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**Ph.D. THESIS**

# The Impact of Long-Acting Injectable Antipsychotics on Neuroinflammatory Biomarkers Associated with Schizophrenia

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# Chapter 1 Schizophrenia

## 1.1 Introduction

During the 1930s, William Dameshek conducted the first investigation on the role of white blood cells in dementia praecox[1]. Hermann Lehmann-Facius, a German neuropathologist, investigated the potential connection between the schizophrenia and the occurrence of an autoimmune response. Specifically, Lehmann-Facius sought to determine if the production of antibodies targeting brain structures may be implicated in this pathological condition. Lehmann-Facius postulated that a specific group of molecules known as "brain lipoids" were particularly impacted since these compounds had biochemical specificity inside brain tissue[2]. Later, Saunders and Muchmore examined the impact of phenothiazine antipsychotics on the immune system [3].

In 1985, some authors reported high titers of interferon in the blood of patients with psychosis [4]. Additional evidence supporting the involvement of the immune system and proposing a viral cause of schizophrenia was documented [5]. This study revealed an increased susceptibility to schizophrenia among individuals born during the winter months. During the 1990s, the first indications of an autoimmune etiology were postulated once again influenced by prenatal viral exposure [6].

Moreover, the autoimmune disorder known as anti-N-methyl-d-aspartate (NMDA) receptor encephalitis is characterized by the presence of immunoglobulin (Ig)G antibodies that specifically target the NR1 subunit of the NMDA glutamate receptor. Psychiatric symptoms, in particular schizophrenia-like psychosis, are seen in around 65% of patients[7].

The presence of immunological alterations found in patients with schizophrenia may potentially contribute to the development of the disease. Moreover, immunomodulatory drugs have the potential to impact the symptoms associated with mental diseases. The therapeutic benefits of antipsychotic drugs may be attributed also to

their ability to modulate the immune system. The comprehension and empirical underpinnings of immunological principles and immunotherapies for schizophrenia illnesses have undergone transformation during medical history [8,9].

Schizophrenia is a multifaceted and chronic psychiatric condition that significantly impacts an individual's thought, perception, cognitive processes, affective states, and behavioral patterns. The condition has a spectrum of symptoms that may exhibit variations in both severity and duration [10].

The historical trajectory of schizophrenia is characterized by its intricate nature and ongoing development. The comprehension and categorization of schizophrenia have seen significant advancements over many centuries. Although the name "schizophrenia" is a relatively recent construct, there are accounts in historical books from ancient civilizations that describe symptoms like those associated with schizophrenia [11]. This literature frequently depicted individuals who encountered hallucinations, delusions, and impaired cognition and, in Old and Middle Ages, these individuals were seen as afflicted by evil entities.

The German psychiatrist, Emil Kraepelin (1856-1926), coined the term "dementia praecox" in the late 19th century to designate a mental disorder distinguished by the onset of cognitive decline and severe mental symptoms, including alteration in thought, perception, and behavior at an early stage. This notion represented a divergence from the dominant perspectives of the era, which frequently correlated mental disorders with immoral or flawed character [12].

Kraepelin was among the pioneers in the classification of mental disorders according to their clinical manifestations. He made a distinction between "dementia praecox" and other mood disorders, including manic-depressive illness [13]. The definition "schizophrenia" was initially proposed by Eugen Bleuler (1857-1939), a Swiss psychiatrist, in 1911. Bleuler employed this terminology to underscore the disintegration and fragmentation of cognitive processes that he observed in patients afflicted with

dementia praecox. [14]. Bleuler contributed to our contemporary understanding of schizophrenia by emphasizing symptoms and the division between thought, emotion, and behavior.

In contrast, Kraepelin had directed his attention towards the clinical course and outcome of the illness. His contributions established the groundwork for a methodical and empirical framework concerning the categorization of psychiatric disorders. Modern diagnostic systems, including the Diagnostic and Statistical Manual of Mental Disorders (DSM) [15,16] were significantly influenced by Kraepelin's mental diseases classification. Kraepelin's contributions were instrumental in shifting from stigmatizing or moralistic perspectives on mental illness to a scientific and medical framework for the diagnosis and treatment of psychiatric disorders, such as schizophrenia (Fig.1). Bleuler's concept of schizophrenia emphasized the core features of the disorder, including disorganized thought processes, impaired reality testing, and disruptions in emotional expression. He introduced the "four A's" to describe symptoms often associated with schizophrenia: Ambivalence (conflicting feelings and thoughts), Autism (self-isolation and withdrawal from reality), Affective disturbances (emotional disruptions), and Associations (thought disorders) [17].

