in subjects, further supporting that inflammation is associated with repeated depressive episodes. Interestingly, a study investigated depression relapse in women undergoing infertility treatment and found that baseline CRP level was higher in relapsers compared to non-relapsers (Freeman et al., 2018) but not IL6. This highlights that the relationship between inflammation and depression may be complex and cytokine-specific.

Fewer studies have explored the relationship between anti-inflammatory cytokines on and depression relapse after ADM treatment. Nonetheless, research has investigated their roles in treatment response by comparing anti-inflammatory activity in antidepressant responders and non-responders. Syed and colleagues compared the

levels of both pro-inflammatory and anti-inflammatory cytokine in responders and non-responders before and after ADM treatment. Like previously mentioned studies, pro-inflammatory cytokines IL6, IL-1ß and TNF increased in non-responders but stabilized in responders after treatment. Interestingly, anti-inflammatory cytokine IL-10 and others increased in both responders and non-responders, suggesting that the anti-inflammatory cytokines of non-responders may be defective in blocking increasing levels of pro-inflammatory cytokines (Syed et al., 2018).

Additionally, one animal research study explored the potential role of immunomodulation in antidepressant discontinuation syndrome. Importantly, the study found that imipramine treatment cessation in rats led to alteration in certain cytokines, including the anti-inflammatory cytokine IL-10, indicating a disrupted immune balance post-antidepressant discontinuation (Kuśmider et al., 2018). Consequently, these findings imply a potential connection between shifts in anti-inflammatory cytokines like IL-10 and the immune response imbalance that might contribute to antidepressant discontinuation, and potentially trigger possibly relapse.

3. On the AIDA study

No established predictors of depression relapse after antidepressant discontinuation are currently available (Berwian et al., 2017). As discussed, cytokines play an important role in depression, yet to our knowledge, the relevance of cytokines in the mechanism underlying relapse after antidepressant discontinuation is under-researched.

The AIDA project (Antidepressiva Absetzstudie) is a longitudinal, two-centre, observational trial designed to identify potential predictors of relapse after antidepressant discontinuation. This dissertation was based on the analysis of data obtained in the AIDA study, particularly plasma levels of pro-inflammatory (IL6, TNF-α and CRP) and anti-inflammatory (IL-10 and TGF-ß1) cytokines before and after ADM discontinuation and the relationship of these to relapse after antidepressant discontinuation. The details of the AIDA study design are the following:

3.1. Study design

An overview of this study design is depicted in Fig. 1.

Eligible patients from the baseline assessment determined by trained staff were randomized to one of two study arms, i.e., 1W2 or 12W (1 and 2 represents the number of the main assessment (MA), "W" represents antidepressant withdrawal/discontinuation). Main assessments consisted of a range of tests ranging from blood samples to various evaluation including neuro-imaging investigations, behavioural assessments, and clinical interviews to assess current depressive symptoms using self-rated and observer-rated reports. For the purpose of this current study, we are solely interested at the blood samples.

1W2 patients underwent MA1, which included a questionnaire to assess stable variables, gradually discontinued their antidepressants over a period of up to 18 weeks and then underwent MA2. To note, the initial 10 patients at each site were assigned to arm 1W2. 12W patients underwent both main assessments before discontinuing their medication and then underwent MA3. Such two-arm design is to allow for the

identification of discontinuation effects while accounting for factors such as time, learning and repetition.

During the discontinuation phase, all patients were contacted every 2 weeks for telephone assessments. After discontinuation, patients were contacted for telephone assessments for 6 months at weeks 1, 2, 4, 6, 8, 12, 16 and 21 to assess relapse status. Those patients with potential relapse indications were invited for an in-person structured clinical interview (SCID-I) to assess criteria for relapse, based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) guidelines (*Diagnostic and statistical manual of mental disorders (5th ed.)*, 2013). Patient who met the relapse criteria proceeded to do their final assessment, finishing up the study and re-referred to their treating physician. Those who finished the follow-up period without relapse undertook final assessment in week 26. Healthy controls matched for age, sex and education underwent MA1 only.

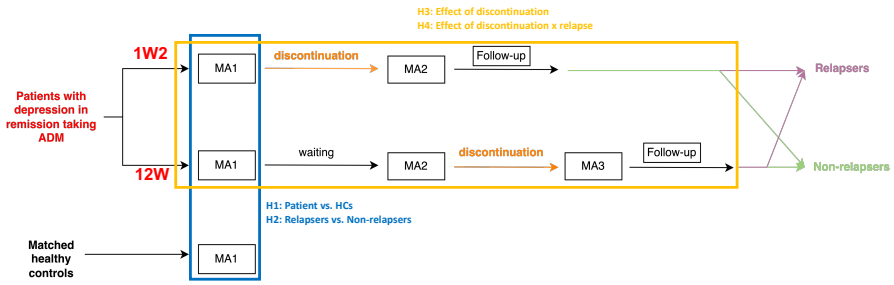

Fig. 1 Overview study design of the AIDA study.

Blood samples were collected from two groups - remitted patients on antidepressant medication (ADM) and matched healthy controls (HCs) at the main assessment MA1 and underwent a comparative analysis for testing hypothesis 1 (blue box). Subsequently, patients were randomized into two arms: one involving medication

discontinuation prior to the second main assessment MA2 (top arm, 1W2 group), and the other entailing a waiting period which medication was continued for a duration equivalent to the anticipated discontinuation interval (bottom arm, 12W group). Patients in the waiting group discontinued the medication after MA2 and underwent a third main assessment MA3. Following discontinuation, a 6-month follow-up period was implemented. Patients experiencing relapse within this period were classified as relapsers, while those who successfully completed the duration without relapse were classified as non-relapsers for the study's scope. Cytokine differences at MA1 of relapsers and non-relapsers were compared to test hypothesis 2 (blue box), which aims to explore whether baseline cytokine levels can predict relapse after ADM discontinuation. Additionally, cytokine changes between MA1 and MA2 for the 1W2 group, and between MA1 and MA3 for the 12W group, were analyzed to test hypotheses 3 and 4 (orange box). This explores the potential contribution of cytokines in the context of medication discontinuation and their involvement in the Discontinuation-Relapse interaction.

4. Analysis plan and hypotheses

A total of four hypotheses were made to answer the two main objectives in this dissertation. A summary of the hypothesis and our expectations are listed in Table 1.

4.1. First aim: clinical course

Firstly, we aimed to investigate whether plasma cytokine levels in depressed patients are related to the course of depression.

To address this, two hypotheses were noted:

1. HC vs remitted patients on antidepressants

Firstly, we tested **whether the baseline cytokine levels differ between healthy controls (HCs) and remitted patients still on antidepressant medication.** According to the cytokine and inflammation theory of depression, depressed patients should have increased plasma levels of pro-inflammatory cytokines IL6, TNF-α, CRP, and these should normalise after successful resolution of antidepressant treatment (Köhler et al., 2017, Yao et al., 2020). Hence, **we hypothesized that the baseline cytokine levels of remitted patients to not differ from HCs.** However, normalisation may be incomplete.

Several studies, including the aforementioned metanalyses by (Köhler et al., 2017), did not observed differences in the serum/plasma level of anti-inflammatory cytokine TGF-β1 between HCs and MDD patients. However, studies did find a significant increase in the level of TGF-β1 after antidepressant treatment in patients (Lee & Kim, 2006; Pallavi et al., 2015). Another study reported that the anti-inflammatory cytokine IL-10 was not only abnormally high at baseline before antidepressant treatment compared to healthy controls but also continued to increase post-treatment, failing achieve the normalization of anti-inflammatory cytokines (Belzeaux et al., 2012). Consequently, it is plausible that remitted patients, even in the medicated state, may exhibit higher levels of anti-inflammatory cytokines IL-10 and/or TGF-β1 compared to HCs.

2. Relapsers vs non-relapsers (relapse prediction)

We next tested whether baseline cytokine levels differ between people who go to suffer a relapse (relapers) and those who remain in remission (non-relapsers) after discontinuing their antidepressant medication.

The literature suggests that pro-inflammatory cytokines levels may predict relapse as lack of therapeutic effect of antidepressants have been associated with overall activation of the inflammatory response. **Therefore, we hypothesized that higher levels of pro-inflammatory cytokines IL6, TNF-α and CRP may predict relapse status.**

As for the role of anti-inflammatory cytokines, we hypothesized that relapsers may have a defective inflammatory response, akin to that treatment non-responders. **We hence tested whether the baseline anti-inflammatory cytokines (IL-10 and TGF-ß1) are lower in relapsers compared to non-relapsers.** This hypothesis was supported by previous research indicating that low levels of anti-inflammatory cytokines are associated with an increased risk of depression and a poor response to treatment (Felger & Lotrich, 2013).

4.2. Second aim: mechanisms

Secondly, given the potential role of cytokines in depression, we sought to understand whether cytokines could mediate the effects of antidepressant medication discontinuation on the course of depression. This includes investigating whether antidepressant discontinuation leads to changes in cytokine levels within patients and whether alterations in cytokine levels contribute to relapse after antidepressant discontinuation. Again, two hypotheses were postulated to address this aim:

Effect of antidepressant discontinuation on cytokine levels

We next examined the effect of antidepressant discontinuation in cytokine levels by comparing the cytokine levels of all patients in both groups (1W2 and 12W), before (MA1) and after discontinuation (MA2 for 1W2 and MA3 for 12W).

Many meta-analyses studies have shown that antidepressant discontinuation increases the risk of depression relapse compared to continuing the treatment (Kato et al., 2021). Considering the aberrant levels of cytokines found in depression, and the potential anti-inflammatory properties of anti-depressants, **we hypothesized that discontinuation could induce alterations (increase/decrease) in cytokine levels.**

Changes in cytokine levels before and after discontinuation by relapse status

Lastly, we asked whether cytokine levels before and after discontinuation vary between relapsers and non-relapsers.

Supposing a role of cytokine in depression relapse after antidepressant discontinuation, we would expect that discontinuation of treatments leads to changes in cytokine levels. If these were causally related to depression, then they should differ between relapsers and non-relapsers. As higher pro-inflammatory cytokine levels have been associated with higher risk of relapse, relapse may follow a greater increase in pro-inflammatory cytokines and greater decrease in anti-inflammatory cytokines after discontinuation. We might still see some changes of cytokines in non-relapsers, but we would expect that the changes in relapsers are greater than those in non-relapsers. Therefore, we hypothesized that there would be a greater change in pro-inflammatory and anti-inflammatory cytokines level after discontinuation in relapsers, but no change or little alterations occurring for non-relapsers.

4.3. Other considerations: covariates

As we progress to address the aforementioned hypotheses, it becomes essential to determine the most appropriate methodologies for elucidating these questions. Given the extensive involvement of cytokines in biological processes, our chosen statistical methods and models must encompass relevant following covariates:

Age and sex: Both age and sex have been shown to influence cytokine level. For instance, low-grade increases in circulating levels of pro-inflammatory cytokines IL6 and TNF-α have been shown to be associated with age (Brüünsgaard & Pedersen, 2003). Likewise, evidence suggest that levels of pro-inflammatory cytokines IL6, and TNF-α are higher in men than in women partly due to hormonal differences (Bernardi et al., 2020).

Body mass index (BMI): Studies have found that individuals with BMI ≥ 25 have higher plasma levels of pro-inflammatory cytokines IL6 and TNF-α compared to normal-weight individuals (Juncal-Ruiz et al., 2018), while anti-inflammatory cytokines IL-10 was found to be negatively correlated with BMI (Charles et al., 2011). (Charles et al., 2011)Increased levels of TGF-ß1 (Alessi et al., 2000)have also been associated with higher BMI.

Table 1 Expected outcome in Cytokine Levels Across Different Conditions: Current Evidence and Study Hypotheses

Cytokine	TNF-α	IL6	IL-10	TGF-ß1	CRP
Existing evidence					
MDD	↑	↑	↑	→	↑
Antidepressant effect	↑ or ↓	↓	↑ or ↓	↑	↓

Our Hypotheses and Expectations					
H1: Remission compared to HCs	→	→	↑	↑	→
*H3: Relapse predictive analysis	yes	yes	?	?	yes
H2: ADM discontinuation	↓ or →	↓ or →	↑ or →	↑ or →	↑ or →
H4: changes after discontinuation in relapsers	↑	↑	↑	↑	↑
H4: changes after discontinuation in non-relapsers	→	→	→	→	→

↑ = increase; → = no change; → = decrease

* ? = unsure

CHAPTER II – Methods

1. Ethical approval

The study received ethical approval from the cantonal ethics commission Zurich (BASEC: PB_2016-0.01032; KEK-ZH: 2014-0355), the ethics commission at the Campus Charité-Mitte (EA 1/142/14), UCL Research Ethics Committee (UCL REC) and conducted in accordance with the Declaration of Helsinki.

