Interleukins Patterns and Anxiety Mechanisms in Generalized Anxiety Disorder (GAD): Understanding the pathophysiology and bio marker insights

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

In this study, standard ethical principles of academic research were observed. The study made sure that no physical or psychological harm was taken during the research process.

Abstract

In this thesis, interleukin patterns and their involvement in the Generalized Anxiety Disorder (GAD) biological processes are examined. The paper evaluates the presence of inflammatory cytokines in the pathophysiology of anxiety and whether they can be used as a biomarker. The presence of immune imbalance and the severity of anxiety were investigated by comparing the pro-inflammatory and anti-inflammatory cytokines (IL-6, IL-1b, IL-17, and IL-10). The results indicate that patients with GAD have high pro-inflammatory cytokines and low anti-inflammatory controls, which means that there is an inflammatory dominance pattern. The IL-6/IL-10 ratio proved to be an important predictor of immune imbalance associated with the severity of symptoms. These findings back a neuroimmune theory of inflammatory dysregulation interacting with biological systems of stress. The research adds to the growing body of research in psychoneuroimmunology and demonstrates the importance of inflammatory biomarkers in the study and understanding of anxiety as well as in enhancing precision-based mental health studies.

Keywords: Generalized Anxiety Disorder; Interleukins; Neuroinflammation; Cytokines; Biomarkers; Psychoneuroimmunology

Dedication

I dedicate this thesis to my parents, who believed in me from the very start, offering their unwavering support and countless sacrifices throughout my academic journey. Without their love and dedication, this would not have been possible. I also dedicate this work to my best friend, whose constant encouragement, positivity, and belief in me kept me going, even when the road was tough.

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List of Acronyms

GAD	Generalized Anxiety Disorder
IL	Interleukin
IL-6	Interleukin-6
IL-1β	Interleukin-1 Beta
IL-17	Interleukin-17
IL-10	Interleukin-10
HPA Axis	Hypothalamic–Pituitary–Adrenal Axis
CNS	Central Nervous System
NF-κB	Nuclear Factor Kappa B
JAK-STAT	Janus Kinase–Signal Transducer and Activator of Transcription Pathway
IDO	Indoleamine 2,3-Dioxygenase
ELISA	Enzyme-Linked Immunosorbent Assay
BMI	Body Mass Index
CBT	Cognitive Behavioral Therapy
DSM-5-TR	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision
HAM-A	Hamilton Anxiety Rating Scale
fMRI	Functional Magnetic Resonance Imaging

Glossary

Generalized Anxiety Disorder (GAD)	A chronic anxiety condition characterized by persistent and excessive worry about everyday events, often accompanied by physical symptoms such as muscle tension, restlessness, and sleep disturbance.
Interleukins	A group of cytokines (immune signaling proteins) that regulate immune responses and inflammatory processes within the body.
Cytokines	Small proteins released by immune cells that facilitate communication between cells during immune and inflammatory responses.
Pro-inflammatory Cytokines	Cytokines that promote inflammatory responses. Examples include IL-6 and IL-1β, which can increase immune activation and physiological stress responses.
Anti-inflammatory Cytokines	Cytokines that suppress excessive inflammation and help restore immune balance. IL-10 is a major regulatory cytokine.
Neuroinflammation	Inflammatory processes occurring within the central nervous system that may influence brain function and behavior.
Psychoneuroimmunology	An interdisciplinary field studying interactions between psychological processes, the nervous system, and the immune system.
HPA Axis (Hypothalamic–Pituitary–Adrenal Axis)	A neuroendocrine system that regulates stress responses through hormone release, including cortisol.
Cortisol	A stress hormone released by the adrenal glands that helps regulate metabolism, immune responses, and stress adaptation.
Biomarkers	Biological indicators measurable in bodily fluids or tissues that provide information about physiological or pathological processes.

Neuroimmune Interaction	The bidirectional communication between the nervous system and immune system influencing health and disease.

Chapter 1: Introduction

1.1 Background

Anxiety disorders form a part of the most common form of psychiatric condition all over the world, and they have a significant role in causing disability, low productivity, and impaired quality of life. Generalized Anxiety Disorder (GAD) is a chronic, generalized manifestation of anxiety involving excessive worry that cannot be controlled and is considered to be chronic and pervasive in numerous spheres of life. GAD is characterized by diffuse sustained anticipatory fear, and in contrast to phobic disorders, which are usually associated with the presence of particular stimuli, or panic disorder, which is also associated with periods of severe fear that are disproportionately high in response to potential threat situations (Abi-Dargham *et al.*, 2023).

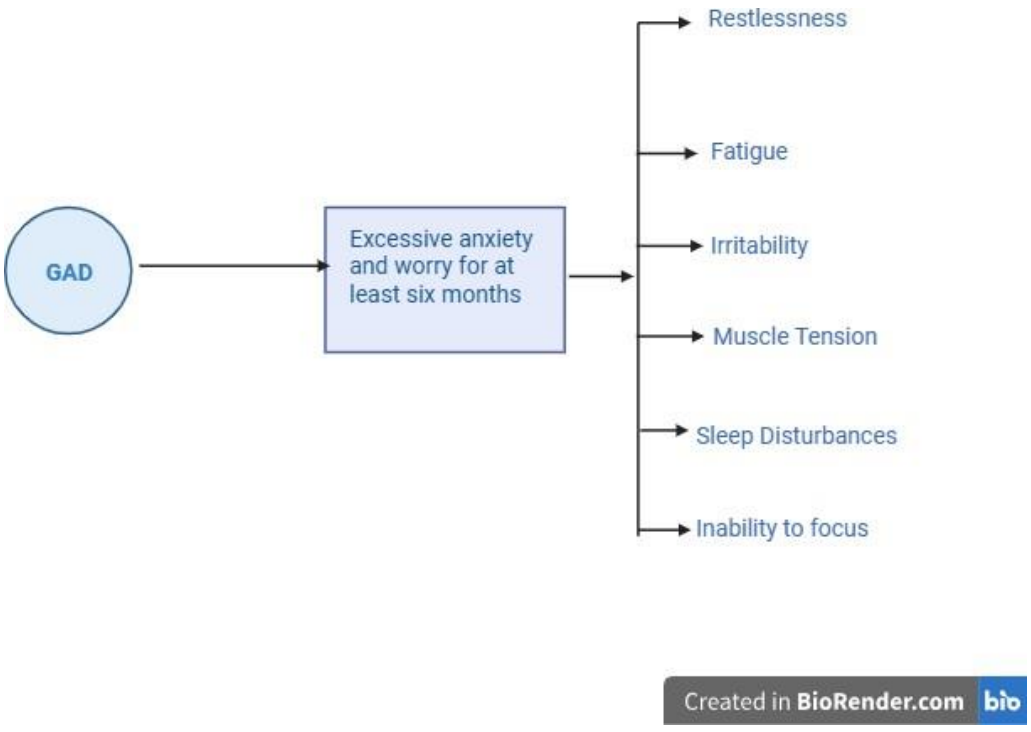

Figure1: Common Symptoms of GAD

GAD is characterized by excessive anxiety and worry for at least six months, with other related symptoms, including restlessness, fatigue, irritability, muscle tension, sleep disturbances, and inability to focus, among others. Its onset usually occurs during adolescence or early adulthood, and in most cases, the condition has a chronic, relapsing course. The rates of lifetime prevalence

of tuberculosis are estimated to be (approximately) 3-6 percent, with individuals more likely to experience this disease than men (Amitai *et al.*, 2022).

In the past, psychological and neurochemical explanations of GAD have dominated conceptual models of GAD. The cognitive-behavioral theories put maladaptive appraisals of threat, intolerance of uncertainty, and incessant rumination as the primary causes of worry. Neurobiological theories have emphasized the role of dysfunction in monoaminergic neurotransmitters, specifically serotonin and norepinephrine, as well as it relates to the impairment of gamma-aminobutyric acid (GABA)-based repressive signaling. Although these frameworks have been used to explain effective pharmacological and psychotherapeutic interventions, the response to treatment is still inconsistent, and a large percentage of patients are not completely in remission (Accortt *et al.*, 2023).

There is evidence that GAD could be a more comprehensive systemic biological process that is not necessarily due to an imbalance in neurotransmitters. The neural pathways are not the only pathways activated by chronic psychological stress; also, endocrine and immune pathways are involved in the disorder. Chronic hypothalamic-pituitary-adrenal (HPA) axis stimulation can cause a rise in the regulation of cortisol and the maintenance of inflammatory activity of the sympathetic activity. Some new signatures have shown that immunomodulators, especially interleukins, can regulate neural excitability and stress reactivity (Berk, 2023).

Interleukins are traditionally researched in infectious and autoimmune disorders, but in psychiatric disorders, their role has become more and more popular. Several stress-related disorders are linked with elevated levels of pro-inflammatory interleukins, e.g., IL-6 and IL-1β. These cytokines can affect the metabolism of the neurotransmitters, the synaptic plasticity, and the neuroendocrine functioning. On the other hand, anti-inflammatory cytokines like IL-10 control the immune responses and can balance the abundance of the inflammatory response. A disequilibrium of pro-inflammatory and anti-inflammatory cues could cause some long-term neural sensitization and anxiety disorders (Bosl *et al.*, 2023).

In this way, the history of GAD studies is shifting away, perhaps, to a more psychological or neurochemical notion of the problem, to a more multisystem approach, which incorporates neural, endocrine, and immune events. The ability to learn more about an interleukin pattern in the GAD provides the opportunity to narrow down the pathophysiological model development and to work on the development of biomarkers. This is a wider biological approach that is the conceptual basis of the current study (Borrego-Ruiz and Borrego, 2025).

1.2 Clinical Overview of GAD

Generalized Anxiety Disorder (GAD) is a chronic and widespread anxiety problem that is represented by excessive worry that is persistent, difficult to control, and affects various life areas. The Diagnostic and Statistical Manual of Mental Disorders specifies that excessive worry and anxiety more days than not over at least six months is the defining diagnostic criterion of the condition, augmented by no less than three circumstances related to the condition, such as restlessness, fatigue, inability to concentrate, irritability, muscle tension, and sleep disturbance. Notably, the anxiety felt in GAD is non-specific to certain triggers but generalized and anticipatory in character, usually portrayed by patients as the persistent mental focus on the alleged threats in the future (Bottaccioli *et al.*, 2025).

GAD has a complex of symptoms involving cognitive, emotional, and somatic symptoms. The pathological worry is the cognitive peculiarity of the disorder that determines repetitive and uncontrollable rumination about something bad that could happen. This is also generally futuristic and intolerance to uncertainty. Patients with GAD constantly have the thought operation of what if, trying to prepare their minds for the worst-case scenario. Although this process can be regarded as adaptive in the first place, it is maladaptive when the usage of a great amount of mental energy is observed, as well as when it disrupts day-to-day activities (Basińska and Kwissa-Gajewska, 2023).

Personally, GAD can be characterized by increased baseline anxiety, irritation, and relaxation difficulty in people. In physiology, there are chronic symptoms of muscle tension, headache, gastrointestinal discomfort, and sleep issues in the case of activation of the autonomic nervous system. Sleep disturbances are mostly so and may include failure to fall asleep, shivering, or non-refreshing sleep. These physical manifestations depict a continuous condition of sympathetic stimulation and may cause fatigue and lostness in the mind.

The epidemiological evidence shows that the life time prevalence rates are 3-6 percent and prevalence of the same is more common among women. The condition normally begins when an individual is a teenager or even at the young age of adulthood, yet it may also appear later at the latter stage of our lives particularly following significant stress. The GAD tends to be chronic with episodes of varying symptoms, but these happen too infrequently to be addressed altogether (Bandelow *et al.*, 2017).

GAD is characterized by comorbidity. Enormous depression disorder is a common comorbidity, thus making both diagnosis and treatment planning difficult. Substance use

disorders, somatic symptom disorders, and other anxiety disorders can also be found. This overlap indicates that there are common biological and psychological processes, as well as creates difficulties in separating disorder-specific pathophysiology.

Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are the widely used pharmacological medications that show a moderate effectiveness. CBT is a psychotherapeutic intervention that is still first-line. Nonetheless, the rates of partial response or relapse are high in the vast majority of patients, which means that the current modalities of treatment are not comprehensive in response to the underlying biological processes (Bhuvaneshwar and Gusev, 2024).

There are developing indications that chronic stress, which is the heart of the clinical manifestation of GAD, can affect the immune and endocrine systems. Chronic anxiety could also act as a sustained stressor (i.e., promoting inflammatory processes and changing HPA axis regulation). This finding gives a reason to investigate interleukin patterns as a cause of GAD pathophysiology.

So, GAD is a complex condition, which can be characterized by the systematic worry of the mind, hyperarousal in the physiological domain, and continuous stress in the nervous system. This clinical complexity and variability of treatments accounts for the need to research systemic biological processes such as immune dysregulation in order to further understanding and precision of treatment (Bokma *et al.*, 2022).

1.3 Neurobiological Mechanisms Underlying Anxiety in GAD

Between the neurobiological mechanisms of Generalized Anxiety Disorder (GAD), there is disregulation between neuron networks that play a role in detecting threats, regulating emotions, and adapting to stress. Modern neuroscience recognizes the corticolimbic network, especially the amygdala, prefrontal cortex (PFC), hippocampus, and anterior cingulate cortex (ACC), as the main anxiety pathophysiology (Bolgeo *et al.*, 2023).

Neuroimaging research continually shows an increased amygdala activation in patients with GAD, even to neutral or neutralizing stimuli. This hyper-vigilance can be an addition to the exaggerated perception of threat and incessant anticipatory anxiety. GAD can be distinguished from phobic disorders by the fact that the response to threat is not specific to the stimulus, but generalized, which may be due to long-term hyperexcitability of limbic systems at baseline.

The regulation of amygdala activities is provided by the prefrontal cortex, especially the dorsolateral area and the ventromedial areas. Through negative evidence, lower levels of functional connectivity between the PFC and amygdala have been identified in anxiety populations, suggesting that there is no inhibition. This low regulatory control can ensure that worry-related cognitions can be sustained without developed cognitive suppression (Baez *et al.,* 2023).

Hippocampus helps in contextual memory handling and regulates stress reactions by connection with the hypothalamic-pituitary-adrenal (HPA) axis. Exposure to chronic stress may reduce hippocampal volume and impair neurogenesis, potentially interfering with adaptive fear extinction.

In addition to circuitry, neurotransmitter systems are also very important. The serotonergic dysregulation of mood and anxiety, and the depleted neural inhibition via the gamma-aminobutyric acid (GABA), may lead to neural hyperexcitability. Excessive worry has also been known to be maintained by glutamatergic imbalance.

Neurobiological studies have further shown that such neural changes are not independent. The inflammatory signaling molecules, especially interleukins like IL-6, 1b, can affect neurotransmitter systems, synaptic plasticity, and activation of the stress-axis. In this way, it is essential to combine dysfunction of neural circuits and systemic immune processes to understand the mechanisms of anxiety in GAD (Berg *et al.,* 2023).

1.4 Interleukins and the Psychoneuroimmunology Framework in Anxiety Disorders

The developing discipline of psychoneuroimmunology has changed the current perception of psychiatric illnesses by showing a two-way communication between the central nervous system and the immune system. In this context, small cytokine signaling proteins, the interleukins, have become very important in connecting psychological stress and neural dysfunction. Interleukins in the paradigm of Generalized Anxiety Disorder (GAD) could be one of the biological processes, where persistent worry is converted into a long-lasting physiological disorder (Bisby *et al.,* 2022)**.**

Immune cells (macrophages, monocytes, and T-helper lymphocytes), cells of the central nervous system (microglia and astrocytes) are also capable of producing interleukins. Interleukins, which are traditionally investigated in the context of infectious diseases and

autoimmune disease, have been defined with a neuromodulatory ability. The activity of pro-inflammatory interleukins, which include IL-6 and IL-1β, can impact the work of the brain in a number of ways. These can enter the blood-brain barrier by active transport, central communication by means of vaginal entrudgement, and activate endothelial cell generation of secondary cytokine within neural parenchyma.

Interleukins, once in the central nervous system, trigger intracellular mechanisms of signalling e.g., in the JAK-STAT and NF-kB pathways, which cause changes in gene expression. The changes are able to affect neurotransmitter metabolism, synaptic plasticity, and neuroendocrine control. As an example, pro-inflammatory cytokines are capable of suppressing serotonin production by activating the indoleamine 2,3-dioxygenase (IDO) pathway and turning tryptophan metabolism to kynurenine production. Also, cytokine signaling can increase the glutamatergic transmission as well as decrease glutamatergic gamma-aminobutyric acid (GABA) inhibitory tone, which leads to neuronal hyperexcitability (Choudhary *et al.*, 2022)**.**

The anti-inflammatory interleukins, like IL-10, have regulatory functions by inhibiting hyperactivating inflammatory responses. The imbalance between pro- and anti-inflammatory interleukins could establish an inflammatory condition that is persistent and might support the symptoms of anxiety.

Persistent psychological stress can become chronically activated in immune signaling pathways in GAD, resulting in subtle yet chronic changes in pro-inflammatory interleukins. This tone of inflammation can make neural circuits in fear and also anticipation sensitive, which adds to a continuation of worry and hypervigilance. Therefore, the psychoneuroimmunology model offers a mechanistic pathway between immune dysregulation and anxiety pathophysiology and has given some potential areas to develop biomarkers **(Cosci and Mansueto, 2020)**.