Kurt Schneider (1887-1967), a German psychiatrist, significantly advanced the knowledge and categorization of schizophrenia through his contribution. Schneider introduced the "first-rank symptoms," and his contributions to the identification and classification of the symptoms of schizophrenia are particularly renowned [18].

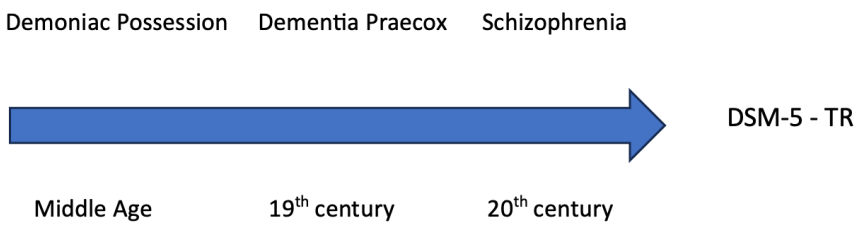
- Auditory hallucinations, particularly those in which an ongoing commentary accompanies the subject's thoughts or actions.

-Individuals who experience thought insertion, withdrawal, or dissemination believe that external forces are monitor or control their thoughts.

- Delusional perception pertains to instances wherein commonplace stimuli are perceived as possessing a distinct, typically malevolent significance.

Schneider postulated that the inclusion of one or more of these primary symptoms might serve as a crucial diagnostic indicator for schizophrenia. His work has had an enduring effect on the field of psychiatry. Nevertheless, it is critical to acknowledge that diagnostic criteria and the comprehension of schizophrenia have transformed throughout history and current diagnostics.

**Figure 1 Evolution of the Schizophrenia**




## 1.2 Epidemiology

Literature data estimates the incidence rates per 100,000 individuals annually, around 15 for men and 10 for females. Additionally, a point prevalence rate of 4.6 per 1000 individuals was reported. Furthermore, the lifetime morbid risk was found to be roughly 0.7%[19]. While there may be minor regional variations, the condition's prevalence is comparatively stable across diverse cultures and countries. The onset of schizophrenia generally occurs during late adolescence or early adulthood, with males experiencing an earlier onset than females. However, any age is susceptible [20]. schizophrenia manifests in both males and females; however, preliminary research indicates that males may experience a marginally earlier onset of the disorder compared to females. The overall prevalence of the condition is comparable in both sexes.

Schizophrenia's prevalence exhibits a relatively stable pattern across diverse geographic regions and cultural contexts [21]. A correlation has been observed between a reduced socioeconomic status and a heightened susceptibility to the development of schizophrenia. Treatment outcomes and the progression of a disease may be influenced by destitution and restricted access to high-quality healthcare [20]. The likelihood of developing schizophrenia is increased if one has a first-degree relative with the disorder. Several research studies have posited that there may be a marginally elevated susceptibility to schizophrenia among individuals raised in urban environments. The underlying causes of this disparity between urban and rural areas remain ambiguous, but they might include social tension, substance abuse, and environmental pollutants. Migration and environmental change have been linked to an increased risk of developing schizophrenia, according to the available evidence. Those who migrate in the opposite direction—from low-risk to high-risk areas—might encounter an elevated risk, whereas those who migrate in the opposite direction might encounter a diminished risk[21].

**1.3 Etiology**

A variety of environmental and genetic factors influence schizophrenia. Although the complete etiology of the condition remains unknown, investigations have yielded valuable insights regarding possible causes and risk factors. Several pivotal factors contribute to the development of schizophrenia (Fig.2).

*Genetic Factors*: Schizophrenia has been found to exhibit a significant degree of polygenicity, as evidenced by genetic investigations, with several unique genetic loci, perhaps numbering in the hundreds or even thousands, implicated in its development.
Genomic-wide association studies (GWAS) have revealed over 100 different genetic loci with common alleles with varying effects. The disease's genetic risk exhibits a notable degree of pleiotropy, as seen by the presence of shared risk alleles among schizophrenia [22]. A