2. Participants

The AIDA study recruited participants diagnosed with Major Depressive Disorder (MDD) who 1) had experienced one severe or multiple depressive episodes, 2) had initiated antidepressant treatment during their last depressive episode and achieved stable remission (scoring less than 7 on the Hamilton Depression Rating Scale 17 for 30 days), and 3) have independently made the decision to discontinue their medication

prior to study participation. Healthy controls matched for age, sex and education were also recruited to identify disease and medication effects. Inclusion and exclusion criteria for the study can be found in (Berwian et al., 2022). Participant recruitment took place between July 2015 and January 2018 in Zurich, Switzerland and Berlin, Germany. All participants provided informed written consent and received monetary compensation for their participation.

Of the 123 remitted patients enrolled to the AIDA study, 19 (15%) dropped out prior to the MA1 and 1 Zurich subject's MA1 sample is missing (but continued the study). 103 remitted patients (28 from the Berlin replication sample and 75 from the main Zurich sample) and 59 matched healthy controls (23 from Berlin and 36 from Zurich) completed MA1.

Of the 104 remitted patients who continued the study, 91 (87%) completed MA2 (26 from Berlin and 65 from Zurich); in which 44 (48%) discontinued ADM before MA2 (1W2) and 47 (52%) discontinued ADM after MA2 (12W). Of the 47 patients in arm 12W, 43 participants completed MA3 (10 from Berlin and 33 from Zurich). An additional Berlin participant who completed MA3 have missing data in MA2.

Of the 87 participants who completed all main assessments in both arms, 84 (43 in 1W2 arm, 41 in 12W arm) reached a study endpoint of either no relapse (in remission for 6 months) or reached criteria for relapse and restarted antidepressants. Of these 84 patients, 30 (36%) relapsed during the follow-up period (15 from 1W2 and 15 from 12W) and the remaining 54 (63%) reached remission (28 from 1W2 and 26 from 12W) in the scope of the study.

3. Measures

3.1. Blood sample collection, processing and storage

Blood samples (60 mL) were taken by venepuncture from the participants by trained staff members at each main assessment and after discontinuation in group 12W. Blood samples were treated with sodium citrate as an anticoagulant. To prepare the platelet-poor citrate plasma, a standardised two-step separation method was used (Reinhold et al., 1997). The citrate plasma was stored as aliquots in Eppendorf reaction tubes at -80°C in a locked cupboard in the archive of the Translational Neuromodeling Unit until measurement. Sample collection commenced in July 2015 and ended on January 31, 2019.

3.2. Determination of cytokine levels using enzyme-linked immunosorbent assay (ELISA)

To process cytokine levels, frozen, pseudonymized samples were sent for analysis to the Institut für Molekulare und Klinische Immunologie, Universitaet Magdeburg. TNF-α, IL6, IL-10, and latent TGF-ß1 concentrations were measured in platelet-poor citrate plasma samples using commercially available specific human Quantikine ELISA kits (bio-techne, Minneapolis, MN, USA). High-sensitivity (HS) Quantikine ELISA kits were used for detecting low minimum detection doses of TNF-α (0.022 pg/ml) and IL6 (0.031 pg/ml). The assays were conducted following the manufacturer's instructions. In order to activate latent TGF-ß1, samples underwent transient acidification before ELISA assessment. CRP analyses were performed by the clinical chemistry lab of the *Universitätsspital Zürich* immediately after blood sampling.

4. Data analysis

Analyses were conducted using *MATLAB* (version 9.14.0.2286388, R2023a) in accordance with a predefined analysis plan of four hypotheses. For the levels of pro-

inflammatory cytokines TNF-α, IL6 and CRP and anti-inflammatory cytokines IL-10 and TGF-ß1, the comparisons conducted were as follows:

A. Baseline cytokine levels comparision:

i. Healthy controls vs. Remitted patients

ii. Relapsers vs. non-relapsers

B. Cytokine changes before and after discontinuation

i. in all remitted patients

ii. Relapsers vs. non-relapsers

C. Prediction analyses for relapsers vs. non-relapsers

4.1. Data preparation

Before the main analysis, the dataset was pre-processed. Outliers were removed using the built-in function *rmoutliers* in MATLAB, which identifies outliers using the Tukey's fences method based on the interquartile range (IQR). This method was chosen because it does not require stringent assumptions about data distribution. After outlier removal, all data were normalized using the min-max scaling to accommodate potential non-Gaussian data distribution, as well as to avoid negative values to preserve interpretability.

According to the current literature, variables including Age, Gender and BMI have potential confounding effect with cytokine levels (refer to 4.3 in introduction). Therefore, we incorporated them into the models to account for their potential confounding impacts. This allows for a more accurate assessment of the cytokine levels with the outcome variable.

4.2. Analysis Plan: Baseline Cytokine Levels Comparison

Hypothesis 1: cytokines in remitted depression - Healthy controls vs. Remitted patients

Baseline cytokine levels were compared between HCs and patients in remission who were still on antidepressants. A multivariate linear regression model was employed, with the **Group** variable (0 for HCs, 1 for remitted patients on antidepressants) as the predictor variable, and the baseline cytokine levels as the outcome variable. This approach controls for potential confounders. We tested whether the difference in baseline cytokine levels (measured at MA1) between remitted patients and never-depressed healthy controls was greater than expected by chance.

The model equation is:

$$\textbf{\textit{MA1 Cytokine levels}} = intercept + ß_k * Covariates + ß * \textbf{\textit{Group}} + \varepsilon$$

$ß_k$ represents the coefficients associated with the covariates i.e., confounders. ß represents the coefficient associated with the predictor variable that quantifies the relationship the relationship between the baseline cytokine levels (outcome) and the patient's status (predictor). ε represents the error term, which captures the unexplained variability in the patient's status that is not accounted for by the predictors in the model.

Hypothesis 2: Relapsers vs. non-relapsers (relapse prediction)

We explored how effective baseline cytokine levels could predict relapse. To achieve this, we employed a logistic regression model using the Zurich sample as the training set. In this model, the Group variable (0 for non-relapsers, 1 for relapsers) served as

the outcome, and baseline cytokine level as predictor. We ran the model both including and not including the covariates.

The model was:

$$\textbf{\textit{Relapse}} = intercept + (ß_k * Covariates) + ß * \textbf{\textit{MA1 Cytokine levels}} + \varepsilon$$

The model performance was evaluated using 5-fold cross-validation with 10 repetitions. In this approach, the Zurich sample was randomly partitioned into 5 equally sized subsets. Four of these subsets were used for training, while the fifth was used for testing. This process was repeated 5 times, ensuring that each subset was used as the test set once. The entire 5-fold cross-validation procedure was then repeated 10 times. By averaging results over these iterations, model variability can be reduced, leading to a more stable and reliable evaluation of the model's performance.

Using the logistic regression coefficients derived from the Zurich model, we then examined its predictive power by applying the Berlin data as an external validation set. This allowed us to assess the model's generalizability and robustness in a new data, ensuring that there are no overfitting problems.

Model performance metrics were reported as:

- **Area Under the Curve (AUC)** which evaluates the model performance to distinguish between relapsers and non-relapsers, with a value closer to 1 indicating better discrimination. AUC = 0.5 means the model's predictive power is no better than random chance.

- **Sensitivity (True Positive Rate)** = the proportion of actual relapsers correctly identified by the model.
- **Specificity (True Negative Rate)** = the proportion of actual non-relapsers correctly identified
- **Accuracy =** the overall proportion of correct classifications by the model.
- **Positive predictive value (PPV)** = proportion of true positives that were truly correct
- **Negative predictive value (NPV)** = proportion of negative identifications that were truly correct.

4.3. Analysis plan: Cytokine Dynamics During Antidepressant Discontinuation and Relapse

Next, we aimed to investigate the role of cytokines in the mechanism underlying discontinuation effects, as well as the intricate interplay of relapse after antidepressant discontinuation.

Hypothesis 3: discontinuation impact on all remitted patients

To investigate whether cytokine levels within patients were affected by antidepressant discontinuation, we compared cytokine levels before and after antidepressant discontinuation among all remitted patients in the study. Given the hierarchal nature of the data involving repeated measurements, we adopted a generalized linear mixed-effect model. This model addressed the inherent correlations and dependencies among observations within each patient, incorporating both fixed and random effects. The fixed effects includes the covariates and the logical variable *offdrug*. The variable *offdrug* indicates whether the patient discontinued (*offdrug* = 1) or not (*offdrug* = 0) at the specific datapoint. With this modelling approach, we can utilize the maximum

number of observations available, rather than being restricted to pairs of data points representing 'before' and 'after' discontinuation. The individual patient-specific differences can be captured random effects. Thus, the model:

Cytokine levels ~ Covariates **+ offdrug + (1|ID)**

And the equation:

$$c^i = a^i + \sum_j b_j x_j^i + ß * D^i + \varepsilon$$

$$c^i = cytokine\ levels\ for\ participant\ i$$

$$a^i = random\ intercept\ for\ participant\ i$$

$$b_j = regression\ coefficient\ for\ j\ covariate$$

$$x_j^i = covariate\ j\ for\ participant\ i$$

$$ß = regression\ coefficient\ for\ \boldsymbol{offdrug}$$

$$D^i = \boldsymbol{offdrug}\ for\ i$$

$$\varepsilon^i = \boldsymbol{error\ term}\ for\ the\ i, which\ captures\ unexplaiend\ variability$$

Hypothesis 4: Discontinuation-Relapse interaction (Relapsers vs. non-relapsers)

Where changes in cytokine levels after discontinuation in patients were statistically significant, we investigated whether these shifts in cytokine levels differed between relapsers and non-relapsers. A linear mixed-effects model was used, incorporating the covariates, the ***offdrug*** variable and relapse occurrence (non-relapse vs. relapse) as

fixed-effects. Random effects capture subject/specific variability. Such model can account for within-subject correlations and individual variability while studying the effects of discontinuation and relapse on cytokine dynamics.

Model:

Cytokine levels ~ Covariates + offdrug * Relapse01 + (1|ID)

Commented [CHK2]: 1. Investigate the influence of discontinuation (denoted as ondrug - indicating whether a patient is on antidepressant medication or not) and relapse (relapse01) on cytokine levels.
2. Examine whether cytokine levels the effect of antidepressant discontinuation differ between relapsers and non-relapsers.

Equation:

$$c^i = a^i + \sum_j b_j x_j^i + ß^R * R^i + ß^D * D^i + ß * D^i * R^i + \varepsilon$$

$$c^i = cytokine\ levels\ for\ participant\ i$$

$$a^i = random\ intercept\ for\ participant\ i$$

$$b_j = regression\ coefficient\ for\ j\ covariate$$

$$x_j^i = covariate\ j\ for\ participant\ i$$

$$ß = regression\ coefficient\ for\ \boldsymbol{offdrug}$$

$$D^i = \boldsymbol{offdrug}\ for\ i$$

$$R^i = \boldsymbol{Relapse\ variable}\ for\ i$$

$$ß^R = regression\ coefficient\ for\ \boldsymbol{Relapse}$$

$$ß^D = regression\ coefficient\ for\ \boldsymbol{offdrug}$$

$$\varepsilon^i = \boldsymbol{error\ term}\ for\ the\ i, which\ captures\ unexplaiend\ variability$$

Here, the coefficient ß represents the relationship between the predictor variables "*offdrug*" and "Relapse" when they interact together in influencing the changes in cytokine levels. This interaction term " *offdrug* **Relapse*" captures how the combined effect of discontinuation and relapse affects the outcome variable (changes in cytokine levels) beyond their individual effects.

4.4. Statistical Analyses

The normality and distribution of the data was assessed through Shapiro-Wilk and Anderson-Darling tests, with p-values ≤ 0.05 taken as indicative of a departure from a normal distribution. This assessment was supplemented by visual examination using Kernel density plots. When necessary, independent or paired t-test were employed if the data approximately adhered to normal distribution. If data deviated from normality, a robust Wilcoxon signed-rank was applied for paired samples and Mann-Whitney U test for independent samples.

4.5. Data Reporting

To assess the effect of our predictor variable while holding all the other covariates constant, we report the standardized coefficient (denoted as ß) with its 95% confidence interval (CI). The standardized coefficient allows us to evaluate the relative strength of our predictor in the model. The mean (μ) and standard error (SE) were reported for data with Gaussian distribution, while the median differences (MD) and interquartile ranges (IQR) were presented for non-Gaussian distributions.