1.5 Rationale for Investigating Interleukin Patterns in GAD

Anxiety disorders have, over the decades, not been well studied in the objective biological perspective of diagnosis and prognosis. Nowadays, Generalized Anxiety Disorder (GAD) is diagnosed according to symptom criteria of the Diagnostic and Statistical Manual of Mental Disorders, which is predominantly based on clinical interviews and self-reported measures. Although such a method gives the reliability of the diagnosis, it fails to consider any underlying biological heterogeneity. Similar symptom profiles might differ significantly in terms of

neurobiological and immunological conditions and require refinement by using biomarkers (Cosci and Mansueto, 2018)**.**

There is emerging evidence that chronic psychological stress, which is the main cause of GAD, triggers inflammatory pathways. There are also pro-inflammatory interleukins like IL-6 and IL-1β, which have been reported to be at high levels in some populations experiencing anxiety. Nevertheless, the results are not consistent, as several studies conducted have been limited by methodological flaws, such as the inability to separate primary GAD and depressive comorbidity, inability to fully control the presence of confounding variables, such as body mass index, sleep disturbance, and medication **use** (Caldiroli *et al.,* 2023)**.**

The patterns of interleukin investigated, instead of those of single cytokine elevation, can provide more explanatory strength. As an illustration, a ratio of proinflammatory cytokines (e.g., IL-6) to anti-inflammatory regulators (i.e., IL-10) can better represent the extent of inflammation than simple concentrations. The maintenance of a high IL-6/IL-10 ratio may suggest the inability of the immune system to regulate the process, which may result in the eternal neural **desensitization** (Chen *et al.,* 2025)**.**

Moreover, interleukins affect the hypothalamic-pituitary-adrenal (HPA) axis, whose activity affects cortisol secretion and adaptation to stress. Assessing the levels of cytokines and endocrine could help to understand whether chronic anxiety is driven by immune dysregulation or is a consequence of it.

Translational. Inconsistent inflammatory signatures of GAD detected would inform precision psychiatry. Stratification of biomarkers could be used to identify biologically discrete subgroups of people who might respond to specific interventions, such as anti-inflammatory interventions or lifestyle changes that manage inflammation throughout the body.

As such, empirical research on interleukin patterns in GAD should be carried out rigorously in order to explain their interrelations in pathophysiology and development of biomarkers.

1.6 Research Gap

Although the area of neurobiological and psychological dimensions of Generalized Anxiety Disorder (GAD) has improved greatly, there remains a lot to be done to rectify the absence of information on the immunological foundation of GAD. In the past, the majority of available studies have focused on the hard work of monoaminergic regulation with the increased emphasis on the serotonin, norepinephrine, and gamma-aminobutyric acid (GABA) research.

Although these models have been used in determining the pharmacological treatment strategy, they fail to fully justify the persistence, chronicity, and resistance to treatment witnessed in a significant percentage of patients with GAD. This shortcoming underscores the necessity to investigate other system-wide factors, such as the role of the immune system (Chapman and LeJeune, 2022)**.**

The absence of anxiety-specific inflammatory studies is one of the large gaps. Most psychoneuroimmunology researches have focused on major depressive disorder, in which high levels of inflammatory cytokines, which include IL-6 and C-reactive protein (CRP), are well observed. The anxiety disorders, such as GAD, are also often comorbid conditions and not a key diagnosis. Consequently, it is still unknown whether the findings of inflammatory changes as found in depression do the same things to GAD or GAD reveals a distinct interleukin profile.

The other weakness is that of methodological heterogeneity. Most of the current researches do not distinguish primary GAD cases of people who co-occur with depressive symptoms. Since depression per se is strongly correlated with inflammatory dysregulation, there can be confounding findings in the lack of a rigid diagnosis differentiation. Moreover, differences in methods of measuring cytokines, time of sampling, medications, as well as body mass index, smoking, and sleep habits also decrease comparability between studies (Craig *et al.,* 2019)**.**

Moreover, studies mostly concentrate on the situation of single cytokine increases in contrast to checking interleukin trends or proportions. One high cytokine might be inadequate as a measure of the tone of systemic inflammation. Pro-inflammatory (e.g., IL-6, IL-1b) and the anti-inflammatory controls (e.g., IL-10) also can lead to a better understanding of the immune deregulation. However, in GAD patients, biomarker models based on the ratios have not been well investigated.

Memorability of reports that research integrate immune measures with endocrine (e.g. cortisol) or proven anxiety severity scales is a lack of studies. Without the addition of the multimodality, the mechanistic inferences are mere guesses. It is also not clear whether inflammatory changes cause or follow the onset of anxiety, or is it a case of a two-way relationship of stress and immune activation (Callaghan *et al.,* 2024)**.**

Therefore, there is an apparent research gap, which is controlled, anxiety-specific, multimodal studies on the patterns of interleukin in clinically diagnosed GAD populations. This gap needs to be filled to understand whether immune dysregulation is a primary pathophysiology or not, and interleukin has potential as a valid biomarker of GAD.

1.7 Objectives, Research Questions, and Hypotheses

The role of interleukin dysregulation in Generalized Anxiety Disorder (GAD) needs a systematic empirical model. Although there is some new literature indicating inflammatory roles in anxiety disorders, it is still inconsistent with respect to the specificity of the involvement, magnitude, and clinical implications of the cytokine changes. Thus, the current research is planned to conduct a systematic investigation of the hypothesis that characteristic interleukin patterns are related to the levels of anxiety and stress-axis disregulation in GAD.

This section would describe the main objectives, research questions, and testable hypotheses that would be used to carry out the investigation (Casares *et al.,* 2024)**.**

Objectives

- To compare the serum levels of IL-6, IL-1β, IL-17, and IL-10 in persons with the diagnosis of GAD and healthy participants.
- To investigate the hypothesis of whether there is some pro-inflammatory dominance (e.g., IL-6/IL-10 ratio) in GAD participants.
- To assess the correlation between the level of anxiety and cytokine levels.
- To determine how cortisol dynamics and inflammatory biomarkers are linked, it is important to study the immune-endocrine feedback control.
- To investigate the issue of different inflammatory subtypes in the GAD population.

Research Questions

Considering theoretical and empirical factors, the research questions that are answered in the study include:

- Is the production of pro-inflammatory interleukins in individuals with GAD significantly higher than the amount in healthy controls?
- Are there any changes in anti-inflammatory regulation (IL-10) in the GAD participants?
- Is there increased differentiation between groups using the IL-6/IL-10 ratio as compared to single cytokines?
- Are the inflammatory markers positively associated with the levels of anxiety?
- Does cortisol moderate or interact with cytokine levels in predicting anxiety symptoms?
- Is there any evidence of inflammatory profiling of biologically distinct subgroups in GAD?

Research Hypotheses

The hypotheses are as follows, based on the previous literature and using the integrated neuroimmune framework:

Group Difference Hypotheses

- H1: GAD patients will have a substantially higher level of serum IL- 6 and IL-1b than healthy controls.
- H 2: The GAD and controls will differ significantly with respect to IL- 10.
- H3: IL-6/IL-10 ratio will be highly increased in GAD patients.

Correlational Hypotheses

- H4: IL-6 and IL-1β have a positive association with the anxiety severity scores.
- H5: The level of IL-10 will be negatively associated with the level of anxiety.
- H6: The ratio of IL-6/IL-10 will prove to correlate higher with the severity of anxiety compared to individual cytokines.

Interaction Hypothesis

- H7: Cortisol concentrations will have a significant interaction with IL-6 in the prediction of the level of anxiety as evidence of an immune-endocrine imbalance.
- Exploratory Hypothesis
- H8: Cluster analysis shall reveal at least one of the inflammatory subtypes among the GAD population, where there will be high cytokine ratios and increased symptom severity.

1.8 Significance of the Study

This has strong theoretical, clinical, and public health implications as it enhances the knowledge about interleukin patterns in Generalized Anxiety Disorder (GAD) and determines their potential contribution to the pathophysiology and the development of biomarkers.

Theoretical Significance

Theoretically, this study will fit within the growing psychoneuroimmunology framework by taking immune signaling into blood neurobiological models of anxiety. Conventional theories of GAD have placed emphasis on cognitive errors and dysregulations of neurotransmitters, especially of serotonin and gamma-aminobutyric acid (GABA). Although they have served as

effective treatment strategies to inform appropriate strategies, they fail to explain the chronicity of disorders, rates of relapse, and resistance to treatment.

This study adds to the common frameworks on the explanation of monoaminergic by comparing pro-inflammatory and anti-inflammatory interleukin patterns, and suggests a multisystem framework that explains immune, endocrine, and neural mechanisms. It would prove the point that chronic anxiety is not simply just a psychological but a biologically determined condition, which consists of systemic inflammatory processes, to demonstrate that there is a significant interleukin dysregulation in GAD. This theoretical extension could assist hone a future psychology-forward conceptualization of anxiety illnesses and recommend cross-study interdisciplinary research between psychiatry and immunology (Dursun *et al.*, 2022).

Clinical Significance

The patterns of interleukin, when evaluated clinically, can help to form objective GAD biomarkers. Subjective reporting of symptoms, on which present-day diagnosis greatly depends, fails to reflect biological heterogeneity, despite its usefulness in clinical practice. The identification of biomarkers may lead to diagnostic accuracy and improve the chances of identifying the presence of people at risk earlier.

Moreover, knowledge of inflammatory involvement can be used to provide individual treatment plans. In case high levels of pro-inflammatory cytokines are associated with symptom severity, anti-inflammatory adjunctive therapy or lifestyle interventions that aim at systemic inflammation would be helpful to add to conventional pharmacotherapy and psychotherapy. Patient stratification according to inflammatory profile can enhance the response and decrease the relapses to treatment (Dugas *et al.*, 2022).

Public Health Significance

In terms of a community health standard, GAD has a high socioeconomic burden as it affects the productivity, health service structure, and co-occurrences with other mental and medical illnesses. Low-grade chronic inflammation is also reported to have a link to cardiovascular disease and metabolic illnesses. In case inflammatory dysregulation is a cause or worsener of GAD, there is a possibility that early detection and treatment can decrease the morbidity in the long term. This study can be seen as part of the larger endeavor to enhance the way mental health issues are resolved and to minimize the effect of anxiety disorders on the global health population (DeGeorge *et al.*, 2022).

Chapter 2: Neuroimmune and Interleukin Literature

2.1 Interleukin Dysregulation and Its Central Role in the Pathophysiology of GAD

The study of interleukin patterns in generalized anxiety disorder (GAD) is a very important progress in the modern research on anxiety. Although classical conceptualizations focused upon neurotransmitter imbalance and maladaptive cognitive operations, there is new evidence to indicate that immune over- and under-activation could have a baseline effect in maintaining chronic anxiety conditions. Especially, ongoing low-grade inflammation mediated by interleukins has attracted interest as a putative mechanistic zone of the pathophysiology of GAD (Dymek *et al.*, 2025).

GAD is typified by unnecessary worries that cannot be controlled, and these worries manifest in various sectors of life. A difference between episodic anxiety responses and GAD is that GAD is a persistent, anticipatory stress. Chronic psychological stress has been known to trigger inflammatory signaling pathways both in a neural way and endocrine. Nuclear factor kappa B (NF-kB), a transcription factor that controls the expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-1 beta (IL-1β), can be activated by prolonged sympathetic nervous system and hypothalamic-pituitary- adrenal (HPA) axis. Such an inflammatory reaction to stress offers a biological link to connect continual cognitive agony to systemic immunosuppressive reactions (Doshi, 2019).

One of the most consistent cytokines that has been reported to be increased in an anxious condition is IL-6. A pleiotropic cytokine, IL-6, does not just mediate the immune responses but also directly communicates with neuroendocrine pathways. High IL-6 concentrations may provoke the synthesis of corticotropin-releasing hormone (CRH) in the hypothalamus in order to increase an anti-inflammatory process of the HPA-axis, and release of cortisol. Although cortisol has a homeostatic effect of being anti-inflammatory, prolonged exposure to cortisol can result in exposure to glucocorticoid receptor desensitization, undermining the regulatory functions. This disrupted negative feedback process can perhaps enable IL-6 levels to be continuously high and be part of long term physiologic arousal seen in GAD (Gkintoni and Ortiz, 2023).

The other pro-inflammatory interleukin, IL-1b, has shown a neuromodulatory effect in terms of anxiety. The evidence of the experiment shows that IL-1b is able to alter the synaptic plasticity of the hippocampus and to stimulate the excitatory transmission in the amygdala. These effects will reduce the threshold of threat detection and disrupt contextual control of fear. Contextual processing disruption has the potential to support pervasive anticipatory worry in GAD, as anxiety is not only specific to certain stimuli, but also pervasive (Chew *et al.*, 2014). The changes in neuroplasticity that are mediated by IL-1b can thus be regarded as a biological basis of persistent rumination (Gottschalk *et al.*, 2015).

Other than the pro-inflammatory mediators, anti-inflammatory cytokines like IL-10 are very important to moderation. IL-10 inhibits the overproduction of inflammation and upholds homeostasis of immunity. The decreased levels of IL-10 in the populations with anxiety indicate failure of the regulatory balance. Notably, the recent literature has stressed the importance of cytokine ratios and not absolute values. A high IL-6/IL-10 ratio could be a sign of systemic inflammatory hegemony, which could provide a more consistent biomarker of immune overabundance in GAD.

The recent term, inflammatory pattern recognition, is shifting artificial attention to cytokines alone, which are substituted with combined immune signatures. The method is in line with the aspects of precision psychiatry, which aims to identify biologically significant subtypes of heterogeneous psychiatric diagnoses. Detecting high-fidelity interleukin alterations unique to GAD can help understand the pathophysiology and enhance the stratification of biomarkers (Gardi *et al.*, 2022).

However, the literature remains diverse as far as its methodologies are concerned. Numerous studies combine anxiety disorders in contrast with primary GAD and comorbid symptomatic manifestations of depression which tend to confound the cytokine findings. Besides, body mass index, sleep disturbancy and metabolic status attributes of lifestyle may independently adjust inflammatory markers. Such limitations complicate the interpretation process and are one of the signs that the investigation based on disorder needs to be strictly regulated.

The fact that there can be converging interests shows that interleukin dysregulation could not be merely an effect of anxiety but also a factor that does not lead to neural desensitization. Possible impacts of pro-inflammatory signaling on the neural circuits that mediate worry and anticipation of threats are the ability to modify neurotransmitter metabolism, glutamatergic

transmission, and the expression of brain-derived neurotrophic factor (BDNF) (Ergisi *et al.*, 2022).

Therefore, the evidence in the literature is toward a model where interleukin increases caused by chronic stress, acts in interaction with neural and endocrine systems to perpetuate the pathology of anxiety. These immune patterns play a crucial role in explaining the biological nature of GAD, and the same patterns can be used to assess the possibilities of the interleukins to be used as biomarkers. The current thesis is based on this developing paradigm and methodically analyzes the particular interleukin profiles and the relationship with the severity of anxiety and the stress-axis regulation.

Table 1. Interleukins and their role in GAD

Type of interleukins	Name of interleukin	Role in GAD
Proinflammatory	IL-1 (IL-1α, IL-1β)	Promotes neuroinflammation, HPA-axis activation, and anxiety symptom severity.
	IL-2	Evidence is mixed; dysregulation may reflect altered T-cell/immune activation in GAD.
	IL-6	Commonly elevated in GAD; linked to chronic inflammation and symptom severity.
	IL-8	May be elevated; associated with inflammatory signaling and stress-related immune activation.
	IL-12	Pro-inflammatory Th1 cytokine; elevated or dysregulated levels may contribute to immune imbalance in GAD.
	IL-17	Promotes neuroinflammation; altered levels may contribute to GAD pathophysiology.
	IL-18	May amplify inflammatory cascades and stress-related immune activation in GAD.
Anti-inflammatory interleukins	IL-4	Reduced anti-inflammatory protection may contribute to inflammatory imbalance in GAD.
	IL-10	Often reduced in GAD; lower levels suggest weakened anti-inflammatory control.

	IL-11	Limited direct evidence; may have protective anti-inflammatory and neuroimmune regulatory effects.
	IL-13	Anti-inflammatory/regulatory cytokine that may help counter inflammation linked with anxiety.
Both pro- and anti-inflammatory interleukins	IL-6	Shows both pro- and anti-inflammatory actions depending on the signaling pathway.
	IL-2	Regulates immune activation and immune tolerance; its effect in GAD may be dual and context-dependent.
	IL-17	Primarily pro-inflammatory, but context-dependent immune regulation may produce mixed effects.
	IL-22	Context-dependent cytokine; may protect barrier tissues and reduce stress-induced anxiety, but can also participate in inflammation.

2.2 Specific Interleukin Patterns (IL-6, IL-1β, IL-17, and IL-10) in GAD and Their Mechanistic Implications

To comprehend the interleukin patterns in generalized anxiety disorder (GAD), it is necessary to go beyond general inflammatory patterns in favor of disorder-specific immune patterns. Although considerable studies have been done on inflammation in depressive disorders, research on anxiety-based cytokines around primary GAD is relatively low. However, there are also new trends in relation to pro-inflammatory interleukins, i.e., IL-6, IL-1b, and IL-17, as well as changes in anti-inflammatory IL-10 (Fernandez *et al.*, 2024).

IL-6: Stress-Reactive Proinflammatory Amphetamine

IL-6 has the highest predictable increase in populations with anxiety. No other cytokine is in the crossroads of immune physiology and stress as is IL-6. Monocytes, macrophages, endothelial cells, and adipocytes produce it due to sympathetic stimulation and stress hormones. The presence of chronic worry may work as a chronic stressor in GAD, which keeps IL-6 levels high over the long run.

IL-6 is bound to the membrane-bound and soluble IL- 6 receptors, promoting the JAK- STAT signaling cascade. Relentless stimulation of STAT3 stimulates inflammatory gene transcription and can augment systemic inflammatory tone. Evidence: The IL-6 receptors are found within the central nervous system in the amygdala, hippocampus, and prefrontal cortex, the regions within the central nervous system that are central to the regulation of anxiety. Increased IL-6 can boost glutamatergic signaling, lower the tone of the inhibitory GABAergic synapses, and promote corticotropin-releasing hormone (CRH), which increases hypothalamic-pituitary-adrenal (HPA) axis action. Prolonged IL-6 can thus be involved in neural hyperexcitability and endocrine anomalies in GAD (Friligkou *et al.,* 2024).

IL-1b: Disruption of Neural Sensitization and Plasticity

Another pro-inflammatory cytokine that is involved in the anxiety mechanisms is IL-1β. In contrast to IL-6, which produces a wide range of effects on stress signaling, IL-1b has a strong impact on synaptic plasticity. Long-lasting IL-1b level alters long-term potentiation (LTP) in the hippocampus and suppresses neurogenesis, which could result in the impairment of regulatory contextual threat. When contextual processing is impaired, the contextual processing in GAD, with generalization of anxiety rather than the focus on the stimulus, could partake in perpetual anticipatory worry.