recent genomic analysis has shown distinct biological processes in which genes are responsible for encoding a diverse range of synaptic proteins, including those found in the postsynaptic density and the voltage-gated calcium channel family of proteins [22]. Additionally, this phenomenon encompasses genetic elements that encode glutamate receptors and dopamine receptor D2. Additionally, a noteworthy discovery in schizophrenia research is the identification of several interrelated variations within the major histocompatibility complex (MHC). Specifically, MHC I have been identified as a regulator of several aspects of brain development, including synapse formation, neurite outgrowth, and homeostatic plasticity [23]. Several studies have revealed a potentially significant correlation with candidate genes, such as COMT, DISC1, RGS4, PPP3CC, ZDHHC8, AKT1, neuregulin, dysbindin, G72/G30, TRAR4, and alpha-7 nicotinic receptor genes [23,24]. These genes are implicated in the modulation of dopamine, hence playing a role in the fundamental etiology of schizophrenia. While it is difficult to determine the mechanism behind these genetic connections, the linkage evidence is particularly robust for two specific genes: dystrobrevin binding protein 1 (DTNBP1) and neuregulin 1 (NRG1). Both DTNBP1 and NRG1 are expressed in synapses within the central nervous system and have a role in modulating glutamate neurotransmission, which is implicated in the pathophysiology of schizophrenia [24].

*Neurochemical Aberrations*: Dopamine dysfunction has been identified as the historical cause of schizophrenia, and treatments that target the dopamine pathway in the central nervous system have been developed. Nevertheless, several shreds of evidence suggest that multiple dysfunctions in neural networks, underpinned by dopaminergic, glutamatergic, serotonergic, and gamma-aminobutyric acid (GABA) signaling, may be at the heart of schizophrenia's pathophysiology [25–27]. This dysfunction could result in aberrant interneural functioning, which manifests as thought, perception, cognitive, behavioral, and social dysfunction via disruptions in a wide

variety of neural networks [28]. Graph theory can be utilized to represent the interactions among neurotransmitters as nodes and edges [29]. In the case of oxidative balance, immune function and neurotransmitter systems can be conceptualized as multiple interlocking nodes centered around a central hub. Any disruption within these nodes could impact the entire system [29]. As a result, novel treatment targets beyond the dopamine hypothesis, such as glutamate, serotonin, acetylcholine, GABA, and inflammatory cytokines, which play a role in the communication of immunity cells, are required [30].

*Brain Structure and Function*: The presence of formal thinking disorder (FTD) is a significant clinical characteristic observed in individuals with schizophrenia. Distinct brain networks for positive and negative FTD were investigated. Both networks included brain areas from the frontal to occipital lobes, including the amygdala [31]. The ventral striatum and ventrolateral prefrontal cortex were associated with negative symptoms. Positive symptoms, specifically persecutory ideation, were associated with the hippocampus/parahippocampal region, amygdala, and medial prefrontal cortex function. While evaluated less frequently, disorganization symptoms were associated with dorsolateral prefrontal cortex function[32].

In addition, ventricular enlargement and generalized brain tissue loss have been observed in schizophrenic patients via CT imaging, in comparison to the control group [33]. The extent of the lateral and third ventricles increases gradually as the disease progresses[34]. Negative symptoms are additionally correlated with thalamic, medial temporal, and superior temporal lobe volume depletion[35]. Furthermore, structural abnormalities in the striatum, thalamus, cerebellum, anterior cingulate gyrus, hippocampus, medial temporal lobe, medial frontal, and posterior parietal cortex have been associated with executive function impairment [36]. In conclusion,

the dysfunction of the brain in schizophrenic patients is not the result of a singular brain region but rather a collection of brain networks.

*Specific prenatal and perinatal factors:* Many prenatal and perinatal factors have been associated with an elevated tendency for developing schizophrenia. Various factors, including stress during prenatal development, malnutrition, maternal infections during pregnancy, and exposure to toxins, may influence the development of the disorder [37].

*Neurodevelopmental Factors*: There is a growing recognition that schizophrenia is a neurodevelopmental disorder, which implies that early abnormalities in brain development may play a role in its initiation. Recent research shows the genetic overlap between schizophrenia and childhood psychopathology syndromes known as "neurodevelopmental disorders" like autism-spectrum disorders, intellectual disability, and Attention deficit and hypermotility disorder [38].