Post-hoc analyses were employed to support the results of the regression model. For non-parametric tests, test statistic *z-value* was reported to reflect the deviation of our coefficient estimate from the null hypothesis. The Spearman's rank correlation

coefficient r_s, which indicates the strength and linear relationship of the two variables, was reported for nonparametric paired data. The equivalent U-statistic was reported for independent data. For parametric tests, Cohen's *d* (for t-tests) and t-statistic were used, with degrees of freedom reported in brackets.

p-values ≤ *0.05* were considered statistically significant.

CHAPTER III - RESULTS

1. Sample sizes and data distribution

Data overview

A total of 296 entries in the dataset was recorded. 59 of which belonged to HCs and 237 were associated with patients. 30 data points had missing Relapse status. Of these, 15 entries were dropouts (from 8 subjects). The remaining 15 without relapse data pertained to 12 patients: 4 from the 1W2 group and 8 from 12W. Depending on the specific hypothesis, these data points with missing information were judiciously excluded. The criteria for inclusion or exclusion depended on both the hypothesis in question and the specific cytokines being investigated.

Commented [CHK3]: From the dropouts, 4 were from 1W2 and 4 were from 12W. 2 from 1W2 had all datapoints from T1 to T2. 1 from 12W [CHK1] had all the datapoints from T1-T3. 3 from 12W dropped out after T2. IL10

[CHK1]3020

The IL10 measurements mostly indicated undetectable levels, with 283 out of 296 entries indicating a measurement of 0 pg/ml**. IL10 was thus excluded from further**

analysis due to insufficient variance. CRP measurements exhibited a high incidence of missing data (71 out of 296). All other cytokines data were complete, except for one patient from group 1W2 missing T1 data.

Data distribution overview

In terms of data distribution, standardised TNF-α and TGF-ß1 cytokine data in HCs followed normal distributions. This was confirmed by the Shapiro normality test ($p_{normal} > 0.05$). Conversely, IL6 and CRP data skewed to the right, which persisted even with log(x+1) transformation ($p_{normal} < 0.01$). **Patient data across all cytokines deviated from a normal distribution, with a mild right skew (Fig.2).**

2. Hypothesis 1: Baseline inflammation of Healthy controls vs. Remitted patients still on antidepressants

We assessed baseline cytokine levels measured at MA1 between HCs and patients in remission still on antidepressants using a multiple linear regression model, controlling for Age, Gender and BMI. 59 HCs and 102 patient entries were utilized for the cytokines. 43 HC and 80 patient entries were recorded for CRP.

Statistically significant differences in baseline inflammation between the two groups were found for TNF-α (***p* < 0.001)** and IL6 **(*p* = 0.019)** (Fig.2, upper panel)**.** Compared to HCs, remitted patients had lower levels of TNF-α (ß = -0.123 [-0.186 to -0.059]) and IL6 (ß = -0.080 [-0.146 to -0.014]). There was no significant difference in baseline TGFß1 (*p = 0.289,* ß = -0.033 [-0.095 to 0.028]) and CRP (*p = 0.797,* ß = 0.012 [-0.082 to 0.107]) concentrations between the groups (Fig.2. lower panel), indicating a minimal relevance of these two cytokines in distinguishing the groups in terms of baseline inflammation. Post-hoc independent comparison tests corroborated the significant

differences observed in both TNF-alpha (r = 0.307; z = 3.855; $p < 0.001$) and IL6 (r = 0.162; z = 1.961; $p < 0.05$).

3. Hypothesis 2: Relapse prediction analysis

For cytokines, 82 out of 102 entries had relapse status at MA1. 69 were recorded for CRP. 41 entries were recorded for each discontinuation group (1W2 or 12W) in cytokines. For CRP, 1W2 had 32 entries and 12W had 31 entries.

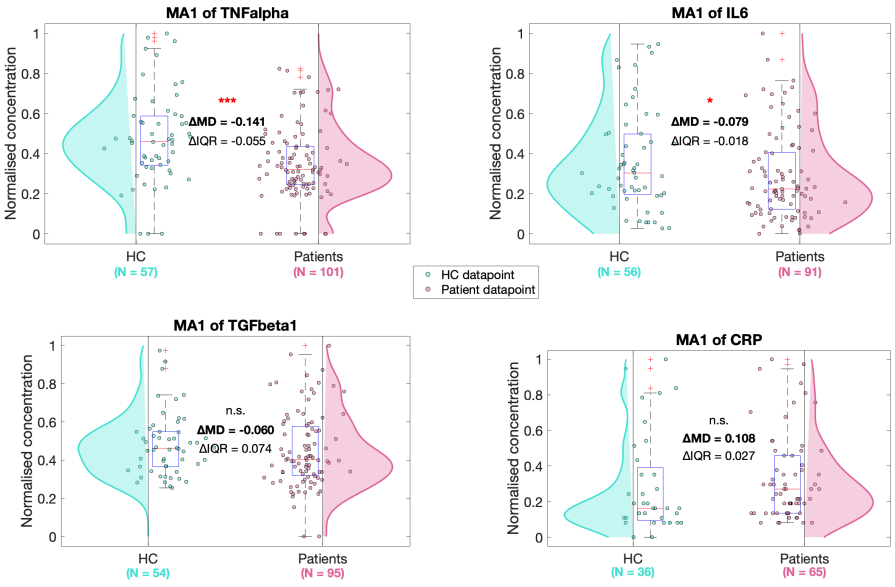

Fig. 2 Comparative Analysis of Normalised Cytokine Levels in healthy controls and remitted Patients on antidepressants.

Boxplots showing the distributions of normalised cytokine levels for HCs (turquoise) and Patients (pink), overlayed by scatter points which indicate the individual data points. Each box represents the

*interquartile range (IQR) with the central red line indicating the median. Red crosses indicate datapoints that lie outside the IQR. Median, IQR and the p-value of the coefficients from the regression model are reported in the centre of each subplot. The statistical significances of the p-values are reported in asterisks (*** for p < 0.001; ** for p > 0.01; * for p < 0.05; n.s. for p > 0.05). Y-axis = cytokine levels or inflammation scores. X-axis = subject status (HC or Patients). Number of samples are indicated below subject status. Upper panel: Cytokines from left to right are TNF-α & IL6. Significant reductions in TNF-α (MD(IQR) = 0.246(0.192); p < 0.001) and IL6 (MD(IQR) = 0.301(0.283); p = 0.021) levels in remitted patients, compared to HCs (MD(IQR) = 0.462(0.320); MD(IQR) = 0.304(0.224)), respectively). TNF-α data in HCs followed normal distribution (p = 0.110) but not in Patients (p = 0.004). IL6 data did not follow normal distribution in neither group (p < 0.001). Lower panel: TGF-ß1 (ΔMD(ΔIQR) = -0.060(0.074)) & CRP (ΔMD(ΔIQR) = 0.108(0.027). No significant differences of these cytokines between HCs and Patients were observed (p > 0.05). Only TGFß1 data of HCs followed normal distribution (p = 0.082). TGFß1 Patient data (p = 0.004), IL6 HC and Patient data did not follow normal distribution (p < 0.001).*

We employed binary logistic regression models to evaluate the possibility of predicting relapse occurrence after antidepressant withdrawal (0 for no relapse and 1 for relapse), using baseline levels measured at MA1 of the corresponding cytokine as predictor variables. After 5 repeated cross-validation, with resampling occurring at each iteration, CRP yielded the best testing model (without include the covariates) performance out of all cytokines (*n* = 69 observations). The model mean area under the ROC curve (AUC) was 0.690, suggestive of a good model performance. From second best to worse performance testing models were TGF-ß1 (AUC = 0.595), IL6 (AUC = 0.408) and TNF-α (AUC = 0.406). The CRP logistic model correctly classified 316 out of 420 resampled observations (mean balanced accuracy = 0.743). Specifically, 76 out of 90 relapsed and 240 out of 390 non-relapsed observations were correctly identified (sensitivity = 0.87, specificity = 0.71). Overall, the model on the training set performed worse with the covariates included in (AUC < 0.5).

Using the regression coefficients of the testing logistic models, we tested how the model would perform on the Berlin validation set. The AUC of the model dropped to 0.462, which was indicative of a much worse model performance. Out of 21 patients in Berlin, the model correctly predicted the relapse-state of 14 patients (balanced accuracy = 0.667), correctly classifying 1 out of 8 relapsed (sensitivity = 0.125) and 13 out of 13 non-relapsed patients (specificity = 1). Notably, TGF-ß1 model's AUC on the validation set was 0.667, with an overall accuracy of 0.682 (15/22). The model correctly classified 6 out of 9 relapsed (sensitivity = 0.667) and 9 out of 13 non-relapsed patients (specificity = 0.692). The discriminative power of TNF-α (AUC = 0.50) and IL6 (AUC = 0.538) models in predicting depressive relapse outcomes were not better than chance.

None of the models' coefficients of in both training and validation sets reached statistical significance ($p > 0.05$).

4. Hypothesis 3: Effect of discontinuation on cytokine levels in all remitted patients

Hypothesis 3 required data of timepoints before and after discontinuation. For cytokines, each discontinuation group (1W2 or 12W) had 86 entries of 43 participants each. For CRP, 70 entries were record in group 12W and 50 in group 1W2.

The base model: the effect of discontinuation on cytokine levels

We aimed to assess whether staying on or coming off antidepressants influences cytokine levels in patients who have achieved remission from their depressive symptoms.

Our multivariate linear mixed-effect model identified a significant association between antidepressant discontinuation and cytokine levels, specifically for TNF-α and IL6. For TNF-α, the results indicated a significant increase in cytokine levels in those who discontinued antidepressants (*offdrug* = 1) with a coefficient ß = 0.097 (SE = 0.023, $t(218) = 4.296$, $p < 0.0001$), and a 95% confidence interval that excludes 0 (CI = [0.053 to 0.142]). Similarly, for IL6, the coefficient was ß = 0.067 (SE = 0.026, $t(207) = 2.569$, $p = 0.011$), which suggests an increase in cytokine levels by 0.067 units for those who stopped antidepressants. The 95% CI of this effect ranged from 0.016 to 0.118 units.

A) Training data model

Cytokine	Coefficient	*p*-value
TNF-α	-0.480 [-3.575 to 2.489]	0.771
IL6	1.879 [-2.908 to 2.641]	0.303
TNFß1	3.695 [-1.711 to 8.069]	0.182
CRP	2.563 [-1.010 to 4.430]	0.134

Cytokine	Mean AUC	StdAUC	Mean Sensitivity	Mean Specificity	Mean PPV	Mean NPV	Mean Accuracy
TNF-α	0.406	0.197	0.485	0.730	0.500	0.765	0.645
IL6	0.408	0.156	0.578	0.617	0.463	0.803	0.603
TNFß1	0.573	0.155	0.619	0.724	0.637	0.815	0.690
CRP	0.690	0.196	0.870	0.710	0.542	0.966	0.743

B) Validation data model

Cytokine	Coefficient	*p*-value
TNF-α	-0.543 [-3.802 to 2.842]	0.720
IL6	-0.133 [-1.792 to 5.549]	0.923
TNFß1	3.179 [1.883 to 9.272]	0.193
CRP	1.666 [-0.922 to 6.047]	0.223

Cytokine	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
TNF-α	0.500	0.889	0.308	0.471	0.800	0.545
IL6	0.538	1.000	0.308	0.500	1.000	0.591
TNFß1	0.667	0.667	0.692	0.600	0.750	0.682
CRP	0.462	0.125	1.000	1.000	0.650	0.667

Table 2 Model parameters and performance metrics of the logistic regression models in predicting relapse of the four tested cytokines.

The model performance was evaluated using both training data from the Zurich site (A) and validation data from the Berlin site (B). The upper panel of each section states the models' coefficients and the associated p-values. While the CRP model showed the highest balanced accuracy and AUC in the training data, its performance dropped in the validation set. Conversely, the TNFß1 model performed relatively consistently across both datasets.

Conversely, for TGF-ß1 and CRP, the results were not statistically significant. TGF-ß1 had a coefficient of ß = 0.024 (SE = 0.020, $t(215) = 1.200$, $p = 0.232$) with a confidence interval from -0.016 to 0.064. Meanwhile, CRP showed an decrease with a coefficient of ß = -0.019 (SE = 0.028, $t(146) = -0.685$, $p = 0.494$) and a CI between -0.074 and 0.036. Therefore, while discontinuing antidepressants might lead to increase in pro-inflammatory cytokines TNF-α and IL6, it does not appear to influence TGFß1 and CRP levels.