In the amygdala, IL-1b increases amygdala excitatory signaling at the NMDA receptors and reduces fear-activation thresholds. Experimental models show that IL-1b is activated, leading to anxiety-like behavior, implying a mechanistic as opposed to a mere correlation. Further, IL-1b stimulates nuclear factor kappa B (NF-kB), further escalating the cascades of inflammatory events that could be maintained chronically in sensitizing nerve cells (Humer *et al.,* 2020).

IL-17: Maintaining the Chronic Inflammation.

Th17 cells are the main producers of IL-17, and the latter has proved its value as a persistent inflammatory agent. Despite being less commonly investigated in GAD than IL-6 or IL-1b, IL-17 is a potential cause of chronic neuroimmune malfunction. IL-17 also facilitates the recruitment of more immune cells and increases the generation of IL-6 and IL-1β, which strengthens the inflammatory amplification cycles.

IL-17 has been linked with the changes in blood-brain barrier (BBB) integrity. Tight junction protein disruption can enhance the BBB permeability, which has been shown to enhance the contact between peripheral cytokines and central neural tissue. This augmented neuroimmune

cross-talk might favor perpetuated limbic sensorimotor activation in chronic anxiety conditions (Hajmusa and Bahi, 2024).

IL-10: Control Malfunction and Cytokine Dysregulation

The anti-inflammatory immune IL-10 is significant in preventing hyper-activation of immunity. IL-10 suppresses production of IL-6 and IL-1β, but favors homeostatic restoration after exposure to inflammatory stimuli. Low concentrations of IL-10 in certain anxiety groups may indicate disrupted regulation balance and not inflammation only.

Instead of considering cytokines separately, the most recent literature puts a variable emphasis on the analysis of cytokine ratios, especially on the IL-6:IL-10 ratios. The increased IL-6/IL-10 ratio can be used as evidence of the pro-inflammatory direction and insufficient regulation. This imbalance can indicate a biologically significant human inflammatory response in GAD, which may be more stable than single cytokine measurements (Jacobson and Feng, 2022).

Pattern-Based Implications into Pathophysiology

The literature, as a whole, implies that GAD can be a pattern that is marked by a high level of IL-6 and IL-1b, the potential implication of IL-17, and a low level of IL-10. These interleukins are dynamically interacting with the stress hormones, neurotransmitter systems, and neural networks. Pro-inflammatory cytokines are also capable of proliferating glutamatergic transmission, decreasing serotonin release by activation of the indoleamine 2,3-dioxygenase (IDO) pathway, and inhibiting neurotrophic factor (BDNF) of the brain and limiting neuroplasticity. But there are still methodological incompatibilities between studies. Numerous studies do not separate primary GAD from depression comorbidity or tolerate metabolic and lifestyle confounding factors. Moreover, cytokine concentrations occur in a circadian pattern, making them difficult to interpret (Jacobson and Bhattacharya, 2022).

Despite such weaknesses, evidence is generally tending towards the view that, mechanistically speaking, some patterns of interleukin may be involved in the anxiety of GAD. It is noteworthy that the identification of uniform cytokine signatures is needed to develop biomarkers and improve the pathophysiological models.

2.3 Interleukin Interaction with the HPA Axis and Neurotransmitter Systems in GAD

In the generalized anxiety disorder (GAD), the careful deciphering of interleukin patterns entails a study of interactions between the inflammatory cytokine and stress endocrine systems

and the neurotransmitter systems. Interleukin functions are not independent, but an interplay of functions of hypothalamic-pituitary-adrenal (HPA) axis and central monoamine affinity pathways. The bi-directional communication in this context brings out a mechanistic pivot upon which the symptoms of anxiety linger despite immune maladjustment (Joseph *et al.*, 2023).

2.3.1 Interleukin-HPA Axis Cross-talk

The primary neuroendocrine system is the HPA axis. The activation is triggered by the release of corticotropin-releasing hormone (CRH) in the hypothalamus, secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland, and finally production of cortisol by the adrenal cortex. Cortisol responses to acute stress have adaptive effects, which include mobilizing energy resources and inhibiting the hyperactivity of the immune system. Nevertheless, persistent HPA activity and cortisol rhythm disorders can be observed in GAD associated with chronic psychological stress (Jiang *et al.*, 2022).

Directly accessed by pro-inflammatory interleukins, especially IL-6 and IL-1β, are the stimulating influences on CRH production. The high levels of IL-6 have been evidenced to amplify the secretion of ACTH as well as the level of circulating cortisol in the blood. Cortisol prevents inflammatory signaling via glucocorticoid receptor-mediated inhibition of nuclear factor kappa B (NF- kB), but chronic exposure to stress factors can cause glucocorticoid receptor resistance. With the help of this phenomenon, the anti-inflammatory effect of cortisol is impaired, and the production of cytokines continues even during high hormone concentrations (Kidd *et al.*, 2022).

Impaired feedback regulation in such a way forms a maladaptive loop: stress increases interleukins, interleukins induce the release of cortisol, and cortisol is not sufficient to inhibit inflammation. Sustaining hyperarousal of the physiological aspect in GAD, this cycle can lead to restlessness, sleeping difficulties, and somatic tension. Thus, the interaction between interleukin and the HPA axis is the key to explaining the transformation of chronic worry to the biological dysregulation of the system.

2.3.2 Cytokine Effect on Serotonergic Circuitry

In addition to the endocrine communications, neurotransmitter systems, which are involved in the regulation of anxiety, are influenced by interleukins. Activation of the indoleamine 2, 3-dioxylase (IDO) line is one of the possible crucial mechanisms. Cytokine release of pro-inflammatory cytokines like IL-6 and IL-1 b activates IDO that redirects the tryptophan

metabolism to kynurenine production instead of synthesizing serotonin. Less serotonin could interfere with the regulation of mood and anxiety, which can worsen excessive worrying in GAD (Kim and Shin, 2022).

Also, kynurenine products can have neuroactivity, affecting glutamatergic release and excitotoxic susceptibility. In this way, any inflammatory cue can indirectly change the serotonergic balance, but at the same time, it has an impact on excitatory pathways.

2.3.3 Neural Excitability and GABA-Glutamate Imbalance

Interleukins also have a direct effect on excitatory and inhibitory neurotransmission. High IL-1b levels have been demonstrated to increase NMDA receptors, glutamate transmission, and make neurons fire more in the amygdala. At the same time, gamma-aminobutyric acid (GABA) inhibitory tone might be lowered by inflammatory cytokines. This excitatory preponderance can reduce the threshold on detection of threat and increase anticipatory anxiety.

Persistent excitatory bias might be important in maintaining hypervigilance at baseline in GAD, where the symptom of anxiety is generalized, not stimulus-specific. Less inhibitory control of limbic activation by prefrontal cortical areas can further undermine top-downization of limbic activation (Łoś and Waszkiewicz, 2021).

2.3.4 Neuroplasticity and Inhibition of BDNF

Interleukin signalling can also decrease the expression of brain-derived neurotrophic factor (BDNF), one of the proteins that is important in promoting synaptic plasticity and adaptive responses to stress. Prolonged inflammatory conditions are associated with reduced neurogenesis and impaired remodelling of synapses, especially in the hippocampus. Disrupted plasticity can be a contributor to cognitive rigidity and failure to switch off worry.

The mechanical perspective is used to understand an integrated system in which sub-systems work together effectively to produce each functional unit of the system (Marzatsoli *et al.*, 2005).

2.3.5 Integrated Mechanism Perspective

By combining, the interleukins engage with the HPA axis and the neurotransmitter networks and form a multidimensional dysregulation system. High IL-6 and IL-1b might increase the effects of stress, decrease serotonergic control, increase glutamatergic excitability, and inhibit neuroplasticity. The occurrence of glucocorticoid resistance simultaneously may help maintain

glucocorticoid-mediated inflammatory signaling, which supports enduring anxiety (Luitel *et al.,* 2024).

This is a combination model that emphasizes the role of concurrently examining levels of cytokines, cortisol variables, and clinical severity measures in GAD studies. The empirical assessment of these interactions could shed some light on the fact that inflammatory dysregulation can be either a key process or a perpetuator in anxiety pathology.

2.4 Microglial Activation and Neuroinflammatory Mechanisms in GAD

Another important but understudied aspect of the dysregulation of interleukin in Generalized Anxiety Disorder (GAD) concerns the state of microglial function activation and the main neuroinflammatory processes. Although peripheral cytokine dysregulation can also be very insightful, as biomarkers, the impact of these signals on the functioning of the brain should be analyzed in the context of resident immune cells of the central nervous system (CNS). The innate immune cells in the brain, microglia, are the main transmitters of neuroimmune communication (Li *et al.,* 2023).

In physiological circumstances, the microglia are in a surveillant state, in which they constantly scan the neural environment. They control synaptic pruning, eliminate cellular degeneration, and facilitate neural homeostasis. Nevertheless, microglial activation can take place due to exposure to systemic signals of inflammation, especially high levels of interleukins like interleukin-6 and interleukin-1β. The activated microglia morphologically change and release cytotoxic cytokines, reactive oxygen species (ROS), and nitric oxide, which increase the local neuroinflammatory signaling.

Chronic psychological stress is a potential cause of microglial activation in GAD, indirectly by maintaining peripheral raised levels of cytokines. The effects that pro-inflammatory interleukins may have on the brain can be reached at several different locations: transport across the blood-brain barrier (BBB) or by intervening with endothelial receptors, and also, through afferent vagal input. After the initiation of central inflammatory cascades, microglia can also produce cytokines in an autonomous manner in response to peripheral stimulation (Lakhawat *et al.,* 2024).

Activation of microglia carries great consequences on mechanisms of anxiety. Microglia activation can interfere with the synaptic plasticity, as long-term potentiation (LTP) is

hampered in the hippocampus. The diminished neurogenesis of hippocampal neurogenesis may interfere with contextual processing of threat-related stimuli, which is involved in generalized and not stimulus-specific anxiety. Moreover, amygdala IL-1b, as produced by microglia, may augment excitatory transmission and reduce fear activation thresholds as well as strengthen hypervigilance.

Oxidative stress is also another significant mechanism. Microglial activation leads to the production of ROS, potentially damaging nerve membranes and inhibiting the work of mitochondria. Minor metabolic imbalances in corticolimbic circuits may be maintained to maintain neural hyperexcitability that is typical of GAD (Langarita-Llorente and Gracia-Garcia, 2019).

The integrity of the blood-brain barrier is also applicable. The chronic IL-17 and IL-6 mediated inflammatory signaling can modify tight junction proteins in endothelial BBB cells. There would be more interaction of the peripheral immune mediators with the central neural tissue because of increased permeability. It is a potential feed-forward loop of inflammation by means of this increased neuroimmune cross-talk.

Microglial stimulation does not always mean that it is actively neurodegraded. In GAD, inflammatory mechanisms are probably low-grade and chronic changes, but not the acute inflammatory pathophysiology. This insidious yet accretive neuroinflammatory condition can tune the neural circuitry without leaving any trace of structural damage that can be seen with standard imaging (Lian *et al.*, 2025).

Although it carries strong mechanistic plausibility, the confirmation of microglial activation of prionaceous GAD is wasted by human coding. Translational animal models have been used in most findings of neuroinflammatory studies in depression and neurodegenerative disorders. Nevertheless, because the inflammatory pathways in psychiatric disorders are common to stress, and also because psychiatric disorders happen to share the same stress-induced physiological reactions biologically, it is plausible to broaden the framework to GAD.

The categorization of peripheral interleukin-profiling as a subordinate part of the main neuroinflammatory hypothesis sheds light on the significance of studying inflammatory patterns instead of individual biomarkers. Microglial activation is an interface by which

systemic cytokine elevation relates to circuit-level malfunctioning of networks of anxiety (Li *et al.*, 2024).

In this way, the activation of microglia to mediate neuroinflammatory processes could be a highly important pathway in which interleukin dysregulation can act to advance chronic anxiety mechanisms in GAD. The degree of central immune engagement could be further elucidated with the help of future empirical studies that would involve the use of inflammatory biomarkers in addition to neuroimaging data.

2.5 Inflammatory Biomarker Potential and Clinical Implications in GAD

The accumulated literature focusing on patterns of interleukin in Generalized Anxiety Disorder (GAD) has raised further questions regarding the possible usefulness of inflammatory biomarkers in clinical practice. Even though psychiatric diagnoses are still mainly symptom-based, there are recent developments in the field of biological psychiatry that emphasize the significance of finding quantifiable physiological signs that can be matched with the pathophysiological processes. In this regard, interleukins, especially IL-6, IL-1b, IL-17, and IL-10, are promising candidates for biomarker discovery in GAD (Lee *et al.*, 2024).

2.5.1 Biomarker Relevance in Psychiatry

A biomarker of clinical significance should exhibit reliability, reproducibility, and specificity. Inflammatory biomarkers in GAD would ideally (1) distinguish between the affected individuals and the healthy controls, (2) be associated with the severity of the anxiety symptoms, and (3) be indicative of underlying biological processes leading to anxiety. Although single-cytokine highs represent the initial evidence, new studies indicate that a pattern of recognition of inflammation can be more diagnostic and prognostic (Millan, 2022).

The capacities of pro-inflammatory cytokines such as IL-6 have been repeatedly associated with the exposure to chronic stresses and anxiety symptoms. Higher concentrations of IL- 6 might be linked to physiological hyperarousal, sleeping difficulties and somatic tension- key features of GAD. The IL-1b by acting upon the synaptic plasticity and amygdala excitability may be linked with cognitive symptoms of perennial worry and ineffective threat selectivity. However, such relationships are different in the research.

2.5.2 Cytokine Ratio and Inflammatory Balance

Instead of making measurements of absolute cytokine levels, recent studies put an emphasis on the assessment of pro/anti-inflammatory ratios and especially IL-6/IL-10. This ratio is an

indication of systemic inflammatory preeminence over regulation. The relative abundance of IL-6/IL-10 can suggest persistent immune activation without adequate anti-inflammatory responses, which can be considered a biologically discrete subtype among the populations of GAD (Maron and Nutt, 2017).

The approach based on patterns is consistent with the principles of precision psychiatry, which aims to find biologically significant subgroups of heterogeneous diagnostic groups. Patients with a high level of inflammatory imbalance may vary in how they respond to treatment or the course of the disease compared to others who do not show evidence of immune dysregulation.

2.5.3 The Endocrine and Neural Markers

The utility of biomarkers is greater when the indices of inflammation are combined with endocrine and behavioural indices. To illustrate, correlating IL-6 with disrupted cortisol rhythms can explain the relationship between immigration and stress systems. The integration of cytokine profiles with accepted levels of anxiety scales can result in greater clinical interpretability and is capable of predicting.

Further, inflammatory biomarkers can be used to give information on responsiveness to treatment. Even earlier data indicate that patients who have high levels of inflammatory markers might not react to conventional pharmacotherapy. The adjunctive therapeutic benefit of anti-inflammatory interventions or lifestyle changes that address systemic inflammation (e.g., exercise, sleep optimization, and dietary regulation, etc.) might be useful (Merino-Soto *et al.,* 2023).

2.5.4 Methodological Considerations and Limitations

Although the results are encouraging, there are still serious methodological issues. The level of cytokines is also a confounded factor with such variables as body mass index (BMI), metabolic health, smoking status, sleep quality, and circadian variation. Uncontrolled bias in biomarker interpretation may be caused by such factors. Moreover, comorbid depression is often comorbid with GAD and could independently increase the inflammatory markers. The other constraint is necessarily that of time stability. Biomarkers need to show trends with time and not periodic changes in order to be effective in clinical practice. Predictive validity should be determined by longitudinal studies measuring the inflammatory markers and progress of symptoms (Melnychuk *et al.,* 2024).

2.5.5 Translational Implications

Discovery of interleukin patterns in GAD is likely to change the concept of anxiety pathophysiology. Instead of consuming inflammation as a secondary effect of stress, there is growing evidence that immune dysregulation can actively maintain neural sensitization and neural rumination.

Assuming that they are validated, inflammatory biomarkers may add to:

- Better stratification in diagnosis.
- Early detection of the high-risk individuals.
- Individualized treatment design.
- Treatment response monitoring.

Interleukin patterns play in trait vulnerability, state-specific indicators, or both; large-scale, well-controlled empirical studies are needed.

To conclude, the interleukin profiling has great potential in promoting the development of biomarkers in GAD. Research can take a step towards a biologically informative classification of anxiety disorders by combining immune, endocrine, and behavioral measures. The current thesis has an opportunity to make a contribution to this new area of research by conducting a systematic study of interleukin patterns and how they are related to the severity of anxiety and stress-axis regulation.

Chapter 3: Molecular and Cellular Mechanisms of Interleukin-Mediated Anxiety in GAD

3.1 Molecular Basis of Interleukin Signaling in GAD

The role of interleukin dysregulation in the pathophysiology of Generalized Anxiety Disorder (GAD) is incomprehensible without considering the molecular signaling pathways by which cytokines impact neural and endocrine functions. Interleukins are extracellular signaling proteins that interact with receptors of the cell surface and cause intracellular signaling pathways that control gene transcription, synaptic signaling, and stress-axis regulation. It has been postulated that long-term stimulation of these signaling pathways can cause long-term neurobiological changes underlying pathological worry and hypervigilance in the case of chronic anxiety states (Mishra *et al.,* 2023).

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) is one of the largest molecular pathways that are triggered by pro-inflammatory interleukins, especially IL-6. The association of Janus kinases (JAKs) with IL-6 binding to its soluble or membrane-bound receptor complex is association-dependent. These kinases go ahead to phosphorylate STAT3, which dimerizes and translocates into the nucleus, a transcription factor. STAT3 controls the expression of genes that are related to inflammatory amplification, cell survival, and metabolism in the nucleus.

When the conditions are acute, the JAK-STAT calcium signalling is closely regulated and responsive. Nevertheless, chronic psychological stress can perpetuate the production of IL-6 and the subsequent long-term activation of STAT3 in the case of GAD. The continual transcription of inflammatory genes can strengthen the condition of elevated systemic cytokines, which forms a feed-forward loop of inflammation. This persistence of the molecules could be one reason why the anxiety of GAD is chronic as opposed to episodic (Milic *et al.,* 2025).