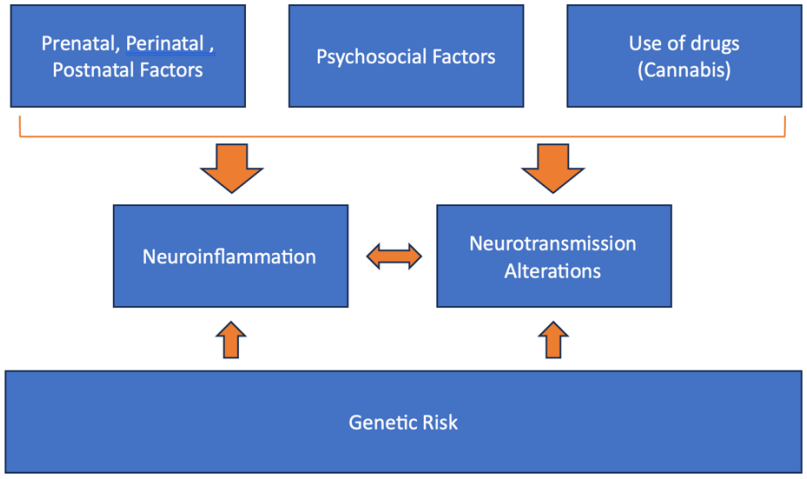
*Substance Abuse*: Cannabis use during adolescence is a recognized risk factor for the development of schizophrenia. The potential adverse effects of cannabis on brain development and function may elevate the susceptibility to psychosis [39].

*Psychosocial factors*: Social determinants of health, including but not limited to childhood trauma, social isolation, and lack of social support, may exert an influence on the trajectory of schizophrenia even though they do not constitute a direct causal link [40].

*Immune alterations*: The relationship between schizophrenia and immune system dysfunction is becoming increasingly supported by evidence. Neuroinflammation, infections, and autoimmune disorders may all contribute to an elevated susceptibility to the disorder[41].

It is critical to recognize that schizophrenia is most likely the result of a complex interaction between these factors and that no single factor can explain its etiology in its entirety. In addition, current research is focused on elucidating the precise mechanisms and chronological progression that initiate the disorder.

**Figure 2 Etiology of Schizophrenia**




### 1.4 Course of Schizophrenia

The progression of schizophrenia is highly unpredictable and varies significantly from individual to individual. Schizophrenia is commonly distinguished by a recurring pattern of relapse and remission, wherein affected individuals undergo episodes of psychotic episodes, which are distinct periods of active symptoms succeeded by intervals of relative stability. The following is a synopsis of the typical progression of schizophrenia [42,43]:

*The prodromal phase:* represents the early stage of schizophrenia, characterized by the development of full-blown psychotic symptoms months or even years later. Individuals may undergo subtle alterations in their thoughts, perception, affectivity and conduct during this phase. Social withdrawal, alterations in sleep patterns, irritability, and

a decline in academic or occupational performance are typical prodromal symptoms.

*Acute phase* is distinguished by the manifestation of active symptoms associated with psychosis. Hallucinations, delusions, disorganized thought processes, and mood and motivational disturbances are some of these symptoms. Diverse are the duration and severity of acute phases.

*Stabilization phase*: After the acute phase, patients may experience a reduction in the severity of their symptoms or even a complete cessation. Specific individuals endure extended phases of stability, whereas others encounter recurring episodes.

*Relapse*: The return of acute symptoms, or relapses, is a frequent occurrence in schizophrenia. Relapses may be precipitated by stress, substance addiction, noncompliance with medication, or other life changes. These risk factors must be closely monitored and managed to decrease the probability of relapse.

*Chronic Phase*: Schizophrenia may progress to a chronic state characterized by enduring symptoms and functional limitations in certain instances. A recent metanalyses found that 24.2 % of patients recovered [44]

**1.5 Clinical Presentation**

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition -Text Revision (DSM-5-TR) [45] provides a comprehensive delineation of the clinical criteria utilized in diagnosing schizophrenia. Presented below are several prominent clinical symptoms associated with schizophrenia [16,45] :

**Positive symptoms**

a. Hallucinations: Auditory hallucinations, specifically the perception of hearing voices, are the prevailing form of hallucination experienced. However, it is worth noting that visual, olfactory, and tactile hallucinations may also manifest.

b. Delusions refer to erroneous beliefs that persist despite being contradicted by logical reasoning or empirical data. Schizophrenia commonly manifests with paranoid delusions, which involve the unfounded assumption that others are engaged in a conspiracy against the individual. Additionally, grandiose delusions are characterized by an inflated sense of self-importance, while odd delusions encompass peculiar and improbable beliefs.

**Disorganized Thinking**

Schizophrenia can present with symptoms of fomal thought, such as disorganized speech, incoherent thinking, and challenges in the organization and articulation of concepts.