The refined model: the effect of discontinuation timing on cytokine levels

Following our initial analysis, we wanted to explore whether the cytokine changed differently depending on whether they waited or not. To explore this idea further, we adjusted our original model to include the 'Discont' variable. This categorical variable differentiates between two discontinuation groups: post-MA1 (denoted as 1W2, Discont = 0) and post-MA2 (12W, Discont = 1). Our refined model was:

cytokine levels ~ Age + Gender + BMI + offdrug * Discont + (1|ID)

Upon revisiting the data with the enhanced model, the influence of the *offdrug* variable on TNF-α levels became even more evident, registering a more pronounced positive effect (ß = 0.212, SE = 0.031, $t(216) = 6.885$, $p < 0.0001$). While the effect of the discontinuation timing (Discont) was not significant on its own ($p > 0.05$), an intriguing interaction surfaced between drug discontinuation and its timing (*Discont x offdrug*): later antidepressant discontinuation (1W2) corresponded to a further decrease in TNF-α levels by 0.210 units (ß = -0.210, SE = 0.041, $t(216) = -5.087$, $p < 0.0001$), with a 95% confidence interval between -0.291 and -0.128 (Fig. 3). Contrarily, previously established notable influence of discontinuation on IL6 levels became statistically indistinct in the refined model (ß = 0.040, SE = 0.040, $t(205) = 1.010$, $p = 0.314$) nor in the other cytokines ($p > 0.05$). Moreover, the discontinuation and timing interaction failed to reach statistical significance in IL6 ($t(205) = 1.055$, $p = 293$).

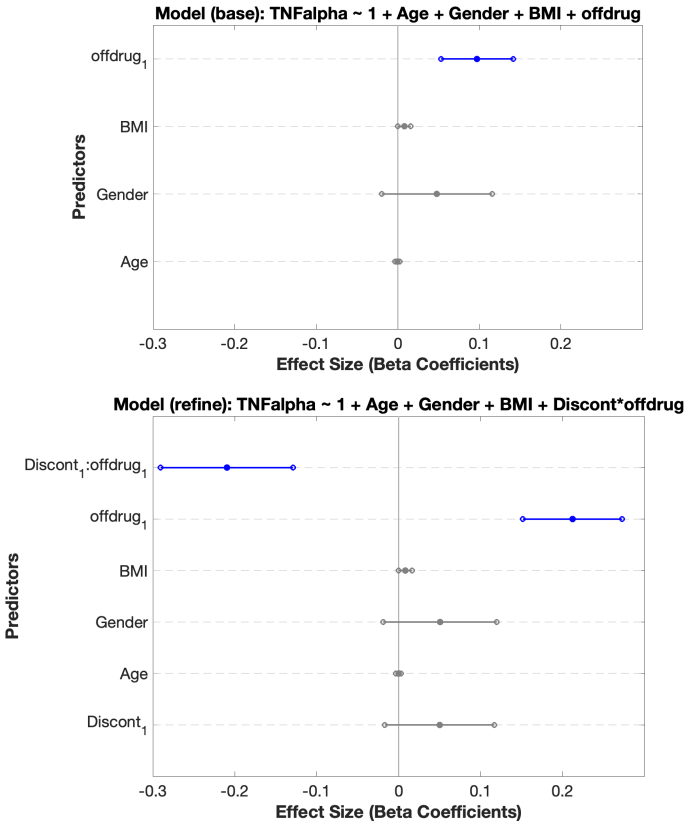

Fig. 3 Forest plots of the base (upper) and refined (refined) regression models comparing the coefficients of the predictors.

Each horizontal line represents a 95% confidence interval for a given predictor's coefficient, with the point indicating the coefficient value. Blue lines and points signify coefficients that are statistically significant at $p < 0.05$, whereas gray lines and points represent non-significant coefficients. The vertical gray line at x=0 serves as a reference, indicating no effect. The order of predictors is derived from their appearance in the regression model, as expressed by the formula shown above each plot. The ß coefficient of offdrug in the base model is 0.097 (CI = 0.053 to 0.142; $p < 0.0001$). Note the pronounced enhancement of the 'offdrug' coefficient upon inclusion of the the 'Discont' variable in the refined model (ß = 0. 212; CI = 0.152 to 0.273; $p < 0.0001$), emphasizing the potential influence of antidepressant

discontinuation timing on cytokine levels. The coefficient of the interaction term 'Discont x offdrug' is negative (ß = -0. 210; CI = -0.291 to -0.128; p < 0.0001).

Subgroup analysis

To further explore the data, we conducted post-hoc subgroup analyses. The dataset was split based on the timing of antidepressant discontinuation (i.e., 1W2 or 12W group).

1W2 Group analyses:

Pairwise Wilcoxon signed-rank tests on the composite vector indicated an overall increase in cytokine levels after discontinuation (r_s = 0.743, z = 4.458, *p < 0.0001*). Aligning with previous findings, the observed increase in cytokine levels due to discontinuation was primarily propelled by TNF-α, showing a marked rise post-discontinuation (r_s = 0.780, z = 5.052*, p < 0.0001*; refer to Fig. 4).

12W Group analyses:

The 12W group, having discontinued antidepressants at the second timepoint, presents an intriguing scenario. These patients possess data of three timepoints, which can be divided into two distinctive periods: one during which the patient does not discontinue (waiting period; MA2-MA1), and another during which the patient discontinues (discontinuation period; MA3-MA2). This distinction offers a unique opportunity for direct comparison on the differences in changes in cytokine levels of two distinct periods within the same individual.

Post-hoc pairwise Wilcoxon signed-rank non-parametric tests (adjusted using Bonferroni) showed that, during the waiting period, these patients experienced an increase in all cytokines' levels; though the rise was only statistically significant for

TNF-α (r_s = 0.752, z = 5.156, $p < 0.001$) and IL6 levels (r_s = 0.390, z = 2.585, $p = 0.015$) (Fig. 4 upper panel). Post-discontinuation painted a different picture: TNF-α levels plummeted (r_s = -0.669, z = -4.583, $p < 0.0001$), while IL6 continued to increase, albeit not statistically significant (r_s = 0.263, z = 1.742, p = 0.081). The variance in the changes of TNF-α levels, between the waiting period and discontinuation period in the 12W cohort, suggests the pronounced sensitivity of TNF-α to antidepressant discontinuation relative to other cytokines.

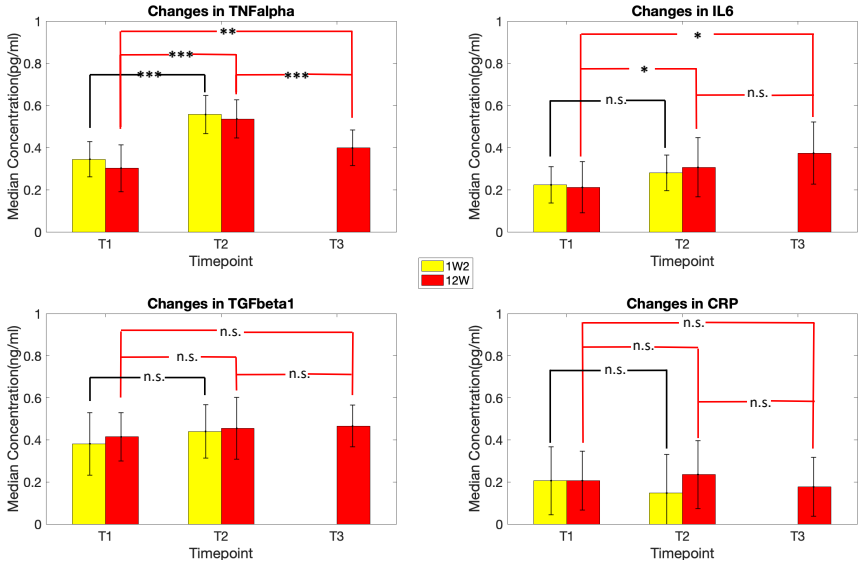

Fig. 4 Temporal profiles of the cytokines TNF-α , IL6, TGFß1 and CRP levels across three primary assessments (T1, T2, T3).

The bar plots compare the median (IQR presented in error bars) cytokine concentrations for two subgroups: 1W2 who discontinued at Timepoint 1 (yellow bars) and 12W who discontinued at Timepoint 2 (red bars). The units of measurement are shown with the y-axis. For the 1W2 group, comparison between T2 and T1 relates to black lines, while three distinct comparisons for the 12W group are linked with red lines. The Y-axis displays cytokine concentrations and x-axis displays the three different timepoints denoted as T1 T2 and T3. ***Upper panel:*** *(Left) TNF-α levels are significantly different for*

pre- and post-discontinuation in both groups 1W2 and 12W. In group 1W2, TNF-α increases post-discontinuation, whilst in group 12W, TNF-α level increase from T1 to T2 but then decreases post-discontinuation to T3. (Right) IL6 levels are not significantly different in group 1W2 post-discontinuation. In group 12W, IL6 levels increases as time progresses. ***Lower panel:*** *from left to right, TGFß1 and CRP. No significant differences between the timepoints are observed for both cytokines.*

5. Hypothesis 4: Effect of antidepressant discontinuation on cytokine levels in Relapsers and Non-relapsers

For hypothesis 4, 80 cytokine entries (of 40 participants) were found in group 1W2. The 12W group had 82 cytokine entries (of 41 participants). For CRP, 60 were recorded in group 1W2 and 75 in 12W.

Before addressing the primary hypothesis, we first examined any potential correlation between the timing of discontinuation and relapse, given that our dataset contains two distinct groups with varying discontinuation timings (1W2 and 12W). The analysis indicated a weak correlation (*rho* = -0.021; *p* = 0.753). Due to the weak correlation, we proceeded without including the timing of discontinuation as a covariate. We then applied the following linear mixed-effect model to assess the changes of cytokine levels after antidepressant discontinuation in relation to patient relapse status:

Results from our multivariate mixed-effects model yielded a significant effect of the discontinuation variable on TNF-α levels (ß = 0.104 [0.046 to 0.163]; *p < 0.0001*). However, both the interaction between discontinuation and relapse, as well as the main effect of relapse, displayed no significant influence on cytokine levels (*p > 0.05) (Fig. 5A)*. This suggests that there is insufficient evidence to reject the null hypothesis of in cytokine levels post-discontinuation concerning relapse status.

Commented [CHK4]: Patients who discontinued ADM (offdrug = 1) had higher odds of high TNF-alpha levels (ß = 0.104; SE = 0.030; t(188) = 3.492). A similar result was observed for the composite component (*p < 0.001*) (ß = 0.681 ± 0.179; t(159) = 3.805), indicating an overall increase in inflammation after antidepressant discontinuation.

To elucidate the direction and magnitude of cytokine level alterations after discontinuation, post-hoc comparisons between the two groups were performed, comparing cytokine level changes pre- and post-discontinuation (Fig. 5B). The tests reiterated the previous results, confirming non-significant difference in cytokine alterations between relapsers and non-relapsers across the four tested cytokines ($p > 0.05$).

Two further noteworthy observations were however made. Firstly, there was a more prominent median shift in TNF-α level for non-relapsers ($r_s = 0.252$; MD (IQR) = 0.13 (0.42)) compared to relapsers ($r_s = 0.171$; MD (IQR) = 0.00 (0.27)). Yet, this was not statistically different to 0 ($p = 0.087$) in both groups, including non-relapsers. Secondly, post-discontinuation changes in IL6 levels were statistically different from no change in relapsers ($p < 0.05$). With an effect size of Cohen's $d = 0.52$ with ($t(24) = 2.600 \pm 0.250$) which indicates a medium effect, the results suggest that IL6 levels rise post-discontinuation among relapsers. In contrast, the change in IL6 levels in non-relapsers seems to be insignificant ($p = 0.101$) and not different to 0 change, with a small effect size r_s of 0.239 ($z = 1.640$).