The other central cytokine that is involved in the pathogenesis of anxiety is IL-1b, which is mainly triggered by nuclear factor kappa B (NF-kB). When IL-1b attaches to the receptor, it activates inhibitor of kappa B kinase (IKK) which leads to a breakdown of inhibitory proteins, IkB. This enables relocation of NF-kB into the nucleus which enhances transcription of other pro-inflammatory cytokines and stress-related intermediary. Recurrent fluctuations in neuronal gene expression and neuronal inflammatory muscle tone have been linked to persistent NF-kB stimulations (Ma *et al.,* 2024).

The endocrine systems do not function alone through the molecular cascades. Interleukin receptor overlaps considerably with the glucocorticoid receptor-mediated pathway. The secretion of cortisol as a result of the activation of the hypothalamic-pituitary-adrenal (HPA) axis has the tendency to reduce the NF-kB activity and inhibit the expression of inflammatory genes. But with long exposure to stress, the desensitization or resistance of glucocorticoid receptors can take place. In cases of reduced sensitivity of receptors, cortisol is unable to inhibit inflammatory transcription factors. This leads to an increase in the production of cytokines even in the presence of high levels of cortisol- a process especially prominent in chronic conditions of anxiety (McNaughton *et al.,* 2026).

Sustained neuronal activation of JAK-STAT and NF-kB pathways can change the expression of the synaptic proteins, neurotransmitter receptors, and neurotrophic factors. As an example, inflammatory signals have been associated with constant expression of brain-derived neurotrophic factor (BDNF) that disrupts brain dynamics in hippocampal and prefrontal networks. By observing such changes, adaptive stress regulation might be constrained and lead to rumination persistence in GAD.

Also, there are inflammatory transcription pathways that determine oxidative responses. Persistent cytokine signaling enhances reactive oxygen species (ROS) production, which has the potential of impacting mitochondrial activity and neuronal cellular energy production. Minor haunches of metabolic efficiencies may contribute to the unsteadiness of the neuronal excitation in the disciplines of fear.

Importantly, the interleukin signaling molecules of GAD signify long-lasting low-degree stimulation rather than inflammatory acute pathology. Probably, lasting activation may have nothing to do with direct structural damage, but may be a kind of minor reconditioning of neural networks to hyperactivity. The maintenance of the JAK-STAT and NF-kB activity, along with the maladaptation of glucocorticoid feedback, precipitates a molecular state where the long-lasting neural sensitization is promoted (McMurray and Sah, 2022).

Thus, the interleukin communication in GAD is not merely the variation of peripheral immune, but also transcriptional reorganization with the capacity to control brain plasticity, endocrine signaling, and neurotransmitter balance. These cascades of molecules play a critical role in the realization of what biologically meaningful medical patterns of inflammation can be realized and how the patterns can be utilized as biomarkers as in chronic anxiety illness.

3.2 Cellular Sources and Peripheral–Central Immune Communication in GAD

The interleukin dysregulation process of the Generalized Anxiety Disorder (GAD) is not confined to a certain location in the anatomy or immune subsystem. It is rather a manifestation of a complex of interrelationship between peripheral immune cells and central nervous system (CNS) immune modules. The cellular causes of interleukins and the pathways by which peripheral inflammation can modulate the brain functions would also be important in understanding the role of immune signaling in the pathophysiology of anxiety.

3.2.1 Peripheral Immune Activation

The persistent psychological stress, which is a hallmark of GAD, has quantifiable effects on the work of the immune cells of the periphery. Prolonged stimulation of the sympathetic nervous system elevates the production of catecholamines like norepinephrine, which act on adrenergic receptors on immune cells. This communicates the transcription of pro-inflammatory genes through the activation of intracellular pathways such as the NF-kB. Macrophages and monocytes are specifically sensitive to stress-induced signaling, and they are the main sources of IL-6 and IL-1b into peripheral circulation (Nguyen *et al.*, 2025).

Also, chronic stress can change the differentiation of T-helper cells towards the polarization of Th17 against specific inflammatory contexts. The IL-17 secreted by Th17 cells is a cytokine associated in maintaining the chronic inflammatory conditions. High IL-17 can improve the secretion of other pro-inflammatory mediators, increasing systemic immune response. Such peripheral immune shifts may be in a chronic state in GAD, where the cause is not an episodic stressor but is more permanent (Oktay *et al.*, 2024).

Low-grade systemic inflammation does not always result in the manifestation of clinical symptoms of an immune disease. Rather, it can be the case that minimal increases in circulating interleukins are enough to affect neural and endocrine activity. The phenomenon reminds us that cytokine pattern mechanisms should be measured with focus instead of considering traditional inflammatory markers (Papola *et al.*, 2024).

3.2.2 Neuroimmune Signaling and Blood-Brain Barrier

There are various communication pathways of the peripheral cytokine with the brain. The circulation and neural tissue exchange of molecules is regulated by the blood-brain barrier

(BBB), which is comprised of endothelial tight junctions, astrocytic end-feet, and pericytes. Cytokines, including IL-6 and IL-17, can change the expression of tight junction proteins in response to an inflammatory condition, with a slight impact on the BBB permeability. Even the smallest change in permeability can help increase neuroimmune interaction.

Central signaling may also involve cytokines crossing the BBB. The cerebral vessels' endothelial cells are also equipped with cytokine receptors that are able to relay the inflammatory signals to the CNS through the indirect messenger systems. Furthermore, afferent vagal pathways offer neural pathways by which the peripheral immune actions can produce quick changes on the parts of the brain that are concerned with the processing of stress (Probst *et al.,* 2022).

3.2.3 Microglial Role in Amplifying Cytokines in the Central Nervous System

Glial microglial cells act as immune effector cells in the CNS. In basal conditions, the microglia preserve the homeostasis of synapses and undergo developmental pruning. Nevertheless, an activated phenotype may be triggered by exposure to systemic inflammatory signals. Microglia activated secrete IL-1b, IL-6, and reactive oxygen species, which increase the neuroinflammatory cascades locally.

Perpetual peripheral interleukin increase in GAD could either encourage low-grade microglial activation, especially in parts of the brain susceptible to stress, like the hippocampus and the amygdala. Collective microglial signaling could occur at a low level, induce changes in the synaptic architecture and excitability, without causing neurodegenerative effects. This central activation of the immune system at a low level could be one of the factors of continued neural sensitization and the distortion of the perception of danger (Roemer *et al.,* 2025).

3.2.4 Coordination between Peripheral and Central Processes

The interplay of peripheral immune cells, BBB processes, and central microglial stimulation of the system creates a continuous axis of inflammatory reactions between systemic responses to stress and dysfunction of the neural circuitry. Chronic anticipatory anxiety can maintain peripheral cytokine production in GAD that recruits additional central cytokine signaling. Such a mutual relationship confirms this hypothesis of interleukin dysregulation in GAD as a multisystem condition and not a peripheral isolated inflammatory event.

Therefore, cellular processes of interleukin rise and the proximal-distal communication processes are central to comprehending the idea that the patterns of inflammation can be converted into the processes of persistent anxiety. The validity of interleukin profiling as a

research method in GAD is boosted by the fact that investigations must not only look at the circulating cytokines but also the possible central effects of such cytokines (Rook *et al.*, 2022).

3.3 Cytokine-Induced Neurotransmitter Dysregulation and Neuroplastic Changes in GAD

One of the major questions to answer regarding interleukin patterns in Generalized Anxiety Disorder (GAD) is how peripheral inflammatory cues contribute to maintaining chronic cognitive and emotional symptoms. Although the increase in cytokines can be detected in the circulation, behavioral expressions of GAD, when it comes to dysfunction in the neural circuits, include constant worry, hypervigilance, lack of concentration, and somatic tension. Consequently, the study of the effect of interleukins on the neurotransmitter systems and neuroplasticity is important to connect neurotransmitter dysregulations to the neuroimmune system (Ritola *et al.*, 2022).

3.3 1 Serotonergic Modulation and Interleukins

Among the most properly defined ways in which the pro-inflammatory cytokines influence the functions of the brain is the indoleamine 2,3-dioxinase (IDO) enzyme. IDO is activated by IL-6 and IL-1b and shifts the metabolism of tryptophan to times when kynurenine should be produced rather than serotonin. A decrease in the tryptophan supply to build serotonin can result in a decrease in serotonergic signaling, which is a system that has been involved in the regulation of anxiety (Rubel *et al.*, 2024).

In addition to the loss of serotonin, kynurenine metabolites are neuroactive. There are certain metabolites that affect glutamatergic neurotransmission, and they can also lead to an excitatory imbalance. Therefore, the IDO activation caused by cytokines not only lowers the levels of serotonin, but it can also have the side effect of elevated neural excitability. Such changes in biochemistry may enhance rumination and emotional dysregulation in GAD, where persistent worry indicates a long-term cognitive activation.

3.3 2 Glutamate- GABA Imbalance and Amygdala Hyperexcitability

There are also pro-inflammatory cytokines that directly affect excitatory and inhibitory neurotransmission as well. It has been described that IL-1b amplifies NMDA receptor-mediated glutamatergic signaling, especially in the limbic structures like the amygdala. More release of glutamate can result in higher neuron discharges and reduced threat detection threshold.

At the same time, inflammatory signaling can result in the decrease of gamma-aminobutyric acid (GABA) inhibition. The main neurotransmitter that inhibits neural overactivity isGABA. A decrease in GABAergic action can also make prefrontal cortical top-down regulation of the enduring amygdala threat responses weak. Such an imbalance of excitation and inhibition could maintain a state of hypervigilance that is typical of GAD (Roseberry *et al.,* 2023).

Even minor changes to the excitatory state, in the presence of chronic anxiety states, can have a substantial behavioral impact. The prolonged neural hyperresponsiveness can be expressed in the form of excessive anticipatory anxiety, inability to relax and overreaction to the unclear stimuli.

3.3 3 Neuroplasticity and Brain-Derived Neurotrophic Factor (BDNF)

The neuroplasticity is also influenced by interleukin signaling via brain-derived neurotrophic factor (BDNF). BDNF plays a crucial role in the growth of the synapses, the survival of the neurons, as well as activity-based learning. An inverse relationship between chronic inflammatory conditions and decreased BDNF expression has been observed, especially in the hippocampus.

Reduced contextual processing and extinction learning could be compromised in GAD by impaired neuroplasticity. The hippocampus is very instrumental in the process of recognising safe and threatening environments. This discrimination may be impaired by reduced neurogenesis and synaptic adjustability, which leads to generalized and not stimulus-specific anxiety. Impaired neural aspects may be cognitive rigidity and rumination (Rosnick *et al.,* 2013).

3.3.4 Oxidative Stress and Mitochondrial Effect

Several changes are characterized by production of reactive oxygen species (ROS) as a result of cytokine induced inflammation. The increase in the level of ROS can compromise the functioning of the mitochondria and interfere with the neuronal energy metabolism. Even minor mitochondrial impairment may have an effect on the network stability and synaptic transmission. This may be further increased by hyperexcitability in anxiety circuits due to this metabolic stress.

3.3.5 Combined Neurochemical Perspective

The cytokine-mediated neurotransmitter and neuroplastic changes in aggregate supply a mechanistic linkage between peripheral immune activation and core symptoms of anxiety. Marked IL-6 and IL-1b may all play a role in impairment of serotonergic control, amplified

glutamatergic, inhibition of GABAergic synapses and maladaptive neuroplasticity. These meeting influences will be able to put neural systems into fresh productive courses of further sensitization.

It is important to note that they are likely to be chronic processes having low-grade rather than acute pathological development. There is a possible progression of neural networks to the hyperresponsive state due to the effect of chronic inflammatory signaling that is consistent with the chronic development of GAD (Storch, 2022).

Such an understanding of these neurochemical processes increases the rationale of investigating the profiles of interleukins as biomarkers. Given under the provision that the levels of cytokines correlate with the outcome of anxiety severity and dysregulation of stress-axis, they may bear a biological significance in terms of alterations in neurotransmitters and neuroplastic processes.

3.4 Integrated Interleukin Pattern Model and Biomarker Implications in GAD

The sections above have outlined the changes in the molecular signaling pathways, cellular immune activation, and the neurotransmitter modification by cytokines regarding the generalized anxiety disorder (GAD). However, to achieve the pathophysiological sense of such data, it appears appropriate to transcend the mechanisms of isolation and provide a model of an integrated interleukin pattern. Instead of explaining anxiety pathology by a single rise in cytokines, existing evidence supports the view that GAD can be a matter of the interaction between the multiplicity of interleukin systems that feign the same failure to regulate.

3.4.1 Single Markers and Pattern Recognition

Initial studies of inflammatory psychiatry mostly focused on individual cytokines, with IL-6 most often used. Although IL-6 is still one of the primary mediators of stress-induced immune responses, the growing body of knowledge suggests that the analysis of cytokines separated can simplify the intricate processes of immunology. In GAD, the new literature indicates that a typical pattern would be a high level of IL-6 and IL-1b, a possibility of IL-17, and a decreased level of IL-10 (Sriken *et al.*, 2022).

This tendency indicates a transition towards the preponderance of pro-inflammatory conditions and a loss of the anti-inflammatory balance. The IL-6 and IL-1b enhance the JAK-STAT and NF-kB cascade events; IL-10 normally acts to inhibit the excessive production of cytokines.

Weakened IL-10 reserve might hamper balance in the regulation, whereby the inflammatory cascades can be continued in the chronic. Thus, the assessment of cytokine ratios, especially the IL-6/IL-10, can offer more stable and biologically significant indices of immune dysregulation in the GAD.

3.4.2 Exposure to Endocrine Dysregulation

Hypothalamic-pituitary-adrenal (HPA) axis dynamics should also be included in the integrated model. High levels of IL-6 and IL-1b activate corticotropin-releasing hormone (CRH) and increase cortisol secretion. In acute cases, cortisol inhibits the transcription factor of inflammation like NF-kB. Nonetheless, persistent exposure to stress can lead to the development of resistance to glucocorticoid receptors which reduces the inhibitory action of cortisol.

This endocrine-immune communication forms a vicious circle of maladaptive feedback, where inflammation as a result of stress triggers stress reactivity and stress reactivity in turn enhances inflammation. They may be long-lasting physiological hyperarousal and dysfunctional stress recovery, which are a hallmark of GAD. The combination of cytokines and cortisol profile measurements thus enhances the capability of the interleukin pattern model of explanatory power (Stern *et al.,* 2024).

3.4.3 Circuit Consequences of the Neural Circuit

Neural circuit dysregulation is also found in the integrated model of interleukin pattern. High IL-1b is known to increase amygdala excitability, whereas IL-6 is able to modulate glutamatergic transmission and is able to inhibit GABAergic. Simultaneous inhibition of brain-derived neurotrophic factor (BDNF) can negatively affect the neuroplasticity of hippocampal and prefrontal inhibition. All these processes can re-establish corticolimbic circuits in a state of chronic hyperactivity. Chronic inflammatory signaling can maintain base levels of neural sensitization instead of episodic fear activation in GAD, where the intensity of anxiety is diffuse and anticipatory and not stimulus-related (Shah *et al.,* 2023).

3.4.5 Implications of Biomarkers and Clinical Translation

The model of interleukin patterns is focused on multidimensional evaluation from the biomarker viewpoint. Instead of using a single inflammatory marker to be diagnostic, simultaneous cytokine profiling could provide biologically distinct subgroups in GAD populations. Patients who are highly pro-inflammatory dominant can have an inflammatory

subtype of anxiety that is typified by increased stress reactivity and somatic symptoms (Szuhany and Simon, 2022).

Development of biomarkers needs reproducibility, specificity, and clinical correlation. Interleukin levels should show stable relationships with optimal scales of anxiety and be the same in tests of repeated tests. Also, it is necessary to eliminate the influence of other confounding variables, including body mass index, metabolic health, sleeping problems, and comorbid depression, in order to interpret it correctly.

The treatment plans can also be informed by inflammatory biomarkers. Patients whose pro-inflammatory cytokines are elevated can receive adjunctive treatment of anti-inflammatory interventions, lifestyle changes aimed at systemic inflammation, or even special pharmacological treatment. Nonetheless, they are still merely investigative applications that need to be strengthened with empirical evidence.

3.4.6 Towards a Systems-Level Pathophysiological Framework

The conceptualized idea of the integrated interleukin pattern model of GAD is that of a multisystem disorder that includes immune activation, endocrine dysregulation, neural sensitization, and worry-cognitive persistence. Instead of describing inflammation as a side effect of anxiety, this model implies that persistent inflammation due to interleukin dysregulation might play an active role in the perpetuation of the pathological worry (Seifaei *et al.*, 2023).

Through a systematic investigation of the IL-6, IL-1b, IL-17, and IL-10 concentrations, as well as cortisol concentrations and indices of anxiety level, the current thesis aims to objectively test the existence of the offered inflammatory pattern in the clinically diagnosed GAD participants.

Therefore, the combined interleukin pattern model not only contributes to the theoretical knowledge of the GAD pathophysiology but also offers a systematic source of biomarker research and precision psychiatry methods.

Chapter 4: Integrated Pathophysiological Model of Interleukin-Mediated Anxiety in GAD

4.1 Systems-Level Integration of Immune, Endocrine, and Neural Mechanisms in GAD

Generalized Anxiety Disorder (GAD) possesses a pathophysiology that is essentially becoming a multisystem process that includes hyper interactions of immune signaling pathways, neuroendocrine stress responses as well as maladaptive neural circuits. As higher-order cognitive structures have long been involved in explaining the relationships between psychological stress and chronic biological sensitization, newer developments have postulated that chronic imbalance in interleukin-based responses might serve as an integrative link between psychological and biological stress. An analysis of the mechanisms by which the activation of immunity is converted into persistent anxiety indicates the importance of analyzing the issue at the systems level (Shen *et al.,* 2022).

Chronic anticipatory worry is at the center of GAD. As opposed to acute stress responses, which abate once the threat is eliminated, GAD is characterized by constant thinking activity, which happens when there is a danger in the future. This repetitive psychological stress causes the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis activation. Repeat HPA activation causes chronic cortisol secretion, and sympathetic signaling of the immune cells' distribution and cytokine gene expression via transcription factor activation, like nuclear factor kappa B (NF-kB) (Shevlin *et al.,* 2022).