Disorganized or abnormal motor behavior encompasses a spectrum of actions, spanning from heightened restlessness and erratic movements to instances of catatonia, characterized by a state of unresponsiveness and physical immobility.

**Negative symptoms** include the absence or diminishment of typical emotional and behavioral reactions:

a. Affective Flattening refers to a condition characterized by limited emotional expressiveness and diminished emotional reactivity. Individuals who are affected may exhibit signs of apathy or emotional detachment.

b. Alogia refers to a condition where patients exhibit restricted speech production, which can manifest as either poverty of speech, typified by brief and monosyllabic responses.

c. Anhedonia refers to a diminished capacity to derive pleasure or exhibit interest in activities that were previously found gratifying.

d. Avolition refers to a diminished inclination to participate in meaningful activities, encompassing tasks related to self-care and the pursuit of individual objectives.

e. Cognitive impairments are frequently observed in individuals diagnosed with schizophrenia, manifesting as deficiencies in several cognitive domains including memory, attention, and executive functioning. Cognitive impairments have the potential to significantly affect an individual's daily functioning and contribute to the development of disability.

Many individuals encounter challenges in maintaining interpersonal connections, sustaining employment, or fulfilling the obligations of their daily routines. In order to receive a diagnosis of schizophrenia, it is necessary for these symptoms to be consistently present for a substantial duration of time, typically lasting six months or longer, and to result in a notable deterioration in overall functioning. It is crucial to acknowledge that schizophrenia can show distinct subtypes, and patients may exhibit diverse combinations of symptoms. Moreover, there exists considerable heterogeneity in the trajectory and intensity of the condition among persons diagnosed with schizophrenia.

Positive symptoms tend to exhibit relapse and remission patterns, while certain individuals may endure lingering long-term psychotic symptoms. The chronic nature of negative and cognitive symptoms is often linked to enduring impacts on social functioning[45].

### 1.6 Prognosis

The prognosis of schizophrenia exhibits considerable variability among individuals, contingent upon various factors such as the timeliness of diagnosis, the efficacy of treatment, and individual attributes. The implementation of early diagnosis and intervention is correlated with improved prognostic outcomes. The prompt highlights the importance of early recognition and treatment of schizophrenia since it significantly increases the likelihood of achieving symptom management and functional recovery. Adherence to prescribed drugs is associated with symptom management and a decreased likelihood of recurrence. Non-adherence to medicine poses a substantial obstacle in the management of schizophrenia and has the potential to result in a less favorable prognosis. Residual symptoms may persist in certain persons diagnosed with schizophrenia, even during periods of relative stability. The presence of residual symptoms, such as minor hallucinations or cognitive deficiencies, can have a significant impact on an individual's everyday functioning and may endure for an extended period [45].

# Chapter 2 Neuroinflammation and Schizophrenia

## 2.1 Neuroinflammation

Neuroinflammation pertains to inflammation inside the central nervous system (CNS). Inflammation is an inherent physiological reaction of the human body in response to various forms of physical trauma, microbial invasion, or noxious stimuli. This process entails initiating immune system mechanisms aimed at safeguarding and restoring compromised tissues [46].

The immune response in the CNS is initiated by the activation of immune cells, (i.e., macrophages) namely microglia [47], that represent just over 10 percent of the total cell population in an adult brain[48]. Under physiological conditions, microglia support the proliferation of neural precursor cells, thereby contributing to CNS homeostasis during brain development. By sculpting synapses, these cells maintain the integrity of neuronal circuits during the postnatal period. Microglia alter their morphology and downregulate genes that support homeostatic functions following a CNS injury. Chronically active microglia do secrete inflammatory proteins such as cytokines, Interleukin- 6 (IL-6), Interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), reactive oxygen species (ROS), chemokines and excitotoxins, including glutamate, that can lead to neurodegenerative processes [49]. Microglial cells are triggered by a variety of factors, such as infections, trauma, autoimmune disorders, psychological or psychosocial stress, aging, lifestyle, diet, sleep pattern, and environmental influences [50–52]. Moreover, microglia are endowed with toll-like receptors (TLRs), which are transmembrane receptors distinguished by an extracellular leucine-rich repeat domain and capable of detecting damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), in order to react quickly to local pathogenic triggers [53,54].

Neuroinflammation is characterized by the following features: activation of microglia, elevated levels of cytokines and chemokines, recruitment of immune cells from the periphery, and injury to brain tissues [55,56] .