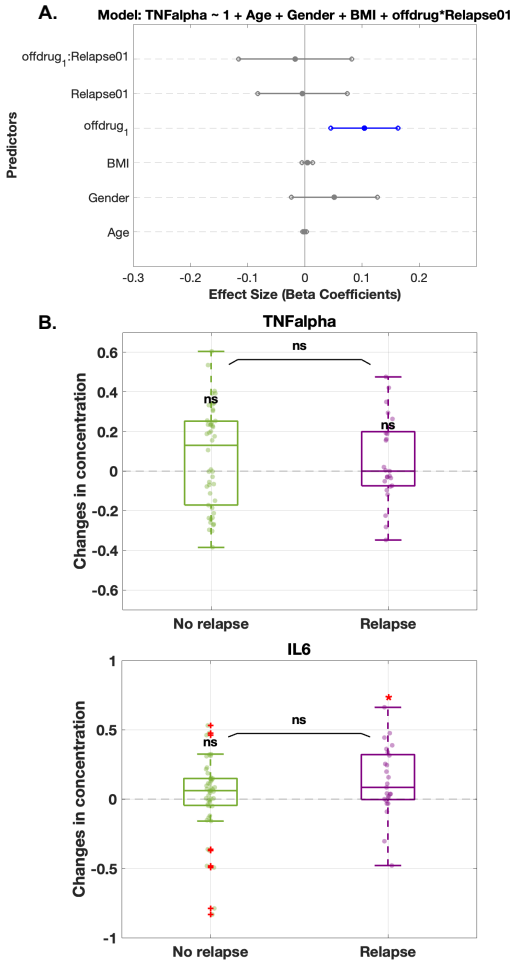

Fig. 5 Comparative plots of hypothesis 4

A) Mixed-effect model forest plot examining the effect of discontinuation on TNF-α levels in relation to relapse status. *The coefficient of the discontinuation variable is denoted as 'offdrug' (ß = 0.104 [0.045 to 0.163]), which shows to be statistically significant as indicated by the blue line (p < 0.0001). Other predictors, including the covariates (Age, Gender and BMI), the main effect of Relapse (ß = -0.004 [-0.083 to 0.075]) and the interaction term of discontinuation and relapse (ß = -0.0168 [0.116 to 0.083]), are non-significant (represented in grey, p > 0.05).* ***B) Boxplots comparing the differential changes in TNF-α and IL6 levels pre- and post- antidepressant discontinuation between relapsers (in purple) and non-relapsers (in green).*** *No statistical differences were detected between the groups cornering the levels of either cytokines. Upper panel: Though not statistically significant, there was a more marked change in TNF-α levels for non-relapsers post-discontinuation (MD (IQR) = 0.13 (0.42); N = 46) than in relapsers (MD (IQR) = 0.00 (0.27); N = 25). One-sample signed rank tests indicate that the post-discontinuation change of TNF-α levels is not significantly different from zero for non-relapsers (p = 0.087) or relapsers (p = 0.212). Lower panel: One sample test showing that the changes in IL6 levels are significant for relapsers (p < 0.05; MD (IQR) = 0.08 (0.32); N = 25) but are non-significant for non-relapsers (p = 0.101; MD (IQR) = 0.06 (0.19); N = 47).*

CHAPTER IV - DISCUSSION

Growing evidence points towards the involvement of the inflammatory system in depression pathology, with research suggesting that activation of the inflammatory response may relate to the therapeutic effects of antidepressants and depression relapse (Liu et al., 2019). The AIDA study collected cytokine data from the blood samples of 103 depressed patients in remission during the gradual withdrawal of their antidepressant treatment (and 59 matched controls), with an overall relapse rate comparable to current epidemiological and clinical evidence (Davies & Read, 2019; Kumagai et al., 2019). Drawing on the cytokine data from the AIDA study, we used multivariate regression models to examine if cytokine levels could predict depression relapse post-antidepressant discontinuation; as well as to investigate the implications of cytokines on the neurobiology of depression and antidepression.

Our regression models revealed that overall levels of pro-inflammatory cytokine exhibit little predictive power of cytokine level in depressive relapse post-antidepressant discontinuation. TGFß1 may have some predictive value but its relevance requires further research. However, our analyses unraveled other insights in relation to antidepressant discontinuation. Specifically, we noted lower levels of pro-inflammatory cytokines (TNF-α and IL6) in remitted patients still on antidepressants compared to never-depressed individuals. Moreover, discontinuing antidepressants led to alterations in these pro-inflammatory cytokine levels, but not in anti-inflammatory cytokines TGFß1 and the inflammation marker CRP. These findings suggest that, while cytokine levels might not directly predict depression relapse, they are implicated in the neurobiological dynamics of depression and its pharmacotherapy.

In this chapter, I will discuss these findings by hypotheses, critically unpack their significance, highlight the study's strengths and limitations, and propose potential avenues for future research.

1. The anti-inflammatory properties of antidepressants

In hypothesis one, we compared the baseline cytokine levels of remitted patients on antidepressants with never-depressed individuals. Initially we expected that depressive remission would correspond with normalisation of the cytokine levels to those of healthy ranges. Consistent with the initial hypothesis, the inflammatory marker CRP and the anti-inflammatory cytokine TGFß1 in patients in remission on antidepressants were not significantly different to never-depressed healthy controls. Contrary to our expectations, we found significantly lower levels of pro-inflammatory cytokines TNF-α and IL6 in the patient group, after controlling for Age, Gender, and BMI. The difference in TNF-α was particularly pronounced ($p < 0.001$) and unlikely to be due by chance.

It is worth noting that our study did not measure baseline cytokines from patients at the onset of their depression, thus it remains unclear if their cytokine levels were normalized to pre-depression states. Yet, the distinct differences in TNF-α and IL6 cytokine levels between the depressed and never-depressed groups indicate inherent disparities in their inflammatory profiles. Notably, the lowered levels of pro-inflammatory cytokines in patients on antidepressants imply a more suppressed inflammatory state than that of healthy controls. This observation aligns to the proposed anti-inflammatory effects of antidepressants. Supporting this notion, a meta-analysis spanning 45 studies revealed a consistent reduction in TNF-α and IL6 post-antidepressant treatment in over 1500 patients' data (Köhler et al., 2018).

However, the proposed anti-inflammatory property of antidepressants is still conflictive in the literature. For instance, an earlier meta-analysis by (Hannestad et al., 2011) indicated that while antidepressant treatment may reduce IL6, it seemingly had no impact on TNF-α levels. Addressing this discrepancy, a more recent meta-analysis of 44 studies by (Liu et al., 2020) noted that only those responding to antidepressant treatment showed a notable decline in TNF-α. This is consistent with the results of this study, as all patients in the AIDA cohort had achieved remission from their depressive symptoms (i.e., they are all responders). This might explain why Hannestad, and colleagues did not detect a reduction of TNF-α levels as their investigation did not differentiate antidepressant responders and non-responders. The fact that TNF-α levels are only involved in antidepressant responders, but not non-responders, further supports the idea that the anti-inflammatory effects of antidepressants might be one of the mechanisms by which these compounds exert their therapeutic effect.

The significant differences found in pro-inflammatory cytokines levels between remitted patients and healthy subject is in contrast with Brietzke et al 2009's findings, which reported no significant differences of IL6 and TNF-α levels between the two groups. However, it is important to note that achieving remission do not necessary imply normalisation of cytokine levels to the range seen in healthy controls. Instead, individuals vulnerable to depression could inherently have baseline cytokine levels different to never-depressed healthy controls. Supporting this, a meta-analysis of 32 studies by (Smith, 2018; Smith et al., 2018) indicated elevated baseline IL6 levels was predictive of later depressive symptoms, highlighting an early inflammatory difference even before depression onset. Therefore, the 'inflammatory set point' might be inherently different for never-depressed and depressed subjects. Future studies should exercise caution when making conclusions about "normalisation" based solely

on comparisons of cytokine levels between depressed to never-depressed individuals. Such comparisons might not offer a precise reflection of a "normal" or baseline state for the individual, given the potential inherent inflammatory differences between the two groups.

2. Impact of Antidepressant Discontinuation on Cytokine Levels

Secondly, we investigated whether antidepressant discontinuation may have an impact on cytokines. The results presented marked changes in the levels of pro-inflammatory cytokine TNF-α, but not others, following antidepressant discontinuation. Specifically, two distinct patterns emerged based on the timing of discontinuation: following discontinuation, TNF-α levels increased in the discontinuation group 1W2 while levels decreased in the waiting group 12W. The changes were statistically significant in our linear mixed-effect model, even after adjustments for confounding variables including Age, Gender and BMI.

The observed pronounced increase in TNF-α levels in the 1W2 group might align with the well-documented phenomena of antidepressant discontinuation syndrome (ADS). ADS usually occurs following the abrupt cessation of antidepressant treatment taken for at least six weeks (Gabriel & Sharma, 2017). One of the most common symptoms of ADS are flu-like symptoms, which are intrinsically linked with inflammation. Consequently, the increase in pro-inflammatory markers like TNF-α could be associated with the increases in cytokine levels observed in group 1W2. Supporting this, Volkmann et al. 2023 in their investigation on discontinuation symptoms using the same AIDA dataset, highlighted that 50% of patients in the discontinuation group 1W2 exhibited symptoms consistent with ADS, whereas only a considerably lower percentage (21%) of patients exhibited ADS symptoms in the waiting group. The

increase in pro-inflammatory cytokines in 1W2, where a higher incidence of ADS is found, may suggest that pro-inflammatory cytokines are key players in the presentation of ADS.

However, while our results offer intriguing insights, these should be approached with caution. Namely, we observed unexpected, statistically significant changes in TNF-α levels during the waiting period (MA1 to MA2) of the 1W2 group. There should have been no alteration in cytokine levels during this phase. One conceivable explanation is that the increase in TNF-α levels is a result of antidepressant effect. However, this contradicts the anticipated anti-inflammatory effects of the antidepressants as discussed earlier. Moreover, the fact that the observed variance aligns the discontinuation changes seen in the 1W2 group suggests more of a measurement error.

Firstly, variations in the timing of sample collections could introduce discrepancies in the data. However, this is unlikely given that the samples were collected intermixed. For some participants, MA1 samples taken in times where others had their MA2 samples already taken. This method ensures that both MA1 and MA2 samples span across various time points, thereby minimising potential time-based biases. Therefore, the possibility of timing as a significant influencing factor to the observed differences between MA1 and MA2 is low.

Secondly, storage conditions could impact the sample integrity. However, both MA1 and MA2 samples of different participants were acquired and stored following the same protocol, and sometimes even on the same day. This means that all samples were subjected to the same conditions, hence the possibility of variation in sample integrity also is slim.

Thirdly, measurement inconsistencies could arise when assaying plates for cytokine level measurements in ELISAs due to non-uniform coating, plate contamination, well-to-well variability, or reagent inconsistency. However, all assays were performed as one single patch, which minimises inter-assay variability. Moreover, the consistency observed in the standard curve—where the measured responses closely aligned with known concentrations across the range of standards—reinforces the notion that the assay should be reliable.

Considering all above, it is difficult to pin down the exact reason to the unanticipated difference in TNF-α levels measured between MA1 and MA2 in 12W group. Regardless, these results highlight the important of rigorous methodologies in examining cytokine levels. Future studies with larger sample sizes might be necessary to elucidate whether the longitudinal time-based effect of TNF-α levels is real. Until then, clinicians should be aware of these findings, but also approach them with the requisite caution when considering clinical applications.

3. Cytokine levels in relapse prediction

In the last two hypothesis, we examined if cytokine levels could serve as predictors of depressive relapse; and whether these levels altered upon discontinuing antidepressants between relapsers and non-relapsers. The logistic regression model generated from the Zurich sample set indicated potential but inconclusive predictive power of cytokine levels in depressive relapse post-antidepressant discontinuation. Similarly, the discontinuation-induced changes in cytokine levels did not reveal any discernible differences between individuals who experienced a relapse and those who remained stable.

From our logistic model using 5 repeats of 10-fold cross validation, CRP emerged as the most promising predictor as it yielded the highest model performance (AUC = 0.690). This aligned with results from Freeman et al 2018, who identified a significant association between CRP levels and depressive relapse in 49 women previously diagnosed with MDD or bipolar depression. However, CRP as a general marker of inflammation biomarker—reactive to various conditions—warrants cautious interpretation of its predictive value. Specifically, CRP's effects associated with psychiatric symptoms have shown to be contingent upon different covariates factored into statistical analyses (Figueroa-Hall et al., 2022). Indeed, the predictive accuracy of our CRP model dropped (AUC < 0.4) in the validation set, suggesting its utility as a depressive relapse predictor may not be consistent across different populations.

Notably, the TGF-ß1 model yielded 68.2% accuracy in predicting relapse in the Berlin replication sample (AUC = 0.667). TGF-ß1 thus could be a potential predictor, possibly reflecting the role of anti-inflammatory processes in depressive relapse. Aligning with this, studies that have found elevated TGF-ß1 levels in MDD patients (Lee & Kim, 2010), indicating a role of TGF-β1 in depression pathology. However, our training set displayed an AUC value of limited performance for TGF-ß1. As the difference in performance in the two sets was not huge, it was unlikely to be due to overfitting. Instead, it was possible that the Berlin sample is more clean or less noisy than the training data. It is important to note that several studies and meta-analyses have found no significant difference of TGF-ß1 levels between HCs and depressed patients (Köhler et al., 2017). Consequently, the predictive power of TGF-ß1 levels is still considered of limited usefulness and warrants further investigation.