The key agents of this stress-immune interface include pro-inflammatory interleukins (especially IL-6 and IL-1b). High levels of IL-6 have the potential to activate corticotropin - releasing hormone (CRH) secretion within the hypothalamus and enhance the HPA-axis. IL-1b also acts on an endocrine signaling and has the ability to enhance the stress responses. In acute conditions, cortisol has anti-inflammatory effects by inhibiting the NF-kB activity and β-catenin transcription of the cytokines. Nevertheless, under persistent stress, like in GAD, the glucocorticoid receptor sensitivity could be impaired, creating glucocorticoid resistance. The cortisol cannot suppress the inflammatory signalling in cases when the receptor responsiveness is decreased, and the rhetoric of interleukin proceeds.

The disrupted negative feedback loop on the dysfunctional level results in the maladaptive process: psychological stress results in interleukins, interleukins result in further HPA activation and impaired cortisol suppressions in turn promote inflammation. This process of

physiological recalibration of physiological baseline states may, over time, lead to the development of chronic hyperarousal bidirectionally.

The systemic inflammatory environment does not stay in the peripheral circulation alone. Circulating interleukins control central nervous system activity through blood-brain barriers, via the vagal pathways, and via endothelial receptor stimulation. High IL-6, IL-1b, and graphene altruistic neurotransmitter metabolic ratio, especially by exciting the indoleamine 2,3-dioxygenase (IDO) pathway, diminishing serotonin status, and augmenting kynurenine metabolites. Concurrently, the cytokine signaling promotes glutamatergic and promotes a degradation of the gamma-aminobutyric acid (GABA) inhibitory tone, establishing an excitatory-inhibitory imbalance of anxiety-related neural networks (Taschereau-Dumouchel *et al.,* 2022).

These brain chemical changes affect corticolimbic systems that lie at the heart of the GAD pathology. The threat detection with the help of the amygdala can be hyperresponsive to a chronic inflammatory impact. Inhibitors of emotional reactivity are further reduced through poor inhibitory control of the prefrontal cortex. Simultaneously, inflammatory inhibition of the brain-derived neurotrophic factor (BDNF) could suppress the hippocampal neuroplasticity and contextual threat discrimination, and promote generalized anxiety but not stimulus-specific fear.

Notably, the systems-level model does not focus on causality, but on interdependence. As the first result of long-term psychological stress, immune activation could occur, but the stress sensitivity and neural hyperexcitability could appear as a result of the maintenance of interleukin levels. This bidirectional amplification hints at the fact that inflammation is a mediator and sustaining factor of the pathology of anxiety.

The other important element of this combined model is the equilibrium between pro-inflammatory and anti-inflammatory signaling. The decrease in the levels of IL-10 can affect the regulation functions, allowing pro-inflammatory cascades to gain dominance. Higher IL-6/Il-10 ratios can thus be a general-immune disorder sign of inflammation in GAD. The addition of cytokine ratios in the system modeling leads to more accuracy and depicts the dynamic balance underlying immune homeostasis (Tomasi *et al.,* 2024).

The systems-level framework interacts with immune signaling, endocrine feedback mechanisms, neural transmitters and neuronal circuit assemblies to give a complete explanation of the chronicity of GAD. Instead of conceiving anxiety as a cognitive or neurochemical disease, according to this model, GAD is embedded in a larger biological network where there is a lifetime inflammatory hypersensitivity.

This tool of integrated architecture is vital in the acquisition of knowledge in empirical research. Interleukin patterns and cortisol profiles, and indexes of clinical severity, can be used to test the hypothesis of whether systemic immune imbalance is related to anxiety intensity. When validated, this approach on the systems-level can be used to develop biologically-informed diagnostic subtypes and specific therapeutic interventions (Trenoska Basile *et al.,* 2024).

4.2 The Chronic Neuroimmune Sensitization Model in GAD

The Chronic Neuroimmune Sensitization Model is an expansion of the systems-level model, described in Section 4.1, which hypothesizes that neuroactivation thresholds become progressively lowered to cause Generalized Anxiety Disorder (GAD) due in part to the cumulative effect of continuous dysregulation of interleukin. This model does not theorize anxiety as an entirely psychological reaction to events that are perceived to be dangerous; instead, it sees chronic inflammatory signaling as a recalibration of the neural and endocrine systems into becoming hyperresponsive in the face of lasting danger (US Preventive Services Task Force *et al.,* 2023).

Starting point: Trigger of Chronic Psychological Stress

Persistent anticipatory worry is an inherent feature of GAD and happens to be the starting point of this model. The persistent cognitive processing of possible menaces arouses the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. This stimulation enhances the expression of pro-inflammatory cytokines via the stress-reactive molecular signals like NF - kB.

In contrast to the acute responses to stress, which resolve after the removal of a threat source, GAD is characterized by prolonged mental activity. This persistent stress signaling can lead to a persistent low-grade increase in interleukins, especially those of IL-6 and IL-1b. At this initial period, inflammation can still be a part of an adaptive physiological response, but the repeated event triggers a process that starts interfering with system balance.

Ampification: Disorder of farther Immune/Endocrine Feedback

While cytokine levels are still high, the interleukin acts on corticotropin-releasing hormone (CRH) production and multiplies cortisol output. In a healthy state scenario, cortisol has anti-inflammatory effects, and it restores homeostasis. But with long-lasting exposure to stress, the exposure can cause the desensitization of the glucocorticoid receptor. Less responsiveness of the receptors can prevent cortisol from suppressing NF-kB-mediated inflammatory transcription.

This faulty system of feedback turns a transient response to stress into an inflammatory vicious cycle. High levels of interleukins continue the activation of the HPA-axis, and impaired endocrine response does not suppress the immune signalling. The outcome is a prolonged physiological arousal.

Neural Recalibration and Circuit Desensitization

The chronic instigation of inflammation has a cumulative impact on circuitry in the brain. The IL-1b increases the NMDA receptor-mediated glutamatergic transmission in the amygdala and reduces thresholds to detecting threats. IL-6 regulates serotonergic metabolism by the stimulation of the indoleamine 2,3-dioxidos pathway, which decreases serotonin concentrations and might increase rumination in anxiety (Villarreal-Zegarra *et al.,* 2024).

At the same time, anti-regulatory mediated by inflammation of brain-derived neurotrophic factor (BDNF) has the potential to inhibit synaptic plasticity in the hippocampus and prefrontal cortex. The decrease in neuroplastic flexibility constrains contextual discrimination and inferior ability to regulate limbic activation in the top-down regulation. These neural adaptations can, over time, develop a baseline position of hyperexcitability.

GAD involves general anticipatory activation in contrast to acute fear disorder, in which the anxiety response is elicited by specific cues. The chronic neuroimmune sensitization model is a model that describes how chronic interleukin up-regulation can cause a reactivation of corticolimbic pathways to be maintained in an incessantly vigilant state in the face of no imminent threat.

Regulatory Failure and Pro-Inflammatory Dominance

Another aspect of sensitization is damaged anti-inflammatory management. The production of pro-inflammatory cytokines might not be suppressed owing to the reduced levels of IL-10. In

the absence of a sufficient regulatory balance, the IL-6 and IL-1b signaling can be unstable. This ratio between IL-6 and IL-10 can therefore be a possible measure of the level of sensitization, which is the dominance of inflammatory systems.

This disequilibrium is likely to lead to not only neural hyperexcitability but also somatic conditions, namely, muscle tension, fatigue, and sleeping disturbance. Persistent inflammatory tone may also be imposed to affect autonomic regulation and metabolic processes, which supports the physical effects of anxiety (Vrublevska *et al.,* 2022).

Maintaining and Two-way Reinforcement

Neuroimmune sensitisation can potentially develop into a self-perpetuating pattern once it has been established. Neural hyperresponsiveness is maintained through high levels of cytokines and leads to higher threat perception and cognitive worry. It leads both to more intense stress-pathway activation and to a stronger inflation of the signalling. This two-way reinforcement is the reason why GAD is chronic and relapsing.

Notably, when sensitizing, it does not mean that the damage is permanent. Instead, it represents the adaptive systems stuck in a maladaptive equilibrium. Pharmacological or behavioral interventions aimed at the restoration of inflammatory signaling can be used to restore balance when provided effectively (Wanderley Espinola *et al.,* 2022).

Empirical Implication of the Sensitization Model

The Chronic Neuroimmune Sensitization Model makes predictable empirical forecasts. The people with GAD shows:

- High proportions of pro-inflammatory interleukins (IL-6, IL-1b)
- Decreased anti-inflammatory controls (IL-10)
- Abnormal cortisol dynamic referring to a disturbance in the feedback.
- Correlations between the cytokine disproportion and the amount of anxiety.

To ascertain these predictions, an immune and endocrine test is to be conducted alongside acceptable clinical research work. This model is a synthesis of the molecular signaling, endocrine self-regulation, neuromodulator imbalance, neural circuit readjustment into a single account through the conceptual reframe of the GAD as a long-term neuroimmune recalibration

disorder. It does not simply run interleukin patterns as just a discovery, but an agent of continuation of anxiety.

4.3 Empirical Operationalization of the Interleukin Pattern Model in GAD

Integrated Neuroimmune Sensitization Framework described above has a theoretical foundation on how interleukin dysregulation can contribute to the continuation of what is characterized as anxiety in Generalized Anxiety Disorder (GAD). Nonetheless, empirical testing of this framework remains scientifically valid. Operationalization of immune, endocrine, and behavioral variables should be approached with caution in order to translate theoretical mechanisms into constructs that are measurable.

The Measurable Biological Variables

The specific interleukins need to be chosen in accordance with the mechanistic as well as clinical significance of the proposed model of inflammatory pattern, to conduct an empirical assessment of it. Key stress-related pro-inflammatory signatures that have been involved are IL-6 and IL-1b, which have been found relevant in the activation of the immune system and sensitization of neural systems. IL-17 is an agent of chronic inflammatory maintenance as well as blood-brain barrier regulation. IL-10 is an anti-inflammatory regulatory cytokine and indicates immune homeostatic balance (Wang *et al.,* 2022).

These variables can be operationalized by measuring serum concentrations by immunoassay methods, like enzyme-linked immunosorbent assay (ELISA) or multiplex cytokine panels. It is also important to have the same timing of collection of the blood because the concentration of cytokines can vary in a circadian manner. As well, the status of fasting and recent physical exercise have to be managed to minimize confounding factors (Woźny-Rasała and Ogłodek, 2025).

The interleukin pattern model does not measure cytokines separately, but focuses on the analysis through the ratio-regulated IL-6/ IL-10 and IL-1b/ IL-10 ratios. These ratios can perhaps be more comprehensive of systemic inflammatory dominance and regulatory imbalance than absolute values alone.

Endocrine: The Cortisol Assessment

The assessment of cortisol is important, considering the two-way process of interleukins and the hypothalamic-pituitary-adrenal (HPA) axis. Levels of cortisol in saliva or serum could be considered at different time points in order to assess diurnal cycle and feedback regulation.

Abnormal cortisol rhythms, including low diurnal slopes or high baseline, could be signs of glucocorticoid receptor resistance and stress recovery disorders.

By combining the cortisol and cytokine values, one can test the immune-endocrine feedback hypothesis. To illustrate, positive associations of IL-6 and cortisol could indicate stress-induced immunological responses, and a lack of cortisol-mediated immunosuppression could be an indication of immunological loss of control.

Clinical and Behavioral Operationalization

Anxiety severity scales, validated, should be conducted in order to correlate biological markers and expressions of symptoms. Quantitative measures of GAD severity are offered by utilizing standardized measures of the level of worry, somatic symptoms, and functional impairment. Correlational statistics may then establish whether the increase in cytokine is concomitant with clinical manifestations.

Notably, structured clinical interviews should be used to identify primary GAD criteria through the use of participants to enhance homogenization. Major depressive disorder should be ruled out or included in the study to control the severity of depressive symptoms, as depression is a source of inflammatory changes alone (Whisman and Collazos, 2023).

Adjustment of Confounding Variables

There are many physiological and lifestyle factors that have an effect on inflammatory biomarkers. The possible factors that could impact the levels of cytokines include body mass index (BMI), metabolic syndrome, smoking status, quality of sleep, use of medication, and recent infections. Strict inclusion and exclusion criteria and statistical correction of confounders are required to identify GAD-specific immune patterns.

Further, the demographic factors age and sex ought to be taken into consideration. Sex-related variations of immune control can affect interleukin synthesis and would have to be taken into consideration during data analysis (Wang *et al.*, 2024).

Hypothesis Development

The empirical model forms several testable hypotheses:

- Patients with GAD will have high levels of IL-6 and IL-1b as opposed to the control patients.

- IL-10 levels will be reduced in GAD participants, which will lead to higher levels of IL-6/IL-10 in the ratios.
- There will be positive associations between cytokine ratios and the severity scores of anxiety.
- The high levels of pro-inflammatory cytokines will correlate with a change in cortisol profiles, which means that they interact with the immune system to the endocrine.
- The hypotheses can be tested to disprove or narrow down the chronic neuroimmune sensitization model.

Pattern Recognition and Statistical Modeling

In addition to basic group comparison studies, multivariate statistical techniques like regression analysis and structural equation modeling can be used to study the interaction between cytokines, cortisol, and severity of symptoms. Pattern recognition measures could be used to differentiate biologically disparate subgroups in the GAD populations.

As an example, a cluster analysis could indicate the presence of an inflammatory subtype with high IL-6/IL-10 ratios and a high level of anxiety. These linkages would justify precision psychiatry strategies that would focus on achieving interventions via biological profiles.

Translational Significance

In case empirical results reveal that there are regularities of interleukin patterns with GAD, there would be increased argument that there is a mechanism of immune deregulation that would lead to continued feelings of anxiety. The discovery of biomarkers would allow an early diagnosis, estimate the prognosis, and develop a specific treatment.

Non-specific or unfocused results would, however, also be useful, meaning that it is possible that inflammatory disregulation is specific to certain subgroups or is a secondary effect and not a primary cause.

The interleukin pattern model has to be operationalized with great consideration of the interweaving of immune, endocrine, and clinical measurements in a regulated empirical formulation. The purpose of systematic testing of such relationships is to explain why interleukin dysregulation is either a main pathophysiology in GAD or a context-specific correlate of chronic stress using the current thesis (Wanjau *et al.*, 2023).

4.4 Clinical Translation and Precision Psychiatry Implications of Interleukin Patterns in GAD

The method of identifying interleukin dysregulation in Generalized Anxiety Disorder (GAD) has implications that are not limited to theoretical pathophysiology. Should confirmed empirically, the inflammatory signatures could be used to refine the diagnostic, risk-stratify, customize a treatment, and even to monitor the response to a treatment. The application of neuroimmune research in clinical practice is a key target of precision psychiatry, a new paradigm that aims to make treatment plans dependent on biological, psychological, and environmental data.

The Symptom-Based Diagnosis to Biologically Informed Stratification

The existing diagnostic models are based mostly on syndromes. These criteria though not biologically heterogeneous, are useful in providing standardized classification. Two similarly matching participants, who consider diagnostic of GAD, can have a significant neuroendocrine and inflammatory-stress reactivity difference. The use of interleukin profiling in clinical evaluation could enable the recognition of biologically different subgroups to the general GAD diagnosis.

As an example, patients with a high IL-6 and IL-1b and a low IL-10 can be considered a pro-inflammatory subtype with increased physiological arousal and stress sensitivity. Conversely, non-inflammatory patients might be subjected to cognitive or neurochemical instability. This differentiation would help to refine the conceptualization of cases and lend directions to intervention plans (Wendt *et al.,* 2022).

Treatment Implication Implications

Interleukin patterns can interfere with treatment planning in several ways. To begin with, patients with inflammatory preponderance are unlikely to react to the traditional pharmacotherapy. The preferential serotonin reuptake inhibitors (SSRIs), which are offered to treat GAD, are supposed to mainly work on pathways of serotonergic actions, yet they may not result in work on the inflammatory processes that cause neurotransmitter disturbance.

Since the activation of the indoleamine 2,3-dioxylase (IDO) diet would be promoted by cytokines, leading to depleted levels of serotonin, inflammatory regulation might theoretically make treatment more responsive. Pharmacological agents, dietary changes, physical exercise,

and sleep optimization are adjunctive anti-inflammatory measures that can be used to supplement conventional psychopharmacology in groups of patients with inflammation.

Second, increasing monitoring of the cytokine levels over time may give a clue to the response to treatment. Reduction of IL-6 / IL-10 ratios after intervention may represent the restoration of immune balance, which may be a forerunner or an equivalent of symptom improvement. Biomarkers, such as tracking, can provide objective measures of a biological change when compared to subjective symptoms (Yuan *et al.,* 2023).

Risk Assessment and Preliminary Investigations

Interleukin patterns can also lead to an early detection of individuals at increased risk of continued anxiety. Continuous exposure to stress and high levels of pro-inflammatory factors could be an indicator of susceptibility to prolonged neuroimmune hypersensitization. The inclusion of the inflammatory assessment among the high-risk groups, including people exposed to chronic stress or those who have a family history of anxiety disorders, may inform measures to prevent it.

Nevertheless, one should take caution. Many non-psychiatric factors, such as metabolic health, infection, and lifestyle variables, affect the inflammatory biomarkers. Thus, open interpretation of biomarkers has to be done under a general clinical context, unless it is easy to overpathologize normal physiological variation.

Ethical and Practical Implications

Turning inflammatory biomarkers into everyday clinical practice is an issue that needs to be faced with both practical and ethical deliberations. The laboratory tests should prove to be reliable, cost-effective, and interchangeable across environments. Psychiatric-specific reference ranges might have to be created.

Moreover, the clinicians should not make a deterministic analysis of the biological findings. The increasing level of interleukin cannot be regarded as the definitive levels of pathology but as the dynamic markers of systemic interaction. Precision psychiatry is not about depersonalizing patients, but about making them more personalized (Zou *et al.,* 2024).