Contrary to our initial expectations, the predictive performance of the two pro-inflammatory cytokines, TNF-α and IL6, was unsatisfactory for both the training and validation sets. This finding is surprising given the consistent evidence linking these pro-inflammatory cytokines to depression as discussed previously. High IL6 have been shown to be predictive of subsequent chronic course of depression in longitudinal studies. For instance, a population-based longitudinal study by Khandaker et al., 2014 involving approximately 4500 individuals demonstrated that children with elevated systemic IL6 levels at age 9 had a heightened risk of developing depression and psychosis by age 18, even after controlling for demographic and psychological confounders (Khandaker et al., 2014). Similarly, high TNF-α levels have also been found to be associated with depression risk. A study involving 1094 patients by (Choi et al., 2021) found that elevated TNF-α levels were predictive of a 24-month relapse in depressed patients who had initially responded to antidepressant medication. Nonetheless, it is crucial to recognise that these studies assessed cytokine levels before patients began antidepressant treatment. Therefore, the low predictive value of these cytokines results in our study may be due to effects of antidepressants. In line with this, our mixed-effect model showed a significant effect of the discontinuation variable on TNF-α levels. Additionally, post-discontinuation changes in IL6 levels were statistically different from no change in relapsers, suggesting that antidepressants influence pro-inflammatory levels. These findings highlight the importance in future research to factor in potential alterations in cytokine levels induced by antidepressants, particularly when the aim is to pinpoint predictors aiding clinicians in deciding the continuation or cessation of antidepressant therapy. Our data suggests that TNF-α and IL6 measurements taken during antidepressant treatment might not serve as optimal indicators for such clinical decisions.

4. Strengths and Limitations

A major strength of this study lies in the unique study design of the AIDA. Specifically, we assessed cytokine levels just prior to patients' decision to discontinue their antidepressants, which are more realistic assessments that can help clinicians making decisions when advising patients on whether to continue or discontinue antidepressant therapy. This method thus allows us to evaluate cytokine levels as practical predictors in real-world settings. Furthermore, the separation of a waiting (12W) and discontinuing (1W2) group in the AIDA study allowed us to pinpoint the effects of antidepressant discontinuation, an aspect rarely explored in prior research. However,

due to the open-label design of the trial, we cannot rule out the potential of nocebo effects or the likelihood of biased reporting influencing the establishment of relapse outcomes. Nevertheless, having certified clinical evaluations in the process likely reduced such biases.

Another strength of our study is the application of the repeated 10-fold cross-validation technique. By repeating the cross-validation process five times and averaging results of the model, the variance associated with the performance measure can be reduced, which allowed us to reproduce a more reliable model evaluation and consistent measures. Furthermore, the availability of a replication/validation set, as demonstrated with the Berlin sample, offers a deeper insight into the generalizability of our findings across diverse settings or populations. Indeed, the CRP model performed well on the training set but poorly on the replication Berlin set. This helped us to identify the potential overfitting issue of the CRP model.

Several important limitations exist in this study. Firstly, the sample size was small, and the distribution of the cytokine sample was highly non-parametric in some cases, which have complicated the interpretation of our statistical findings and may have potentially mask subtle yet clinically significant effects. Additionally, this small sample size restricted our capacity to confidently identify heterogeneously presented subgroups. Moreover, missing values, particularly in the CRP dataset compromised the robustness and reduced the overall power of our analyses, impacting our ability to draw definitive conclusions with confidence. Importantly, IL-10 data was not considered in this study due to invalid results from measurement, which has unfortunately compromised our research in understanding the potential role of IL-10 in the study context. These issues stem largely from the methodologies used in cytokine assay, which their problems and complexities have already been risen in several reviews (Hubertus Himmerich et al., 2019; Liu et al., 2021), including sensitivity limitations in detecting low-abundance cytokines or inherent variability across assays. This points toward a crucial area of improvement and future research endeavours should prioritise refining and enhancing the consistency and accuracy of cytokine level acquisition.

5. Implications and Future directions

The results of this study have important clinical implications. Firstly, our findings add to the existing literature on the potential involvement of cytokines in depression, as well as the anti-inflammatory properties of antidepressants. Our results support that specific cytokines like TNF-α and IL6 may offer potential therapeutic target in treating or managing depression. From a clinical perspective, understanding the inflammatory

pathways influenced by antidepressants may help in tailoring treatments for patients, especially those who don't respond to conventional therapies.

Secondly, if the notable alterations in pro-inflammatory levels are indeed due to antidepressant discontinuation, clinicians should be aware of these inflammatory effects when considering tapering or discontinuing medication. Such inflammatory changes may correspond with ADS withdrawal symptoms. In fact, Volkmann's analysis from the same patient sample supports this, as significant correlation between antidepressant discontinuation and depressive was found. Volkmann's findings suggest that clinicians may mistakenly interpret ADS symptoms as a relapse of depression. Future research could investigate the potential physiological link between pro-inflammatory cytokine changes and ADS discontinuation symptoms. Such insights may improve the diagnostic precision, minimising the risk of misinterpreting discontinuation symptoms with relapse.

Thirdly, despite the overall low predictive value of cytokine levels in depressive relapse; the fact that cytokine TGF-ß1 exhibited some potential predictive power regarding depression relapse in the validation set, suggest that the role of cytokines in depression relapse might not be completely out of the picture. Instead, TGF-ß1 alone may not be sufficient to forecast the risk of a patient experiencing depression relapse, but their predictive value may be improved with additional parameters. For instance, overactivity of the HPA-axis have been suggested to involve in depression neurobiology (Mikulska et al., 2021), and pro-inflammatory cytokines can influence the HPA-axis by disturbing the negative feedback inhibition of circulating corticosteroids (Schiepers et al., 2005). Furthermore, the discrepancies in the cytokine results in depression studies may be in part explained by genetics, in particular, the

heterogeneity of depression immunophenotype (Barnes et al., 2017). In fact, twin studies have shown a hereditable link between depression and inflammation (Su et al., 2009). That is, future endeavours may focus on building a more complex predictive model encompassing genetics, physiological markers, and neurobiological markers for understanding depression relapse risk.

Importantly, refining methodologies for cytokine measurements will be crucial to bridge discrepancies and solidify conclusions. This applies not just to cytokines but to all physiological metrics that are employed in understanding depression. With this, future research with larger and more diverse cohorts should be applied to substantiate these findings.

CHAPTER V - CONCLUSION

The journey to treat depression extends beyond merely prescribing antidepressants. Once a patient reaches remission, clinicians are met with a critical decision: to continue or discontinue the antidepressant regime. Discontinuing antidepressant may increase the chances of relapse. As such, identifying biomarkers that can guide this pivotal decision is imperative. More importantly, we need to understand what are the mechanisms that make some people more prone to relapse and others less so.

Among many potential biomarkers, one of them are cytokines. Long-standing theories suggest the inflammatory system's role in depression's pathology, but the precise nuances of its involvement remain elusive. This study explored the roles of cytokines, in specific TNF-alpha, IL-6, TGF-ß1 and CRP on the psychopathology underlying depression. Drawing from the extensive, two-center AIDA study, we analyzed cytokine levels of patients who have achieved remission from MDD and monitored their cytokine levels along their journey from remission to potentially relapse.

Using powerful analytical tools, our robust logistic model indicated that these cytokines offer limited predictability regarding depression relapse after discontinuing antidepressants. However, our results unraveled other significant insights. Importantly, our results suggest that the changes in pro-inflammatory cytokines TNF-alpha and to a less extent, IL-6 are highly correlated with antidepressant discontinuation. This is suggestive to a role of inflammation in antidepressants. Further supporting this statement, remitted patients on antidepressants exhibit lower pro-inflammatory cytokines (TNF-α and IL6) when compared to their never-depressed counterparts. This reinforces the notion that while these cytokines may not serve as straightforward predictors for relapse, they play a significant role in the broader neurobiological

landscape of depression and its treatment. As the field moves forward, understanding these nuances could hold the key to refining therapeutic strategies, guiding research towards a more holistic understanding of depression's underpinnings, and fostering patient-centric approaches for management and intervention.

REFERENCES

A. John Rush , M. D., Madhukar H. Trivedi , M. D., Stephen R. Wisniewski , P. D., Andrew A. Nierenberg , M. D., Jonathan W. Stewart , M. D., Diane Warden , P. D., M.B.A. ,, George Niederehe , P. D., Michael E. Thase , M. D., Philip W. Lavori , P. D., Barry D. Lebowitz , P. D., Patrick J. McGrath , M. D., Jerrold F. Rosenbaum , M. D., Harold A. Sackeim , P. D., David J. Kupfer , M. D., James Luther , M. A., & Maurizio Fava , M. D. (2006). Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. *American Journal of Psychiatry*, *163*(11), 1905-1917. https://doi.org/10.1176/ajp.2006.163.11.1905

Alessi, M. C., Bastelica, D., Morange, P., Berthet, B., Leduc, I., Verdier, M., Geel, O., & Juhan-Vague, I. (2000). Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes*, *49*(8), 1374-1380. https://doi.org/10.2337/diabetes.49.8.1374

Angst, J., Gamma, A., Sellaro, R., Lavori, P. W., & Zhang, H. (2003). Recurrence of bipolar disorders and major depression. A life-long perspective. *Eur Arch Psychiatry Clin Neurosci*, *253*(5), 236-240. https://doi.org/10.1007/s00406-003-0437-2

Banks, W. A., Kastin, A. J., & Broadwell, R. D. (1995). Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation*, *2*(4), 241-248. https://doi.org/10.1159/000097202

Barnes, J., Mondelli, V., & Pariante, C. M. (2017). Genetic Contributions of Inflammation to Depression. *Neuropsychopharmacology*, *42*(1), 81-98. https://doi.org/10.1038/npp.2016.169

Bauer, M., Pfennig, A., Severus, E., Whybrow, P. C., Angst, J., & Möller, H. J. (2013). World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of unipolar depressive disorders, part 1: update 2013 on the acute and continuation treatment of unipolar depressive disorders. *World J Biol Psychiatry*, *14*(5), 334-385. https://doi.org/10.3109/15622975.2013.804195

Belzeaux, R., Bergon, A., Jeanjean, V., Loriod, B., Formisano-Tréziny, C., Verrier, L., Loundou, A., Baumstarck-Barrau, K., Boyer, L., Gall, V., Gabert, J., Nguyen, C., Azorin, J. M., Naudin, J., & Ibrahim, E. C. (2012). Responder and nonresponder patients exhibit different peripheral transcriptional signatures during major depressive episode. *Transl Psychiatry*, *2*(11), e185. https://doi.org/10.1038/tp.2012.112

Bernardi, S., Toffoli, B., Tonon, F., Francica, M., Campagnolo, E., Ferretti, T., Comar, S., Giudici, F., Stenner, E., & Fabris, B. (2020). Sex Differences in Proatherogenic Cytokine Levels. *Int J Mol Sci*, *21*(11). https://doi.org/10.3390/ijms21113861

Berwian, I. M., Walter, H., Seifritz, E., & Huys, Q. J. (2017). Predicting relapse after antidepressant withdrawal - a systematic review. *Psychol Med*, *47*(3), 426-437. https://doi.org/10.1017/s0033291716002580

Berwian, I. M., Wenzel, J. G., Kuehn, L., Schnuerer, I., Seifritz, E., Stephan, K. E., Walter, H., & Huys, Q. J. M. (2022). Low predictive power of clinical features for relapse prediction after antidepressant discontinuation in a naturalistic setting. *Scientific Reports*, *12*(1), 11171. https://doi.org/10.1038/s41598-022-13893-9

Brådvik, L. (2018). Suicide Risk and Mental Disorders. *Int J Environ Res Public Health*, *15*(9). https://doi.org/10.3390/ijerph15092028

Brüünsgaard, H., & Pedersen, B. K. (2003). Age-related inflammatory cytokines and disease. *Immunol Allergy Clin North Am*, *23*(1), 15-39. https://doi.org/10.1016/s0889-8561(02)00056-5

Burcusa, S. L., & Iacono, W. G. (2007). Risk for recurrence in depression. *Clin Psychol Rev*, *27*(8), 959-985. https://doi.org/10.1016/j.cpr.2007.02.005

Carboni, L., McCarthy, D. J., Delafont, B., Filosi, M., Ivanchenko, E., Ratti, E., Learned, S. M., Alexander, R., & Domenici, E. (2019). Biomarkers for response in major depression: comparing paroxetine and venlafaxine from two randomised placebo-controlled clinical studies. *Transl Psychiatry*, *9*(1), 182. https://doi.org/10.1038/s41398-019-0521-7

Charles, B. A., Doumatey, A., Huang, H., Zhou, J., Chen, G., Shriner, D., Adeyemo, A., & Rotimi, C. N. (2011). The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. *J Clin Endocrinol Metab*, *96*(12), E2018-2022. https://doi.org/10.1210/jc.2011-1497

Choi, W., Kang, H. J., Kim, J. W., Kim, H. K., Kang, H. C., Lee, J. Y., Kim, S. W., Stewart, R., & Kim, J. M. (2021). Predictive values of tumor necrosis factor-α for depression treatment outcomes: effect modification by hazardous alcohol consumption. *Transl Psychiatry*, *11*(1), 450. https://doi.org/10.1038/s41398-021-01581-7

Copeland, W. E., Shanahan, L., Worthman, C., Angold, A., & Costello, E. J. (2012). Cumulative Depression Episodes Predict Later C-Reactive Protein Levels: A Prospective Analysis. *Biological Psychiatry*, *71*(1), 15-21. https://doi.org/https://doi.org/10.1016/j.biopsych.2011.09.023

D'Mello, C., Le, T., & Swain, M. G. (2009). Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. *J Neurosci*, *29*(7), 2089-2102. https://doi.org/10.1523/jneurosci.3567-08.2009

Davies, J., & Read, J. (2019). A systematic review into the incidence, severity and duration of antidepressant withdrawal effects: Are guidelines evidence-based? *Addict Behav*, *97*, 111-121. https://doi.org/10.1016/j.addbeh.2018.08.027

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *J Clin Psychiatry*, *61 Suppl 6*, 7-11.

DeRubeis, R. J., Siegle, G. J., & Hollon, S. D. (2008). Cognitive therapy versus medication for depression: treatment outcomes and neural mechanisms. *Nat Rev Neurosci*, *9*(10), 788-796. https://doi.org/10.1038/nrn2345

Diagnostic and statistical manual of mental disorders (5th ed.). (2013). American Psychiatric Association. https://doi.org/https://dsm.psychiatryonline.org/doi/book/10.1176/appi.books.9780890425787

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. *Biol Psychiatry*, *67*(5), 446-457. https://doi.org/10.1016/j.biopsych.2009.09.033

Eller, T., Vasar, V., Shlik, J., & Maron, E. (2008). Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*, *32*(2), 445-450. https://doi.org/10.1016/j.pnpbp.2007.09.015

Felger, J. C., & Lotrich, F. E. (2013). Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience*, *246*, 199-229. https://doi.org/10.1016/j.neuroscience.2013.04.060

Figueroa-Hall, L. K., Xu, B., Kuplicki, R., Ford, B. N., Burrows, K., Teague, T. K., Sen, S., Yeh, H.-W., Irwin, M. R., Savitz, J., & Paulus, M. P. (2022). Psychiatric symptoms are not associated with circulating CRP concentrations after controlling for medical, social, and demographic factors. *Translational Psychiatry*, *12*(1), 279. https://doi.org/10.1038/s41398-022-02049-y

Foley, É. M., Parkinson, J. T., Kappelmann, N., & Khandaker, G. M. (2021). Clinical phenotypes of depressed patients with evidence of inflammation and somatic symptoms. *Comprehensive Psychoneuroendocrinology*, *8*, 100079. https://doi.org/https://doi.org/10.1016/j.cpnec.2021.100079

Fornaro, M., Carvalho, A. F., Fusco, A., Anastasia, A., Solmi, M., Berk, M., Sim, K., Vieta, E., & de Bartolomeis, A. (2020). The concept and management of acute episodes of treatment-resistant bipolar disorder: a systematic review and exploratory meta-analysis of randomized controlled trials. *J Affect Disord*, *276*, 970-983. https://doi.org/10.1016/j.jad.2020.07.109

Frank, E., Prien, R. F., Jarrett, R. B., Keller, M. B., Kupfer, D. J., Lavori, P. W., Rush, A. J., & Weissman, M. M. (1991). Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. *Arch Gen Psychiatry*, *48*(9), 851-855. https://doi.org/10.1001/archpsyc.1991.01810330075011

Freeman, M. P., Lee, H., Savella, G. M., Sosinsky, A. Z., Marfurt, S. P., Murphy, S. K., & Cohen, L. S. (2018). Predictors of Depressive Relapse in Women Undergoing Infertility Treatment. *Journal of Women's Health*, *27*(11), 1408-1414. https://doi.org/10.1089/jwh.2017.6878

Friedrich, M. J. (2017). Depression Is the Leading Cause of Disability Around the World. *Jama*, *317*(15), 1517. https://doi.org/10.1001/jama.2017.3826

Gabriel, M., & Sharma, V. (2017). Antidepressant discontinuation syndrome. *Cmaj*, *189*(21), E747. https://doi.org/10.1503/cmaj.160991

Geddes, J. R., Carney, S. M., Davies, C., Furukawa, T. A., Kupfer, D. J., Frank, E., & Goodwin, G. M. (2003). Relapse prevention with antidepressant drug treatment in depressive disorders: a systematic review. *The Lancet*, *361*(9358), 653-661. https://doi.org/https://doi.org/10.1016/S0140-6736(03)12599-8

Goldsmith, D. R., Haroon, E., Woolwine, B. J., Jung, M. Y., Wommack, E. C., Harvey, P. D., Treadway, M. T., Felger, J. C., & Miller, A. H. (2016). Inflammatory markers are associated with decreased psychomotor speed in patients with major depressive disorder. *Brain Behav Immun*, *56*, 281-288. https://doi.org/10.1016/j.bbi.2016.03.025

Greenberg, P. E., Fournier, A. A., Sisitsky, T., Simes, M., Berman, R., Koenigsberg, S. H., & Kessler, R. C. (2021). The Economic Burden of Adults with Major Depressive Disorder in the United States (2010 and 2018). *Pharmacoeconomics*, *39*(6), 653-665. https://doi.org/10.1007/s40273-021-01019-4

Gueorguieva, R., Chekroud, A. M., & Krystal, J. H. (2017). Trajectories of relapse in randomised, placebo-controlled trials of treatment discontinuation in major depressive disorder: an individual patient-level data meta-analysis. *Lancet Psychiatry*, *4*(3), 230-237. https://doi.org/10.1016/s2215-0366(17)30038-x

Haapakoski, R., Mathieu, J., Ebmeier, K. P., Alenius, H., & Kivimäki, M. (2015). Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive

protein in patients with major depressive disorder. *Brain Behav Immun*, *49*, 206-215. https://doi.org/10.1016/j.bbi.2015.06.001
Hannestad, J., DellaGioia, N., & Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology*, *36*(12), 2452-2459. https://doi.org/10.1038/npp.2011.132
Hiles, S. A., Baker, A. L., de Malmanche, T., & Attia, J. (2012). A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: Exploring the causes of heterogeneity. *Brain, Behavior, and Immunity*, *26*(7), 1180-1188. https://doi.org/https://doi.org/10.1016/j.bbi.2012.06.001
Himmerich, H., Patsalos, O., Lichtblau, N., Ibrahim, M. A. A., & Dalton, B. (2019). Cytokine Research in Depression: Principles, Challenges, and Open Questions. *Front Psychiatry*, *10*, 30. https://doi.org/10.3389/fpsyt.2019.00030
Himmerich, H., Patsalos, O., Lichtblau, N., Ibrahim, M. A. A., & Dalton, B. (2019). Cytokine Research in Depression: Principles, Challenges, and Open Questions [Review]. *Frontiers in Psychiatry*, *10*. https://doi.org/10.3389/fpsyt.2019.00030
Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med*, *71*(2), 171-186. https://doi.org/10.1097/PSY.0b013e3181907c1b
Janssen, D. G., Caniato, R. N., Verster, J. C., & Baune, B. T. (2010). A psychoneuroimmunological review on cytokines involved in antidepressant treatment response. *Hum Psychopharmacol*, *25*(3), 201-215. https://doi.org/10.1002/hup.1103
Johnston, G. R., & Webster, N. R. (2009). Cytokines and the immunomodulatory function of the vagus nerve. *Br J Anaesth*, *102*(4), 453-462. https://doi.org/10.1093/bja/aep037
Juncal-Ruiz, M., Riesco-Dávila, L., de la Foz, V. O.-G., Ramírez-Bonilla, M., Martínez-García, O., Irure-Ventura, J., Leza, J. C., López-Hoyos, M., & Crespo-Facorro, B. (2018). The effect of excess weight on circulating inflammatory cytokines in drug-naïve first-episode psychosis individuals. *Journal of Neuroinflammation*, *15*(1), 63. https://doi.org/10.1186/s12974-018-1096-6
Kappelmann, N., Lewis, G., Dantzer, R., Jones, P. B., & Khandaker, G. M. (2018). Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry*, *23*(2), 335-343. https://doi.org/10.1038/mp.2016.167
Kenis, G., & Maes, M. (2002). Effects of antidepressants on the production of cytokines. *International Journal of Neuropsychopharmacology*, *5*(4), 401-412. https://doi.org/10.1017/S1461145702003164
Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., & Jones, P. B. (2014). Association of Serum Interleukin 6 and C-Reactive Protein in Childhood With Depression and Psychosis in Young Adult Life: A Population-Based Longitudinal Study. *JAMA Psychiatry*, *71*(10), 1121-1128. https://doi.org/10.1001/jamapsychiatry.2014.1332
Köhler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C. L., Miller, B. J., Lanctôt, K. L., & Carvalho, A. F. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*, *135*(5), 373-387. https://doi.org/10.1111/acps.12698

Köhler, C. A., Freitas, T. H., Stubbs, B., Maes, M., Solmi, M., Veronese, N., de Andrade, N. Q., Morris, G., Fernandes, B. S., Brunoni, A. R., Herrmann, N., Raison, C. L., Miller, B. J., Lanctôt, K. L., & Carvalho, A. F. (2018). Peripheral Alterations in Cytokine and Chemokine Levels After Antidepressant Drug Treatment for Major Depressive Disorder: Systematic Review and Meta-Analysis. *Mol Neurobiol*, *55*(5), 4195-4206. https://doi.org/10.1007/s12035-017-0632-1

Köhler, O., Benros, M. E., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., & Krogh, J. (2014). Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry*, *71*(12), 1381-1391. https://doi.org/10.1001/jamapsychiatry.2014.1611

Kraus, T., Haack, M., Schuld, A., Hinze-Selch, D., Koethe, D., & Pollmächer, T. (2002). Body weight, the tumor necrosis factor system, and leptin production during treatment with mirtazapine or venlafaxine. *Pharmacopsychiatry*, *35*(6), 220-225. https://doi.org/10.1055/s-2002-36390

Kubera, M., Maes, M., Holan, V., Basta-Kaim, A., Roman, A., & Shani, J. (2001). Prolonged desipramine treatment increases the production of interleukin-10, an anti-inflammatory cytokine, in C57BL/6 mice subjected to the chronic mild stress model of depression. *Journal of Affective Disorders*, *63*(1), 171-178. https://doi.org/https://doi.org/10.1016/S0165-0327(00)00182-8

Kumagai, N., Tajika, A., Hasegawa, A., Kawanishi, N., Horikoshi, M., Shimodera, S., Kurata, K. i., Chino, B., & Furukawa, T. A. (2019). Predicting recurrence of depression using lifelog data: an explanatory feasibility study with a panel VAR approach. *BMC Psychiatry*, *19*(1), 391. https://doi.org/10.1186/s12888-019-2382-2

Kuśmider, M., Faron-Górecka, A., Pabian, P., Solich, J., Szlachta, M., Kolasa, M., Żurawek, D., Wójcikowski, J., Daniel, W., & Dziedzicka-Wasylewska, M. (2018). Effects of imipramine on cytokines panel in the rats serum during the drug treatment and discontinuation. *Neurochemistry International*, *113*, 85-91. https://doi.org/https://doi.org/10.1016/j.neuint.2017.11.016

Lee, D. W., Lee, S., Oh, S. S., Youn, H. M., Choi, D. W., Jung, S. J., Jang, S. I., & Park, E. C. (2022). Risk of suicide death in psychiatric patients according to the level of continuity of care and area deprivation: A population-based nested case-control study. *J Psychiatr Res*, *151*, 279-285. https://doi.org/10.1016/j.jpsychires.2022.04.024

Lee, H. Y., & Kim, Y. K. (2010). Transforming growth factor-beta1 and major depressive disorder with and without attempted suicide: preliminary study. *Psychiatry Res*, *178*(1), 92-96. https://doi.org/10.1016/j.psychres.2009.03.023

Lee, K. M., & Kim, Y. K. (2006). The role of IL-12 and TGF-beta1 in the pathophysiology of major depressive disorder. *Int Immunopharmacol*, *6*(8), 1298-1304. https://doi.org/10.1016/j.intimp.2006.03.015

Lépine, J. P., & Briley, M. (2011). The increasing burden of depression. *Neuropsychiatr Dis Treat*, *7*(Suppl 1), 3-7. https://doi.org/10.2147/ndt.S19617