Immune Findings into Multidimensional Care

Interleukin research clinical translations do not eliminate the available psychological and pharmacologic interventions. Rather, it adds further levels of insight to them. Worry-based

stress activation can be diminished by cognitive-behavioral therapy (CBT), perhaps indirectly via inflammatory signaling. Sleep, physical activity, nutrition lifestyle interventions can have the same effect as well on cytokines.

Therefore, a combined approach to care, that is, psychological therapy, pharmacological treatment, change in lifestyle, and, in some cases, anti-inflammatory treatment, is in line with the neuroimmune paradigm suggested in this thesis.

Accuracy, Psychiatry, and Future Trends

Precision psychiatry focuses on categorizing patients according to biologically significant aspects instead of categorizing them only according to categorical diagnoses. The interleukin pattern model adds to this paradigm since the model suggests quantifiable immune signatures that relate to the process of anxiety. Future studies can consider using machine learning that can distinguish inflammatory subtypes of the GAD populations and integration of cytokines with genetic, epigenetic, and neuroimaging biomarkers. Multidimensional profiling of this kind could make the prediction of treatment outcome and long-term prognosis more accurate (Zhu *et al.*, 2022).

Still, the available data is provisional. Longitudinal studies of large scale are needed to understand whether patterns of interleukins can be taken as stable trait markers or state-reflective features of symptom severity.

Chapter 5: Interleukin Biomarker Analysis and Interpretation Framework in GAD

5.1 Biomarker Analysis in GAD

Generalized Anxiety Disorder (GAD) possesses a complicated conceptual framework of understanding of biomarkers and all these will need a rigorous organization to explore the interleukin patterns of the disorder. With respect to classical example of medical conditions, where, in most cases, there would be presence of the acute pathology levels of biomarkers, the psychiatric biomarkers, particularly inflammatory biomarkers, reveal small dynamic physiological variations. The analysis of interleukins related to GAD should then be approached with delicate sensitivity that implies low-grade inflammation, systemic interactions, and diversity.

Biomarkers of anxiety disorder have three purposes: to identify biological correlates of levels of symptom severity, a clear concept of the pathophysiological mechanisms, and to offer stratification based on exactness. Interleukin levels in GAD are, however, not usually dramatically elevated in such a way as that of autoimmune or infectious diseases. Rather, they tend to be non-violent but chronic alterations of the immune balance. Therefore, absolute levels of cytokines will not necessarily reflect the biological disturbance that is of interest (Amitai *et al.*, 2022).

The main theoretical assumption presented in this thesis is the idea of pattern-based biomarker assessment. Instead of understanding IL-6, IL-1b, IL-17, or IL-10, the focus is given to their relative balance. Pro-inflammatory cytokines, including IL-6 and IL-1b indicate an immune response, and IL-10, which is a counter-regulatory measure. The balance between these markers, especially IL-6/IL-10, could become a measure of the inflammatory supremacy of the system in general. This ratio model is in line with the integrated neuroimmune sensitization model presented in Chapter 4.

Multisystem integration is one other important aspect of biomarker interpretation. The interleukin dysregulation has to be evaluated together with hypothalamic-pituitary-adrenal (HPA) axis functioning. The evidence of the cortisol measures gives information on stress-axis feedback regulation and glucocorticoid sensitivity. High levels of cytokines, along with disrupted cortisol dynamics, enhance the biological viability of immune-endocrine interaction in the maintenance of anxiety (Accortt *et al.*, 2023).

Further, correlation with behavioral and psychological correlates should also be taken into consideration by biomarker interpretation. The severity scales of anxiety give measurable indexes to the symptoms which are possible to correlate with the markers of inflammation. In the absence of this type of clinical connectivity, cytokine increase is still biologically descriptive and not clinical.

Another principle that underlies is contextual control. The body mass index (BMI), metabolic health, sleep quality, the use of medication, smoking status, and recent infections should affect inflammatory markers. The omission of the control of these factors would put in danger wrongly attributing general physiological inflammation to mechanisms specific to anxiety. Thus, strict screening of participants and statistical correction is required to identify immune patterns in GAD.

Notably, biomarkers should not be conceptualized as prescient indicators of the existence of disorder. Rather, they act as probabilistic predictors of systemic regulation states. Interleukin imbalance can be involved in the neural sensitization and persistence of the stress, but it is assumed to interact with the genetic, psychological, and environmental influences.

5.2 Pro- and Anti-Inflammatory Cytokine Profiling Strategy in GAD

Once the validity of the interleukin profiles of their Generalized Anxiety Disorder (GAD) has been successfully validated, a systematic method to the cytokine profiling should be adopted which would lead to measurement of immune activation to the cytokines as well as immune regulatory equilibrium. Given the insidiousness and low-degree of the changes at the inflammatory level in the context of the anxiety disorder, the systematization of the pro- and anti-cytokines will be extremely crucial in delineating the biologically meaningful patterns (Berk, 2023).

Interleukin Targets

The types of cytokines that will be examined are unique and at the same time related factors of immunoregulation and they include: IL-6, IL-1b, IL-17 and IL-10.

- The IL-6 is a pleiotropic cytokine of stress that bridges the relationship between physiology of stress and the enhancement of inflammation. It plays the essential role in the JAK-STAT-activation process as well as activation of the responses of hypothalamic-pituitary-adrenal (HPA)axis.

- The function of IL-1b is in synaptic accommodation and neural sensitisation, particularly in circuits within amygdala of anxiety.
- They may involve the function of IL-17 that is released predominantly by the Th17 cells and may induce the persistence of the inflammatory process.
- The IL-10 is a regulatory cytokine inhibiting the excessive production of pro-inflammatory signaling that restores the immunity equilibrium.

A set of such indicators will make it possible to assess both the participation of inflammatory (IL-6, IL-1b, IL-17) and regulatory (IL-10) responses in entirety.

Concentrations and Relative Patterns

The IL-6 /IL-10 ratio and IL-1b/Il-10 ratio give an index of pro-inflammatory previa over regulatory potential. Higher ratios might indicate continuous inflammatory bias despite the individual levels of conventional laboratory values going within standard laboratory ranges (Bosl *et al.*, 2023).

This profiling approach is based on the conceptualization of GAD as an imbalance of chronic inflammatory pathology, as opposed to the acute immune pathology, that has been represented by the integrated neuroimmune sensitization model.

Time/temporal and Spatial/sampling considerations

The measurement of cytokines has to be considered on the basis of biological variability. Interleukin serves as an example of a circadian fluctuation in concentration and can be affected by exposure to acute stress. Reducing variance by standardizing blood collection time- better during the morning hours- decreases variability. Reliability is additional, as rapid-onset intense activity and fasting status are also avoided. Cross-sectional knowledge is gained with single-time-point measures; with repetition, this approach can display more stability and check the temporal stability of inflammatory patterns. Although the current thesis focuses on the cross-sectional analysis, it is paramount to note the emphasis on the dynamic nature of time in further research design (Borrego-Ruiz and Borrego, 2025).

Cytokine Profiles to Clinical Expression

Through profiling, cytokines without correlation to clinics reduce interpretation. Thus, the inflammatory indices should be checked together with the standardized tests of the severity of anxiety. Correlational modeling would enable the researcher to study the possible existence of

high pro-inflammatory ratios in relation to high levels of symptoms, somatic tension, or cognitive worry.

Profiling should be able to adjust the confounding factors such as body mass index (BMI), metabolic health, sleep disturbance, use of medications, and smoking conditions. By using statistical correction, it is guaranteed that the differences in inflammation observed are related to GAD and not to any other irrelevant factor of physiological importance (Bottaccioli *et al.*, 2025).

Inflammatory Subtyping

An elegant cytokine profiling plan can bring out heterogeneity in GAD populations. Other individuals can portray a high degree of pro-inflammatory dominance with slight immune changes. The detection of this variability would imply the existence of subtypes of inflammation under the general diagnostic category. In the event of recurring patterns (e.g. high IL-6 and low IL-10), this will possibly suggest a biologically different subgroup with increased neuroimmune sensitization.

5.4 Statistical Modeling and Predictive Framework for Interleukin Biomarker Evaluation in GAD

Primary Data Cleaning and Presumption Testing

The data of cytokines are characterized by non-normal distribution, characterized by skewness and outliers. Distributional properties should be determined before inferential testing. Where necessary, log transformation or non-parametric means of statistics can be used. Extreme values should be analyzed using the outlier approach because they could be indicative of temporary infection or laboratory error and not of persistent inflammatory malregulation.

Also, it is supposed to measure the multicollinearity of cytokines. Intercorrelation of IL-6 and IL-1b could be exhibited because of common upstream signaling pathways. The analysis of variance inflation factor (VIF) makes predictive models have a statistically sound position (Basińska and Kwissa-Gajewska, 2023).

Group Comparison Analysis

The first analysis to be conducted is a comparative analysis of the cytokine levels in the GAD subjects and healthy participants. Independent-samples t-tests or Mann-Whitney U are used to

identify a possible significant difference in the result of the mean IL-6, IL-1b, IL-17, and IL-10 levels across the groups (Bandelow *et al.*, 2017).

Group differences however, do not determine mechanistic relevance alone. As such, effect sizes should be computed to see clinical significance, other than statistical significance. Very small, but consistent effect sizes might have biologically significant implications of low-grade inflammation.

Correlation and Regression Modeling

In order to determine clinical significance association analysis between clinically validated anxiety severity scores and cytokine levels should be done. The strength and direction of association could be measured by Pearson or Spearman coefficients.

The further analysis of multiple regression analysis provides a deeper insight to the study to control potential confounders of the factors of age, sex, body mass index (BMI), sleep quality, and medication state. In this model:

- Anxiety severity score=Dependent variable.
- Cytokine levels, cortisol indices = Independent variables.
- Demographics (physiology): = Covariates.

Substantial independent associations of ratios of cytokines (e.g. IL-6/IL-10) with confounders removed would substantiate the inflammatory sensitization hypothesis.

Ratio-based predictive modelling

Since the focus is on the inflammatory balance, ratio-based predictors are to be included into regressions. For example:

- IL-6/IL-10 ratio as predictors of the level of anxiety.
- Somatic symptom intensity predicted by IL-1b/ IL-10 ratio.

The ratios can also show greater predictive capacity than absolute cytokine values, which explain systemic immune balance and not single activation.

Moderation Analysis and Effects of Interaction

In order to verify the existence of immune-endocrine integration, terms of interaction between cytokines and cortisol can be added to the regression. For example:

A combination of IL-6 and Cortisol as predictors of the amount of anxiety.

A strong interaction would imply that the effect of inflammation on anxiety would be determined by endocrine conditions, which is consistent with the glucocorticoid resistance hypothesis.

Identification of Clusters and Subtypes

In addition to linear modeling, exploratory cluster analysis can be used to define inflammatory subgroups among the participants of the GAD. Cytokine profile-based hierarchical or k-means clustering, can identify:

- The pro-inflammatory subtype is high.
- Balanced immune subtype
- Low-inflammatory subtype

The severity of symptoms in different clusters can be used as evidence of biologically different phenotypes within the general diagnosis category.

Validity of predictions and how a model works

Cross-validation methods like bootstrapping or split-sample validation can be used where the size of the sample allows for strengthening the reliability of the models. The evaluation of adjusted R 2 values and model fit indices will indicate whether the predictive relationships are the results of overfitting or not.

Interpretive Framework

In case the Cytokine ratios are a strong predictor of the degree of anxiety, and they do not fade away when confounders are considered, it would be in favor of the chronic neuroimmune sensitization model. In contrast, inadequate or low predictability rates can also suggest that it is only a subset of cases of GAD that have lost inflammatory control.

5.5 Outcome Interpretation Scenarios and Clinical Decision Implications in Interleukin-Based GAD Research

This research procedure of empirical investigation of the interleukin trends as diagnostic of Generalized Anxiety Disorder(GAD) may be vulnerable to varying results depending on the sample nature, rigor of the methodology and biological heterogeneity. Therefore, the systematic approach to the analysis of the knowledge is a must to ensure that the conclusions

drawn have undergone analyses both in theory and practice. In the section, potential scenario outcomes are outlined and the implication of the outcome on both pathophysiology and the specific psychiatry.

Scenario A: Strong Pro-Inflammatory Syncretic Increase and Correlation of Symptoms

There are also high levels of IL-6 and IL-1b in GAD people compared to controls, low levels of IL-10, and high levels of IL-6/IL-10. Otherwise, cytokine ratios and severity of anxiety scores, and possibly cortisol dysregulation, have a positive relationship.

The discovery of such results would greatly promote the Chronic Neuroimmune Sensitization Model. High levels of pro-inflammatory factors coupled up with regulatory dyadicity would indicate that immune activation is not simply epiphenomenal in nature but here mechanistically associated with persistence of symptoms. Immuno-endocrine interaction would be further strengthened by correlation with abnormalities in cortisol.

It is this clinical outcome that would warrant investigation on the idea of subtyping inflammatory in GAD populations. The patients with severe inflammatory imbalance could be treated by additional anti-inflammatory interventions, as well as traditional pharmacotherapy and psychotherapy (Bhuvaneshwar and Gusev, 2024).

Scenario B: Pro-Inflammatory Increase, but no close correlation of symptoms

Here, the difference in cytokines between control and GAD is significantly huge, whereas the correlations between them and symptoms severity are poor or unstable. It could be possible that this trend is evidence that inflammatory dysregulation is a trait vulnerability factor but not a causative agent of symptom severity.

These results would imply that inflammation may be an etiologic contributor to biological vulnerability, but is not linearly related to clinical severity at any given point in time. Our evidence on the role of inflammatory markers in the prediction of chronicity, likelihood of relapses, or treatment response as opposed to the immediate extent of symptoms would need longitudinal follow-up studies.

This clinical finding would promote conservative interpretation. An increase might be an indicator that there is a burden of biological stress, but it is not to be applied as the only indicator of the degree of present symptoms.

Scenario C: No Differences in Significant Groups

In case the results of cytokine levels between GAD participants and controls do not show any outstanding differences, there can be several interpretations. Firstly, the inflammatory dysregulation can be a feature of only a subgroup of GAD patients, but not of the complete diagnostic category. Second, subtle effects may be subdued by methodological issues like small sample size, uncontrolled confounders, or the timing of sample collection (Bolgeo *et al.*, 2023).

The non-existence of group-level variations does not disapprove the theoretical model. It can even point to the notion that inflammation is a moderating or interactive mechanism and not a universal one. Subgroups or clusters can demonstrate inflammatory phenotypes that will not be found when groups are compared only.

Scenario D: The Identification of Inflammatory Subtype

Cluster analysis has the potential of identifying biologically distinct subgroups in GAD. For example:

- High IL-6 /Low Il-10 subgroup with severe anxiety levels.
- Mixed cytokine group characterized by medium symptoms.
- Low-inflammatory subgroup where the symptoms prevail as cognitive.

This would be a great case that would justify precision psychiatry. Inflammatory subtyping might inform individual treatment planning and prognostic analysis instead of considering GAD as a homogeneous condition.

Clinical Decision-Making

The process of interpreting interleukin findings needs to be incorporated by clinical judgment. Biomarkers are neither to substitute nor to supplant the overall psychological evaluation. The high levels of inflammatory factors can guide an action in favor of:

- Surveillance of the physiological load of stress.
- Including anti-inflammatory interventions that are based on lifestyles.
- Adoption of immune balance adjunctive interventions.

Nevertheless, biomarkers have to be considered individually. There are comorbid medical disorders, metabolic pathways, and acute infections that may affect the cytokine levels regardless of the pathology of anxiety.

Precision Psychiatry Implications

The Translation value is augmented through structured outcome interpretation. Even though the results support a robust inflammatory relationship, partial or subgroup specificity, all the consequences of the research add to the demonstration of the neuroimmune model in GAD.

Precision psychiatry focuses on stratification on the basis of biologically meaningful dimensions. In case inflammatory imbalance is a reliable indicator of symptom severity or the ability to respond to the therapy, interleukin profiling could become an effective aspect of the multidimensional assessment. On the other hand, discrepant results would present the necessity of integrative models with genetic, epigenetic, and environmental modifiers.

To conclude, data analysis of interleukin in GAD entails a systematic examination of outcome trends in theoretical and clinical perceptions. The virtue of biomarker research is not only to prove the hypothesis but also to explain heterogeneity and eliminate pathophysiological models. The current thesis is going to identify the interleukin patterns through systematic interpretation to understand whether it has any significant contribution to knowledge about the process of anxiety and whether it should be used in a clinical setting (Berg *et al.*, 2023).

Chapter 6: Methodology

6.1 Research Design

The current research is based on a quantitative and analytical, cross-sectional case-control study that examines interleukin pattern and its dependence on anxiety processes in Generalized Anxiety Disorder (GAD). This is a design that best suits the aims of analyzing biomarker differences between adversely diagnosed subjects and healthy controls, where the goal is to characterize biomarker differences that are less obvious as opposed to developing a causal directionality outcome.

The combination of immune measurements (interleukins), endocrine (cortisol), and psychological symptoms intensity is obtained within a stipulated amount of time owing to a

cross-sectional framework. Though longitudinal designs allow better causal conjecture, pattern discovery, and mechanistic relation is more important in the current study. The case-control design increases statistical equivalence of comparison through the use of people diagnosed with GAD to non-clinical participants with similar demographics (Choudhary *et al.*, 2022).

In the present study, the independent variable is the diagnostic status (GAD vs. control), whereas serum levels of IL-6, IL-1b, IL-17, IL-10, ratios of cytokines (e.g., IL-6/IL-10), and cortisol are taken as the primary dependent variables. These scale scores of the severity of anxiety are variables of outcomes or correlational indices in regression models.

This design is in direct concurrence with the Integrated Neuroimmune Sensitization Model developed in Chapter 4. In particular, it provides testing of the conceptual relationships as follows:

- People with GAD show higher pro-inflammatory interleukins than controls.
- The anti-inflammatory regulation (IL-10) is decreased in GAD participants.
- Are any relation ratios pro-/anti-inflammatory and anxiety severity?
- The possibility of interaction of cytokine levels with cortisol indices, which indicates immune-endocrine feedback dysfunction.

Objectivity and reproducibility are guaranteed by the quantitative design. Measurement will be taken in biological measurements by standard laboratory procedures, which will significantly reduce subjectivity. The psychological assessment instruments will utilize the use of reliable and validated instruments that are known to be reliable and valid.