Liu, C., Chu, D., Kalantar-Zadeh, K., George, J., Young, H. A., & Liu, G. (2021). Cytokines: From Clinical Significance to Quantification. *Adv Sci (Weinh)*, *8*(15), e2004433. https://doi.org/10.1002/advs.202004433

Liu, C.-H., Zhang, G.-Z., Li, B., Li, M., Woelfer, M., Walter, M., & Wang, L. (2019). Role of inflammation in depression relapse. *Journal of Neuroinflammation*, *16*(1), 90. https://doi.org/10.1186/s12974-019-1475-7

Liu, J. J., Wei, Y. B., Strawbridge, R., Bao, Y., Chang, S., Shi, L., Que, J., Gadad, B. S., Trivedi, M. H., Kelsoe, J. R., & Lu, L. (2020). Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. *Mol Psychiatry*, *25*(2), 339-350. https://doi.org/10.1038/s41380-019-0474-5

Liu, Y., Ho, R. C., & Mak, A. (2012). Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J Affect Disord*, *139*(3), 230-239. https://doi.org/10.1016/j.jad.2011.08.003

Lotrich, F. E. (2009). Major depression during interferon-alpha treatment: vulnerability and prevention. *Dialogues Clin Neurosci*, *11*(4), 417-425. https://doi.org/10.31887/DCNS.2009.11.4/felotrich

Maes, M. (1999). Major depression and activation of the inflammatory response system. *Adv Exp Med Biol*, *461*, 25-46. https://doi.org/10.1007/978-0-585-37970-8_2

Maes, M., Van Der Planken, M., Stevens, W. J., Peeters, D., DeClerck, L. S., Bridts, C. H., Schotte, C., & Cosyns, P. (1992). Leukocytosis, monocytosis and neutrophilia: Hallmarks of severe depression. *Journal of Psychiatric Research*, *26*(2), 125-134. https://doi.org/https://doi.org/10.1016/0022-3956(92)90004-8

Manoharan, A., Rajkumar, R. P., Shewade, D. G., Sundaram, R., Muthuramalingam, A., & Paul, A. (2016). Evaluation of interleukin-6 and serotonin as biomarkers to predict response to fluoxetine. *Hum Psychopharmacol*, *31*(3), 178-184. https://doi.org/10.1002/hup.2525

Mesquita, A. R., Correia-Neves, M., Roque, S., Castro, A. G., Vieira, P., Pedrosa, J., Palha, J. A., & Sousa, N. (2008). IL-10 modulates depressive-like behavior. *Journal of Psychiatric Research*, *43*(2), 89-97. https://doi.org/https://doi.org/10.1016/j.jpsychires.2008.02.004

Mikulska, J., Juszczyk, G., Gawrońska-Grzywacz, M., & Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sci*, *11*(10). https://doi.org/10.3390/brainsci11101298

Min, X., Wang, G., Cui, Y., Meng, P., Hu, X., Liu, S., & Wang, Y. (2023). Association between inflammatory cytokines and symptoms of major depressive disorder in adults [Original Research]. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1110775

Monroe, S. M., & Harkness, K. L. (2011). Recurrence in major depression: a conceptual analysis. *Psychol Rev*, *118*(4), 655-674. https://doi.org/10.1037/a0025190

Mosiołek, A., Pięta, A., Jakima, S., Zborowska, N., Mosiołek, J., & Szulc, A. (2021). Effects of Antidepressant Treatment on Peripheral Biomarkers in Patients with Major Depressive Disorder (MDD). *J Clin Med*, *10*(8). https://doi.org/10.3390/jcm10081706

Mössner, R., Mikova, O., Koutsilieri, E., Saoud, M., Ehlis, A. C., Müller, N., Fallgatter, A. J., & Riederer, P. (2007). Consensus paper of the WFSBP Task Force on Biological Markers: biological markers in depression. *World J Biol Psychiatry*, *8*(3), 141-174. https://doi.org/10.1080/15622970701263303

Mueller, T. I., Leon, A. C., Keller, M. B., Solomon, D. A., Endicott, J., Coryell, W., Warshaw, M., & Maser, J. D. (1999). Recurrence after recovery from major depressive disorder

during 15 years of observational follow-up. *Am J Psychiatry*, *156*(7), 1000-1006. https://doi.org/10.1176/ajp.156.7.1000

Myint, A. M., Leonard, B. E., Steinbusch, H. W., & Kim, Y. K. (2005). Th1, Th2, and Th3 cytokine alterations in major depression. *J Affect Disord*, *88*(2), 167-173. https://doi.org/10.1016/j.jad.2005.07.008

Narita, K., Murata, T., Takahashi, T., Kosaka, H., Omata, N., & Wada, Y. (2006). Plasma levels of adiponectin and tumor necrosis factor-alpha in patients with remitted major depression receiving long-term maintenance antidepressant therapy. *Prog Neuropsychopharmacol Biol Psychiatry*, *30*(6), 1159-1162. https://doi.org/10.1016/j.pnpbp.2006.03.030

NICE guideline [NG222]. (2022).

O'Brien, S. M., Scully, P., & Dinan, T. G. (2008). Increased tumor necrosis factor-alpha concentrations with interleukin-4 concentrations in exacerbations of schizophrenia. *Psychiatry Res*, *160*(3), 256-262. https://doi.org/10.1016/j.psychres.2007.11.014

Olfson, M., Marcus, S. C., Tedeschi, M., & Wan, G. J. (2006). Continuity of antidepressant treatment for adults with depression in the United States. *Am J Psychiatry*, *163*(1), 101-108. https://doi.org/10.1176/appi.ajp.163.1.101

Orsolini, L., Pompili, S., Tempia Valenta, S., Salvi, V., & Volpe, U. (2022). C-Reactive Protein as a Biomarker for Major Depressive Disorder? *Int J Mol Sci*, *23*(3). https://doi.org/10.3390/ijms23031616

Osimo, E. F., Baxter, L. J., Lewis, G., Jones, P. B., & Khandaker, G. M. (2019). Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. *Psychol Med*, *49*(12), 1958-1970. https://doi.org/10.1017/s0033291719001454

Osimo, E. F., Pillinger, T., Rodriguez, I. M., Khandaker, G. M., Pariante, C. M., & Howes, O. D. (2020). Inflammatory markers in depression: A meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain, Behavior, and Immunity*, *87*, 901-909. https://doi.org/https://doi.org/10.1016/j.bbi.2020.02.010

Pallavi, P., Sagar, R., Mehta, M., Sharma, S., Subramanium, A., Shamshi, F., Sengupta, U., Pandey, R. M., & Mukhopadhyay, A. K. (2015). Serum cytokines and anxiety in adolescent depression patients: Gender effect. *Psychiatry Research*, *229*(1), 374-380. https://doi.org/https://doi.org/10.1016/j.psychres.2015.06.036

Pérez-Sánchez, G., Becerril-Villanueva, E., Arreola, R., Martínez-Levy, G., Hernández-Gutiérrez, M. E., Velasco-Velásquez, M. A., Alvarez-Herrera, S., Cruz-Fuentes, C., Palacios, L., de la Peña, F., & Pavón, L. (2018). Inflammatory Profiles in Depressed Adolescents Treated with Fluoxetine: An 8-Week Follow-up Open Study. *Mediators of Inflammation*, *2018*, 4074051. https://doi.org/10.1155/2018/4074051

Roohi, E., Jaafari, N., & Hashemian, F. (2021). On inflammatory hypothesis of depression: what is the role of IL-6 in the middle of the chaos? *Journal of Neuroinflammation*, *18*(1), 45. https://doi.org/10.1186/s12974-021-02100-7

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L., Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S., Chattopadhyay, J., Cogen, R. M., Collins, J. K., . . . Ferrari, A. J. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, *398*(10312), 1700-1712. https://doi.org/10.1016/S0140-6736(21)02143-7

Schiepers, O. J. G., Wichers, M. C., & Maes, M. (2005). Cytokines and major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(2), 201-217. https://doi.org/https://doi.org/10.1016/j.pnpbp.2004.11.003

Smith, J. K. (2018). IL-6 and the dysregulation of immune, bone, muscle, and metabolic homeostasis during spaceflight. *npj Microgravity*, *4*(1), 24. https://doi.org/10.1038/s41526-018-0057-9

Smith, K. J., Au, B., Ollis, L., & Schmitz, N. (2018). The association between C-reactive protein, Interleukin-6 and depression among older adults in the community: A systematic review and meta-analysis. *Exp Gerontol*, *102*, 109-132. https://doi.org/10.1016/j.exger.2017.12.005

Solomon, D. A., Keller, M. B., Leon, A. C., Mueller, T. I., Lavori, P. W., Shea, M. T., Coryell, W., Warshaw, M., Turvey, C., Maser, J. D., & Endicott, J. (2000). Multiple recurrences of major depressive disorder. *Am J Psychiatry*, *157*(2), 229-233. https://doi.org/10.1176/appi.ajp.157.2.229

Sutcigil, L., Oktenli, C., Musabak, U., Bozkurt, A., Cansever, A., Uzun, O., Sanisoglu, S. Y., Yesilova, Z., Ozmenler, N., Ozsahin, A., & Sengul, A. (2007). Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy. *Clin Dev Immunol*, *2007*, 76396. https://doi.org/10.1155/2007/76396

Syed, S. A., Beurel, E., Loewenstein, D. A., Lowell, J. A., Craighead, W. E., Dunlop, B. W., Mayberg, H. S., Dhabhar, F., Dietrich, W. D., Keane, R. W., de Rivero Vaccari, J. P., & Nemeroff, C. B. (2018). Defective Inflammatory Pathways in Never-Treated Depressed Patients Are Associated with Poor Treatment Response. *Neuron*, *99*(5), 914-924.e913. https://doi.org/10.1016/j.neuron.2018.08.001

Topic, R., Milicic, D., Stimac, Z., Loncar, M., Velagic, V., Marcinko, D., & Jakovljevic, M. (2013). Somatic comorbidity, metabolic syndrome, cardiovascular risk, and CRP in patients with recurrent depressive disorders. *Croat Med J*, *54*(5), 453-459. https://doi.org/10.3325/cmj.2013.54.453

Valkanova, V., Ebmeier, K. P., & Allan, C. L. (2013). CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. *Journal of Affective Disorders*, *150*(3), 736-744. https://doi.org/https://doi.org/10.1016/j.jad.2013.06.004

Więdłocha, M., Marcinowicz, P., Krupa, R., Janoska-Jaździk, M., Janus, M., Dębowska, W., Mosiołek, A., Waszkiewicz, N., & Szulc, A. (2018). Effect of antidepressant treatment on peripheral inflammation markers - A meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*, *80*(Pt C), 217-226. https://doi.org/10.1016/j.pnpbp.2017.04.026

Worthen, R. J., Garzon Zighelboim, S. S., Torres Jaramillo, C. S., & Beurel, E. (2020). Anti-inflammatory IL-10 administration rescues depression-associated learning and memory deficits in mice. *Journal of Neuroinflammation*, *17*(1), 246. https://doi.org/10.1186/s12974-020-01922-1

Wysokiński, A., Socha, K., Sołtysik, B. K., Kłoszewska, I., Sobów, T., & Kostka, T. (2016). Levels of C-reactive protein (CRP) in elderly patients with unipolar depression - case control analysis. *Nord J Psychiatry*, *70*(7), 503-507. https://doi.org/10.3109/08039488.2016.1174298

Xu, Y., Wei, H., Zhu, Y., Zhu, Y., Zhang, N., Qin, J., Zhu, X., Yu, M., & Li, Y. (2019). Potential serum biomarkers for the prediction of the efficacy of escitalopram for treating depression. *Journal of Affective Disorders*, *250*, 307-312. https://doi.org/https://doi.org/10.1016/j.jad.2019.03.008

Yao, L., Pan, L., Qian, M., Sun, W., Gu, C., Chen, L., Tang, X., Hu, Y., Xu, L., Wei, Y., Hui, L., Liu, X., Wang, J., & Zhang, T. (2020). Tumor Necrosis Factor-α Variations in Patients With Major Depressive Disorder Before and After Antidepressant Treatment [Original Research]. *Frontiers in Psychiatry*, *11*. https://doi.org/10.3389/fpsyt.2020.518837

Yoshimura, R., Hori, H., Ikenouchi-Sugita, A., Umene-Nakano, W., Katsuki, A., Atake, K., & Nakamura, J. (2013). Plasma levels of interleukin-6 and selective serotonin reuptake inhibitor response in patients with major depressive disorder. *Hum Psychopharmacol*, *28*(5), 466-470. https://doi.org/10.1002/hup.2333