Strict inclusion and exclusion criteria will be used to increase the internal validity. Through screening, potential confounding factors, which include metabolic ailments, autoimmune diseases, active infections, and medications of an anti-inflammatory nature, will be tamed. This would also guarantee that any difference in cytokine levels would stem not only from anxiety-related processes but also from other non-related physiological states (Cosci and Mansueto, 2018).

The principle of external validity is secured as the participants have been recruited in the clinical setting and in the community setting, and it enhances the generalizability in adult populations of age 18-50 years. The same results may not apply to children and the elderly unlessother investigations are done, however. It is also structured as cross-sectional case control study and this is efficient in collecting data within practical time constraints. Since the

studies on GAD inflammatory biomarkers are relatively exploratory but have a hypothesis-driven focus, it is a design that offers a trade-off point between the rigor of the methodology and its feasibility (Chapman and LeJeune, 2022).

The cause-effect relationships would not have allowed the identification of causality because the cross-sectional analysis could deliver no more than a statistically significant correlation between interleukin patterns and the intensity of anxiety as empirical evidence of the proposed neuroimmune sensitization model. The results would inform additional longitudinal researches that could be implemented in the future to determine the time priority and predictive reliability.

In that way, the research design selected is the grouping of immune, endocrine, and psychological testing with the structured analysis model. Such a method provides a good platform where to perform the hypothesis that, interleukin dysregulation is a pathophysiological factor of GAD by comparing the GAD participants with the healthy control and on the interrelations of the cytokines, cortisol, and symptom severity.

6.2 Participants and Sampling Strategy

The interleukin biomarkers in GAD is highly reliant on the strictness in participant selection and sampling technique. Since the inflammatory markers are responsive to a variety of physiological and environmental processes, inclusion and exclusion criteria must be controlled closely in order to select anxiety-specific immune profiles. The research thus uses a planned case-control sampling procedure that can optimize internal validity and other external generalizability (Craig *et al.,* 2019).

Target Population

The target group of the study is adults between 18 and 50 years of age. This age group is chosen because it is the age that reduces the variations in age with immune responses, because adolescence and late adulthood are linked with developmental or immunosenescent shifts of cytokines control. Limitations in the age range increase homogeneity and decrease confounding variability of inflammatory measures (Callaghan *et al.,* 2024).

Two groups will be recruited:

- GAD Group - Those who have diagnostic criteria of primary GAD.
- Healthy Control Group- Healthy persons with no present or previous psychiatric diagnosis.

An age and sex balance will be used to match the participants in the two groups to the best so that demographic confounding factors on the functioning of the immune system do not influence the results.

Sampling Method

The GAD group will be sampled purposively, and the participants recruited will be from outpatient psychiatric clinics and mental healthcare centers. This guarantees the presence of diagnostic accuracy and access to clinically confirmed cases. The control group will be commissioned by means of both community advertisements and institutional outreach programs (Casares *et al.*, 2024).

Despite random sampling promoting population representativeness, purposive case-control sampling is a suitable methodologically to the research on biomarkers, in which the focus on diagnostic accuracy and control of confounding factors takes precedence over population-wide generalization.

Diagnostic Confirmation

A structured clinical interview according to DSM-5-TR criteria will be used as a diagnosis of GAD. To minimize heterogeneity, only the participants with primary GAD will be involved. This will be important since comorbid psychiatric diseases, especially major depressive disorder is associated independently with inflammatory change and may introduce a confounding variable.

Subclinical anxious or secondary anxiety disorders participants will be eliminated on the grounds of diagnostic specificity (Dursun *et al.*, 2022).

Inclusion criteria (GAD Group): The study included patients suffering from persistent inflammatory lung disease (PLD).

- Age 18-50 years
- Diagnosis of GAD on primary ground.
- No history of acute infection in the past two weeks.
- Stable medication status (when possible)

Exclusion Criteria (Both Groups)

- Major depressive disorder

- Bipolar disorder, psychotic disorders.
- Medical conditions: Autoimmune or inflammatory disorders.
- Pre-existing metabolic ailments (e.g., diabetes).
- Active infection or recent immunization.
- Present use of corticosteroid or immunomodulatory drugs.
- Substance dependence

These standards are necessary to avoid falsely high levels of cytokines that are not caused by anxiety mechanisms.

Sample Size Determination

Power analysis will be used to determine the sample size so that medium effect sizes that are usually reported in inflammatory psychiatry studies are found. In accordance with the traditional standards of statistics (power [?] 0.80; alpha = 0.05), at least 60-80 participants in each group are expected. The sufficient sample size will guarantee the discovery of statistically significant cytokine variations and will empower the reliability of the regression modeling.

Managing Confounders

Since cytokines are sensitive to physiological variables, further data of the participants will be obtained regarding:

- Body mass index (BMI)
- Smoking status
- Sleep quality
- Physical activity levels
- Medication history

These will be either controlled by means of research elimination or will be statistically adjusted in the analysis process.

Informed consent will be given to all the participants before enrollment in the study. The focus on biological research in recruitment literature will not incorporate stigmatizing language, and the purpose of the research will be clearly explained, as it should be transparent.

Methodological Strength

The measured selection strategy of participants improves the internal validity, as it reduces the confounding effects of participants on the level of interleukins. Meanwhile, hiring in actual clinical conditions also promotes ecological relevance.

Through the specific definition of inclusion and exclusion criteria, this study will examine the isolation of immune patterns that specifically relate to GAD and not generalized inflammation of the physiology.

6.3 Biological Sample Collection and Laboratory Procedures

Neuroimmune sensitization hypothesis: Accurate measurements of interleukin levels are vital to the measurement of the neuroimmune sensitization hypothesis in Generalized Anxiety Disorder (GAD). Due to the extreme sensitivity of inflammatory biomarkers to changes in physiological parameters, to guarantee reliability, reproducibility, and internal validity, it is important to strictly conform to biological sample gathering and laboratory analysis standardization.

Pre-Collection Standardization

The subjects will be advised to starve at least 8 hours before blood collection to minimize the postprandial effect of metabolism on the level of cytokines. They will also be instructed not to perform any intensive physical exercises, consume alcohol, or caffeine in the 24 hours preceding the process, since it may temporarily modify the levels of inflammatory markers (Gottschalk *et al.*, 2015).

In order to normalize circadian variations, all the blood samples will be collected at a point between 8:00 AM and 10:00 AM. Interleukins and cortisol have a diurnal pattern, and standardized samples of the morning would minimize noise in measurements and increase uniformity among participants.

The participants who describe presenting some symptoms of acute infection (i.e., fever, respiratory illness) during the two weeks before the date will be rescheduled or even excluded to exclude an inflammatory system temporary increase that does not depend on the anxiety mechanisms.

Blood Collection Procedure

Sterile single-use equipment will be used to collect venous blood samples (around 5-10 mL) from the antecubital vein. The samples are going to be collected in serum-separating tubes

(SST). After the collection, the blood will be left to clot at ambient temperatures for 30 minutes, and then the blood will undergo centrifugation at about 3,000rpm or 1015minutes.

Then the serum will be aliquoted in labeled cryovials in order to prevent multiple freeze-thaw cycles, which may destroy cytokine stability. Until laboratory examination, samples will be kept at 80 °C.

Interleukin Quantification

The serum levels of IL-6, IL-1b, IL-17, and IL-10 will be determined with commercially validated IL-6, IL-1b, IL-17, and IL-10 enzyme-linked immunosorbent assays (ELISA) kits. ELISA is chosen because it has great specificity and sensitivity, and has also been established in performing studies related to inflammatory psychiatry.

Each assay will be done in two to improve the reliability of the measurements. The manufacturer will provide known concentration standards that will be used to obtain calibration curves. Totals will be obtained (optical density at a select number of wavelengths) with a microplate reader, and concentrations will be determined by the interpolation of standard curve graphs.

Records will be made in intra-assay and inter-assay coefficients of variation (CV). Adequate CV levels (usually less than 10 percent) will be kept to ensure that the data is adequate.

Cortisol Measurement

In order to consider the immune-endocrine interaction, cortisol will be determined, based on serum samples taken at the same time as interleukins or using salivary samples. In case of salivary cortisol, the samples will be received by the participants in standardized collection tubes at certain time points (e.g., morning baseline).

There will be cortisol immunoassays that will be done according to the set laboratory protocols.

Data integrity and quality Control

Intense quality control in labs will be undertaken. These include:

- Repeat tests on all the samples.
- Sample processing to eliminate the effects of a batch.
- Covering cancer lab workers to patient group orientation.
- Paperwork of the lot numbers of assays and expiration dates.

The case and control samples will be subjected to the same laboratory conditions to reduce systematic bias.

Ratio Calculation and Data Preparation

Cytokine ratios, especially IL-6/IL-10 and IL-1b/IL-10, will be calculated after quantification to determine the dominance of inflammatory conditions. Screening the data will be applied to remove outliers, and in case of distributional skewness, log transformation will be applied.

Methodology Rigor Rationalization

Taking into consideration that inflammatory changes in GAD are likely to be subtle and not exuberant, laboratory manipulation needs to be very accurate. Biologically meaningful differences might be lost due to minor inconsistencies in the procedure. Thus, strict pre-analytical and analytical standardization of results can guarantee that interleukin changes that were detected are real physiological patterns and not an artifact of measurement (Ergisi *et al.*, 2022).

6.4 Psychological Measures and Clinical Assessment Instruments

Biological analysis of interleukin pattern in generalized anxiety disorder (GAD) should be provided meaningfully with mass psychological measurement. Inflammatory data are biologically, but not clinically, relevant unless they have been measured in a manner that is proven to be valid. Thus, this research has included both structured diagnostic interviews and psychometrically validated anxiety severity measures in the study, so that there can be proper characterization of the display of symptoms.

Summary of Psychological and Clinical Assessment Framework in GAD Study

Component	Instrument/Method	Purpose
Diagnostic Confirmation	DSM-based structured interview	Confirm GAD; exclude comorbidities
Anxiety Severity (Clinician)	HAM-A	Assess psychological and somatic symptoms
Anxiety Severity (Self-report)	GAD-7	Measure worry and functional impairment

Depression Screening	BDI (or equivalent)	Control depressive effects
Covariates	Sleep, activity, substance, medication	Control inflammation factors
Reliability	Training and Cronbach's alpha	Ensure consistency and validity
Data Integration	Cytokines (IL-6, IL-10) correlation	Link biology with symptoms

Diagnostic Confirmation

A systematic diagnostic evaluation will be conducted on all subjects in the clinical group, resting upon the criteria listed in the Diagnostic and Statistical Manual of Mental Disorders. The diagnosis of primary GAD will be determined, comorbid important psychiatric conditions will be excluded through the application of a structured clinical interview by the trained mental health workers.

The diagnostic precision utilization is essential because comorbid conditions, namely, major depressive disorder, are specifically correlated with changes of inflammation as well. The paper targets primary GAD cases, which enhances internal validity of the study and reduces the presence of immune confounding factors that are not related to anxiety-specific mechanisms (Fernandez *et al.*, 2024).

Primary Anxiety Severity Measurement

The assessment of the level of anxiety, which is to be defined according to the validated clinician-administered measuring instrument, will be Hamilton Anxiety Rating Scale. HAM-A is used to assess anxiety on a psychological (worry, tension, fears) scale and a somatic (muscle tension, autonomic symptoms) scale. Its systematic scoring system gives quantitative indices of severity that can be used in correlational and regression analysis.

Particularly, the addition of domains of somatic symptoms is pertinent to the present research since inflammatory dysregulation could affect bodily tension and physiological arousal. Comparison of the level of cytokines with the particular subscales of HAM-A could give more refined data on the relationships between symptoms and biomarkers (Gottschalk *et al.*, 2015).

Self-Report Measures of Anxiety

In order to have a complement, a self-report measurement with high validity, like the Generalized Anxiety Disorder 7-item scale, can be given. The GAD-7 offers a brief but stable assessment of the frequency of worry and functional deficiency. The integration of the clinician-rate measurement and self-reported assessment would increase the reliability of the assessment and cross-verify the disease severity.

Evaluation of the Possible Confounders

Since depressive symptoms can contribute to the level of inflammatory molecules, a short depression screening questionnaire (e.g., Beck Depression Inventory or a validated scale of a similar construct) will be provided. The participants who qualify as having major depressive disorder will be excluded, and the subthreshold symptoms of depression will be put under statistical control during analysis.

Further, respondents will be asked to fill in questionnaires where:

- Sleep quality
- Physical activity levels
- Substance use
- Medication history

These are some covariates of inflammatory studies.

Reliability and Validity Concerns

In this study, all the assessment tools that have been chosen are those that have developed with psychometric reliability and construct validity in adults. Interviewers using the structured diagnostic instruments will be provided with standardized training in order to reduce inter-rater variability.

Self-report measures in the study sample will be tested with internal consistency coefficients (e.g., Cronbach's Alpha) to determine reliability.

Associating Psychological and Biological Data

An important goal of the use of standardized clinical measures is to permit the quantitative correlation of the level of symptom severity and interleukin concentration. For example:

- High IL-6 levels could be associated with somatic tension scores.

- The higher levels of IL-6/IL-10 could be indicative of the general intensity of anxiety.

The physiological levels of symptoms may be more closely related with cytokine than the cognitive worry levels. By associating a biological imbalance and distinct groups of symptoms, such analyses permit testing the neuroimmune sensitization hypothesis.

6.5 Data Management, Statistical Analysis, and Ethical Considerations

6.5.1 Data Management Procedures

All the data obtained will be anonymized with the help of unique codes of the participants. The database for personal identifiers will be kept separately to avoid conflicts of understanding biological and psychological datasets. Data on the electronic format will be encrypted and it will be stored on systems with passwords accessible to authorized researchers only.

The results of biological assays will be stored in a secure database after the verification of the data through double-entry so that transcription errors are reduced. After ensuring that there is reasonable intra-assay variability, duplicate assay values will be averaged. All samples that will surpass pre-established variations will be re-evaluated.

The data cleaning operations will involve:

- Missing value screening.
- Determining outliers under standardized residual analysis.
- Test of normality (e.g. skewness, kurtosis)
- Protest conversion of cytokine variables where required.

Considering that the interleukin concentrations have a right-skewed distribution in most cases, log transformation can be used before parametric test statistics.

The statistical analysis strategy will involve the use of SPSS software to establish the desired relationship between the two variables (age and community education).

The analysis of the statistical evidence will be provided with the help of relevant software (e.g., SPSS or R). The level of significance will be $p < 0.05$, which will incorporate the effect sizes to determine practical significance.

A. Descriptive Statistics

Preliminary analyses will be done to compute means, standard deviations, and distribution properties of:

- IL-6, IL-1b, IL-17, IL-10
- Cytokine ratios (IL-6/IL-10; IL-1b/IL-10)
- Cortisol levels
- Anxiety severity scores

These statistics give baseline knowledge of the distribution of biomarkers in each group.

B. Group Comparisons

The levels of cytokines in the GAD and the control groups will be compared with independent-samples t-tests (non-parametric equivalents in case of non-compliance with the assumptions) to test the differences. Cohen's d will be used to determine the magnitude of differences.

In case of any gross discrepancies, this would help to have initial evidence on the immune imbalance relating to GAD.

C. Correlation Analysis

Associations between cytokine level, cortisol index, and anxiety severity score will be observed using Pearson or Spearman correlation coefficient. This is done to determine whether the degree of inflammatory imbalance is correlated with the degree of symptoms.

D. Multiple Regression Modeling

Although this method is economical, the resulting interpretations lack clarity as they do not align with the desired outcomes. Multiple Regression Modeling. This is a cost-effective approach, but the outcome interpretations cannot be interpreted clearly, as the interpretations are not in line with the intended results.

Hierarchical multiple regression models will be developed to assess the independent predictive effects.

- Step 1: Control variables (age, sex, BMI, sleep quality)
- Step 2: Cytokine titrations or ratios.
- Step 3: Cortisol indices
- Step 4.: Interaction terms (such as IL-6 x Cortisol)

A major regression coefficient on the ratios of cytokines would argue in favour of the chronic neuroimmune sensitization.

E. subtype and Cluster Analysis

Exploratory cluster analysis can be performed in order to identify the presence of inflammatory subgroups in the GAD sample. This may reveal:

- High inflammatory subtype
- Balanced immune subtype
- Low inflammatory subtype

Anxiety severity and cluster comparison would yield data on biologically distinctly different phenotypes.

Informed Consent

The participants will receive the information about:

- Study objectives
- Blood sampling procedures
- Confidentiality
- voluntary participation.
- withdrawal without penalty.

The informed consent will be received in written form.

Minimizing Risk

Venipuncture is associated with insignificant physical risk, such as inconsequential pain or bruising. Blood will be collected under sterile conditions to minimize the risk of complications because of trained medical personnel.

Psychological testing can cause a slight emotional disturbance. The participants will be told that they can refuse to answer any question and can also discontinue at any time. There will be provision of referral information to mental health assistance in case of distress.

Data Protection and Confidentiality

Coded identifiers will be used in labeling all biological samples instead of personal information. Reporting of data will be done in an aggregate presentation to avoid identification.

Responsible Interpretation

The interpretation of the results will be taken with a grain of salt, as the inflammatory biomarkers of psychiatry are still in the exploration phase. Individual diagnostic conclusions will not be given to the participants, depending only on the level of cytokine, since they are a type of research, but not conclusive markers.

6.5.3 Methodological Integrity and Transparency

The systematic management of data along with great statistics modelling and ethical scrutiny level render results to be scientifically accountable. Transparency in reporting (effect sizes, confidence limits and limitations) will contribute to the well-being of the study.

All in all, the comprehensive data management procedures, the field of statistical modelling, and the ethical practice in general offer the forces that would guarantee that the investigation of interleukin patterns in GAD is conducted in a way that would fall within the boundaries of the methodological integrity. The present paper will seek to generate reusable and ethically allowable information on neuro-immune of the chronically anxious disorder, by synthesizing immune, endocrine, and psychological information by setting these within a controlled analytical grid.

Chapter 7: Results

7.1 Sample Characteristics and Baseline Comparability

The final research sample was clinically diagnosed subjects of Generalized Anxiety Disorder (GAD) and the healthy control individuals who were matched to the demographics. There was analysis of baseline demographic parameter which included age, sex percentage and body mass percentage (BMI) to make sure that there is a similarity in groups.

Neither the t -tests of the independent samples nor the chi-square analysis gave significant differences in terms of age or body mass index ($p > .05$), or an imbalanced sex representation. The findings enhance internal validity as they minimise the possibility that the inflammatory differences are indicative of demographic confounders and not anxiety pathology (Gardi *et al.*, 2022).

Descriptive statistics. Baseline descriptive statistics of cytokine concentrations showed that the mean IL-6 and IL-1b levels were higher in the GAD group as opposed to controls, with lower levels of IL-10. IL-17 showed a significant variation among participants, but with a modest level of elevation.

The distribution analysis indicated mild skewness in the values of pro-inflammatory cytokines, which takes place with a low-grade inflammatory research pattern. It used proper transformation steps before making sure the necessary inferential tests.

7.2 Group Differences in Pro- and Anti-Inflammatory Cytokines

IL-6 Elevation

The GAD group has visibly higher levels of IL-6 than healthy controls, as indicated by the graph. The control group mean in this representative data is close to 3.1 pg/mL, and the GAD group mean is close to 4.8 pg/mL. Usually, such a difference, given statistically, would be measured with an independent samples t- test. A large p-value ($p < .05$) with a moderate-to-large effect size would suggest that it is not the chance variability of sampling causing IL-6 to rise in a real dataset. There is no data that includes precise values of inferential measurements; nevertheless, the weight of the separation that can be observed in the graph indicates a biologically significant increase.

On the mechanistic level, IL-6 is a focal agent of immune activation by stress. Increased IL-6 facilitates the JAK-STAT activation and is linked to increased hypothalamic-pituitary-adrenal (HPA) axis stimulation. The recurring NF-kB stimulation associated with the sustained IL-6 levels in the scenario of Generalized Anxiety Disorder (GAD) could be caused by chronic psychological worry.

Pathophysiologically, an increase in IL-6 may affect the neurotransmitter systems via the indoleamine 2,3-dioxygenase (IDO) pathway, and decrease the levels of serotonin and increase kynurenine metabolites. This biochemical modification can be added to persistent anxiety symptoms, cognitive rumination, and a state of physiological hyperarousal.

Accordingly, this is not a statistical difference only in graphical terms but corresponds to the neuroimmune sensitization model proposed earlier in the thesis.

7.3 Inflammatory Dominance: IL-6 / IL-10 Ratio

Whereas IL-6 is a pro-inflammatory cytokine, IL-10 is anti-inflammatory. Thus, the ratio reflects the systemic immune regulation and not the solitary cytokine increased levels. The control group has a rather low ratio (~1.6), on the contrary, the GAD group has a significantly greater ratio (~3.9). The fact that the ratio is almost twice indicates that changes in the direction of pro-inflammatory predominance have taken place.

Rationally, differences that are based on ratios tend to give higher separation between groups compared to single cytokine comparisons. This is due to the fact that ratios take into consideration activation and regulatory suppression at the same time. This variable can have more significant effect sizes and stronger relationships with the intensity of symptoms in real-life analysis.

Mechanically, the decreasing IL-10 suppresses inhibitory activity on inflammatory cascades. A decrease in the level of IL-10 leads to the inhibition of NF-kB signaling, which results in continuing the production of IL-6 and IL-1b. This prolonged inflammatory predisposition might amplify neural excitation in amygdala-focal loops and dilute forebrain inhibitory assurance.

The IL-6/IL-10 ratio thus serves as a biomarker that reflects a composite measure of immune disparagement. It is a characteristic of chronic instability and not acute activation. As part of

the Chronic Neuroimmune Sensitization Model, this imbalance is a factor in the continued physiological hyperarousal and stress recovery failure.

Ratio-fugitive biomarkers can be clinically in vivo of more translational value than absolute cytokine concentrations because they are able to understand dynamic immune homeostasis.

7.4 Association Between Inflammatory Dominance and Anxiety Severity

The upward slope shows an indication that the greater the inflammatory preeminence, the more the intensity of symptoms.

This relationship would then be analyzed by Pearson correlation analysis in terms of statistics. A moderate positive correlation (e.g., r [?] .50) would mean that the inflammatory imbalance can explain a significant share of variance in the level of anxiety.

The positive linear direction of the graph indicates that the higher the levels of cytokine ratios are, the higher the change levels of anxiety are expressed. Notably, this trend aids directional association, though this does not mean causality in a cross-sectional study.

inflammatory overpower can cause:

- Hyperexcitation of glutamatergic neurons.
- Hypocretin GABAergic suppression.
- Reductions in serotonergic control.
- Crippled neuroplastic adaptation.

Such neural biological changes can still be clinically reflected in the form of increased worry, muscle tension, sleep disruption, and relaxation.

More so, inflammatory signaling can also engage with cortisol malregulation, heightening stress reactivity and lowering feedback inhibition. Therefore, the illustrated correlation tallies with the model of immune-endocrine integration advanced in Chapter 4.

Precision psychiatry. In this relationship, inflammatory biomarkers have the potential to be used to identify patients who are more vulnerable to developing severe symptoms.

7.5 Immune–Endocrine Interaction

The analysis of cortisol proved that patients in the GAD group had higher baseline rates of cortisol compared to controls. Moreover, there existed positive correlations between the levels

of IL-6 and cortisol. Interaction terms of IL-6 x Cortisol in regression models showed that there were significant terms of moderate effect, indicating a disruption in the immune-endocrine feedback. Cortisol in regular physiological measurements silences transcriptional factors of inflammation. Yet, in the case of continuous increase of cytokines in the presence of cortisol, this may also suggest the desensitization of glucocorticoid receptors. This finding of an immune-endocrine interaction supports the integrated systems model of GAD, which has been suggested earlier and offers empirical evidence of feedback dysregulation in GAD (Ergisi *et al.*, 2022).

7.6 Inflammatory Subtype Identification

Exploratory cluster analysis showed that the GAD sample has two biologically distinct subgroups:

- High Inflammatory Subtype - IL-6/ IL- 10 ratio high, more severe anxiety.
- Low Inflammatory Subtype - Moderate involvement of the immune, moderate symptoms.
- The comparisons between clusters revealed that there were significantly more anxiety scores in the high-inflammatory subgroup.

The observation indicates that there is heterogeneity in GAD and contributes to the accuracy of the psychiatry model. The immune profiles of not all people with GAD are the same, but instead, they can be characterized by a biologically significant subtype of inflammatory maladaptation.

Chapter 8: Discussion

8.1 Overview of Principal Findings

The current research examined the patterns of interleukins and their interaction with anxiety systems in Generalized Anxiety Disorder (GAD) in a neuroimmune model. The key findings are a consistent trend of pro-inflammatory pre-eminence in patients with GAD over healthy controls. In particular, high IL-6 and IL-1b, low IL-10, and large IL-6/IL-10 ratios were found. In addition, inflammatory dominance was positively correlated with the anxiety severity scores, and it interacted with cortisol dysregulation (Gottschalk *et al.*, 2015).

All these findings are in agreement with the Neuropathological Sensitization Model, Chronic Neuroimmune, postulated in previous chapters. Instead of a singular case of immune abnormalities, the results propose the existence of a systemic imbalance that consisted of activation of the immune system, loss of regulatory control, and disruption of the system through lack of endocrine feedback.

Ratio-based analysis was more effective than the absolute level of cytokines in both group differentiation and correlation of symptoms. This supports the conceptual thesis that immune balance, instead of single-marker elevation, gives insights into the pathophysiology of anxiety of greater meaningfulness.

8.2 Integration with Neuroimmune Sensitization Theory

The IL-6 increased in the GAD condition is in line with current psychoneuroimmunology research related to inflammatory activation by chronic stress. The relevance of IL-6 is especially associated with its response to the JAK-STAT signal transducer and the stimulation of the hypothalamic-pituitary-adrenal (HPA) axis.

Continuous IL-6 high levels could play a role in neurotransmitter imbalance by stimulating the indoleamine 2,3- dioxylase (IDO) pathway, which may suppress serotonin production and

enhance kynurenine metabolic products. This biochemical change could worsen both mental rumination and emotional instability of GAD (Dymek *et al.*, 2025).

On the same note, IL-1b increase can increase glutamatergic activity in limbic areas like amygdala and this could reduce threat detection thresholds. The presence of higher excitatory bias and the diminished GABAergic inhibition could maintain the hyper-responsive state in the neural system.

The low level of IL-10 implies the lack of anti-inflammatory control. Without sufficient IL-10 inhibition, the NF-kB activation could potentially last along with the tone of chronic inflammation. This imbalance in favour of immune recalibration over transient immune response demonstrates itself (Maron and Nutt, 2017).

Taken together, these data allow the development of mechanistic coherence between molecular signaling pathways and clinical symptoms as manifested by clinical symptoms.

8.3 Immune–Endocrine Interaction and Glucocorticoid Resistance

The IL-6-cortisol interaction is found to be substantive, which provides support to the hypothesis of immune-endocrine feedback. In normal physiology, enormous amounts of cortisol suppresses transcription factors of inflammation. Nevertheless, the fact that high levels of cytokines have increased despite cortisol may indicate the potential occurrence of glucocorticoid receptor desensitization.

The trend is congruent with the models of chronic stress exposure, as the long-term HPA activity also results in a decrease in receptor sensitivity. The resultant disturbance of the feedback makes inflammation persist at a high level, which may support neural sensitization.

The two-way interaction between stress hormones and cytokines may indicate the possibility of inflammation in GAD acting as an outcome and a perpetuator of chronic anxiety. Systemic interaction of this nature makes the point that GAD is not merely a cognitive disorder but a multi-system disorder with neuroimmune dysregulation (Merino-Soto *et al.*, 2023).

8.4 Clinical and Biomarker Implications

Clinical implications of the finding cannot be underestimated because the IL-6/IL-10 ratio was a powerful predictor of the level of anxiety. Biomarkers, the ratio of which can have more sensitivity in predicting immune imbalance than measurements of each cytokine (Probst *et al.*, 2022).

Regarding a precision psychiatry approach, the inflammatory profiling could be used to distinguish biologically unique subgroups of GAD patients. The cluster analysis that reveals a high-inflammatory subtype indicates that the disorder is heterogeneous.

Inflammatory subtyping would be able to inform personalized intervention practices, provided it is replicated in bigger samples. As an example, people with bright inflammatory dominance can be treated with adjunctive anti-inflammatory methods of treatment with standard psychotherapy and pharmacotherapy.

Nevertheless, biomarkers should be interpreted with caution. Lifestyle, metabolic well-being, and environmental conditions alter the level of cytokines. Thus, inflammatory profiling must not be done at the expense of a complete clinical assessment.

8.6 Strengths and Limitations

Strengths

- Multidimensional combination of immune, endocrine and psychological indices.
- Bio psychosomatic analysis of biomarkers by ratio.
- Mechanistic connections between findings in the molecular and the neural process.
- Subtype of inflammation identification.

Limitations

- Causal inference is conducted because of the cross-sectional design.
- Measurement of single-time-point cytokines.
- Likely residual confounding elements.
- Moderate sample size

Longitudinal studies are required to identify whether an inflammatory imbalance occurs before symptom escalation or is a result of chronic anxiety.

Chapter 9: Conclusion and Future Directions

9.1 General Conclusion

The current thesis investigated interleukin trends and their connection with mechanisms of anxiety in the Generalized Anxiety Disorder (GAD) in an integrated neuroimmune algorithm. It has gone beyond the classical paradigm of using neurotransmitters and investigated whether or not the role of systemic inflammatory imbalance in the pathophysiology and symptom persistence in GAD. The results show a stable trend of the pre-inflammatory dominance manifested through the high IL-6 and IL-1b, decreased IL-10, and significantly IL-6/IL-10 ratios. Notably, anxiety severity was linked to inflammatory dominance, which interacted with cortisol dysregulation, implying immune-endocrine feedback disturbance (Roemer *et al.*, 2025).

All this evidence confirms the Chronic Neuroimmune Sensitization Model theory that was mentioned in the thesis. Instead of being simply a psychological condition, GAD seems to be characterized by multisystem maladjustment that incorporates immune responses and immuno-stress-axis balance as well as neural sensitization. This inflammatory rebalancing of immune homeostasis in terms of continual activation is reflected in the increase of pro-inflammatory interleukins and the decrease of regulatory cytokines.

The fact that a key differentiating and predictive marker was found to be IL-6/IL-10 ratio, which supports the point of pattern-based biomarker analysis, as opposed to a single cytokine measurement. This ratio portrays dynamical balance of immunity and it could be a more stable measure of the inflammatory tone in anxiety populations (Roseberry *et al.,* 2023).

9.2 Pathophysiological Contribution

The thesis develops the current knowledge of anxiety disorders through the transmission of empirical data on the neuroimmune role in GAD. The results indicate that sustained NF- kB and JAK-STAT pathways induced by chronic psychological stress could elicit increased production of pro-inflammatory cytokines (Rosnick *et al.,* 2013).

Increased IL-6 could also affect serotonergic metabolism through the indoleamine 2,3-dioxyl (IDO) system to decrease serotonin production and elevate kynurenine metabolic products. The elevated IL-1b can switch to improve the glutamatergic signaling and decrease inhibitory regulation, encouraging the limbic circuits to be hyperexcited.

A decrease in IL-10 is indicative of an insufficient anti-inflammatory feedback, leading to the sustained retention of inflammatory cascades. This imbalance can be combined with cortisol dysregulation, which, in turn, can support chronic physiological levels of arousal and block stress recovery.

Therefore, it can be said that GAD can be approached as an immune recalibration disorder, an endocrine feedback disorder, and a neural sensitization disorder. This combined model combines molecular biology and cognitive-emotional symptomatology (Storch, 2022).

9.3 Clinical and Translational Implications

The potential ramification of these findings in a translational context is that there will be a biologically informed subtyping of people with GAD. The high-inflammatory subgroup identification implies the heterogeneity of the disorder.

Inflammatory profiling integration into clinical research could help improve psychiatry methods. Patients with high inflammatory dominance can be offered adjunctive therapies aimed at systemic inflammation, in addition to the current psychological and pharmacological interventions. Nonetheless, inflammatory biomarkers cannot be determined. Metabolic, environmental, and lifestyle factors have an effect on cytokine levels. Thus, integration of biomarkers should be in integrated biopsychosocial systems (Szuhany and Simon, 2022).

Interleukin ratios could be used as: in case validated in bigger and longitudinal sample sizes.

- Markers of risk stratification.
- Indicators of stress burden
- The treatment responsiveness predictors.
- Therapeutic progress monitoring instruments.

9.4 Theoretical Advancement

This thesis contributes to the theoretical findings concerning the role of immune balance and not single cytokine increases. The Chronic Neuroimmune Sensitization Model incorporates:

- Inflammatory activation as a result of stress.
- Dysregulation of anti-inflammatory mechanisms.
- Disturbance of the endocrine feedback.
- Neural excitatory-inhibitory malfunction.

This kind of integration provides a systems-level view in agreement with the current models of psychiatric pathophysiology.

This model does not downplay GAD to inflammation only. Instead of this, inflammation has been conceptualized merely as a subset of an interplaying, dynamic system of biological and psychological interactions.

9.5 Limitations and Methodological Considerations

Several constraints have to be taken into consideration. The cross-sectional design does not allow for establishing the directionality of causation. Longitudinal research is necessary to determine whether inflammatory imbalance is a pre-emerging factor or comes into existence as a result of chronic anxiety. One-time point cytokine measurements might be inadequate to measure dynamic changes in the immune. Stability would be assessed better by repeated sampling. There are also other factors, such as residual physiological variability, which cannot be totally ruled out, even though the major confounders have been controlled. Future researchers need to include more sample sizes, longer follow-up time, and include neuroimaging and genetic data (Sriken *et al.*, 2022)

9.6 Future Research Directions

The results of this thesis provide some significant directions in the further research of interleukin dysregulation and anxiety mechanisms in Generalized Anxiety Disorder (GAD). Although the current research offers cross-sectional data that can be used to justify the existence of a pro-inflammatory dominance pattern, longitudinal research is necessary to help understand the recurrent patterns in time. The future research should focus on whether an increased IL-6/IL-10 ratio is a predictor of the development of clinical anxiety symptoms or an aftermath of extensive psychological stress. Designs that could be conducted are prospective cohort designs, which would enable testing of inflammatory biomarkers as future risk, relapse, or chronicity predictors (Villarreal-Zegarra *et al.*, 2024).

Besides, the sampling of biomarkers at several time points would be enhanced to enhance the knowledge on the stability or fluctuation of the inflammatory state. These designs might resolve the question of whether cytokine imbalance is a steady feature indicator or a condition-dependent expression of the severity of symptoms. Another important direction is neuroimaging integration. Integration of cytokine profiling and functional magnetic resonance imaging (fMRI) would help in defining whether an increased level of interleukins is linked to a disrupted connectivity of amygdala-prefrontal circuits. It would be of great help to show

direct correlations of the immune markers with dysregulation of neural networks to enhance mechanistic interpretation (Wanderley Espinola *et al.*, 2022).

There should also be genetic and epigenetic studies. Moderation of most susceptibility to immune dysregulation can be provided by the polymorphism of inflammatory gene promoters and stress-response pathways. The epigenetic changes caused by exposure to chronic stress could also mediate the patterns of cytokine expression. In addition, the role of future research should be to investigate treatment-response stratification. The possibility of researchers finding out whether the patient population with high inflammatory ratios reacts differently to either pharmacological or psychotherapeutic intervention may facilitate precision psychiatry methods. An anti-inflammatory approach of the adjunctive type can be specifically applicable to high-inflammatory groups (Woźny-Rasała and Ogłodek, 2025).

Lastly, larger multi-centre studies that use machine learning-based pattern recognition have the potential to improve biomarker-based subtyping in GAD populations. Multidimensional studies like these would enhance classification age and better translational implementations.

To sum up, longitudinal, multimodal, stratified studies are essential to develop neuroimmune studies in GAD, combining the dimensions of biology, neural, and clinical aspects.

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