The morphological characteristics of microglia may be broken down into three distinct stages: the resting phase (M0) and the activated phases (M1, pro-inflammatory; M2, anti-inflammatory) [57] (Fig.3). Persistent inflammation induces microglia to adopt the activated proinflammatory phenotype M1, which fosters inflammation and results in elevated levels of proinflammatory cytokines, such as TNF-α, IL-4, IL-6, IL-12, and IL-18 [57] .

**Figure 3 States of Microglia**


*Red-colored cytokines induce Microglia in a pro-inflammatory shape (M1), while cytokines in black induce anti-inflammatory Microglia (M2).*

In addition, microglia have only recently become known for their extraordinary diversity in structure and function, suggesting that they play an even more crucial role in regulating brain development, plasticity, behavior, and cognition. A multitude of environmental factors, in conjunction with an individual's genetic predispositions, contribute to a heightened lifetime risk of developing schizophrenia [58]. Microglia are exceptionally susceptible to protracted psychological stress, malnutrition, bacterial and viral infections, pollution, and inadequate or disrupted sleep, not only during critical

developmental phases but also over the course of an individual's lifetime [58].

Neuronal circuits are subject to constant refinement, promoted by microglia, primarily through the formation, modification, and elimination of synaptic structures. This mechanism underpins learning, memory, and adaptation to an ever-changing environment. [59,60]. The coexistence of genetic susceptibilities and environmental risk factors contributes to the development of a variety of neuropsychiatric disorders[58] .

Moreover, microglial involvement in the maintenance of blood vessels and the formation of the blood–brain barrier (BBB) has been suggested by findings from recently published studies [61,62]

The CNS is safeguarded by a physiological mechanism known as the BBB. This barrier serves to restrict the passage of numerous immune cells and substances from the bloodstream into the brain. During the occurrence of neuroinflammation, the integrity of the BBB may be impaired, hence facilitating the enhanced infiltration of immune cells and chemicals into the brain [63,64]. The increased BBB permeability observed with advancing age and in pathological states like chronic inflammation suggests that a form of microglia, renamed as dark microglia may play a role in disrupting the BBB [65,66].

According to postmortem examination, in the prefrontal cortex of patients with schizophrenia, microglial cells resemble the dark microglia [67]. These microglia, which are characterized as "dystrophic," contain vacuoles-filled cytoplasm and an electron-dense nucleus [67]. It is noteworthy that these cells were situated near oligodendrocytes in the white matter. Based on the discovery of the dark microglia adjacent to oligodendrocytes in the white matter[67], it is probable that they influence oligodendrocyte functions, such as myelin formation, either directly or indirectly [58].

The involvement microglia in neuroinflammation have been observed in various neurological and neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and stroke[68].

## 2.2 Neuroinflammation and Schizophrenia

Patients diagnosed with schizophrenia have impairments in white matter and cortical grey matter volume and show neuroinflammation. Some studies have revealed a notable decrease in cortical grey matter and superior frontal gyrus volumes among those diagnosed with schizophrenia and classified as having a 'high inflammation' status compared to those with schizophrenia and classified as having a 'low inflammation' status in the prefrontal cortex[69]. There was a substantial correlation seen between the expression of inflammatory-related mRNAs in the orbitofrontal cortex and the dorsolateral prefrontal cortex [70]. A dysfunctional immune system and chronic inflammation have both been linked to schizophrenia [71–73].

Inflammatory markers were present at greater levels in the extensive CATIE study. In fact, in patients with schizophrenia, several molecules have been linked to inflammation, including C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin [74].

Positron emission tomography (PET) imaging and postmortem investigations have provided evidence indicating a potential association between schizophrenia and an elevated quantity of activated microglia cells [75,76]. Among the hypothesized mechanisms are disruptions in neurotransmitter pathways, diverse inflammatory mechanisms, environmental risk factors, and genetic components. The dopaminergic hypothesis has been the most widely accepted explanation for the disease's pathophysiology. It establishes a correlation between striatal hyperdopaminergic and frontal hyperdopaminergic with symptoms of schizophrenia [77]. Dopamine exerts its effects not only as a neurotransmitter but also on peripheral immune cells and dopamine receptors located in the microglia of humans. A wide range of immune cells exhibit the presence of dopamine receptors and other proteins associated with dopaminergic activity. Additionally, numerous immune cells are involved in the uptake, production, storage, and release of dopamine. These

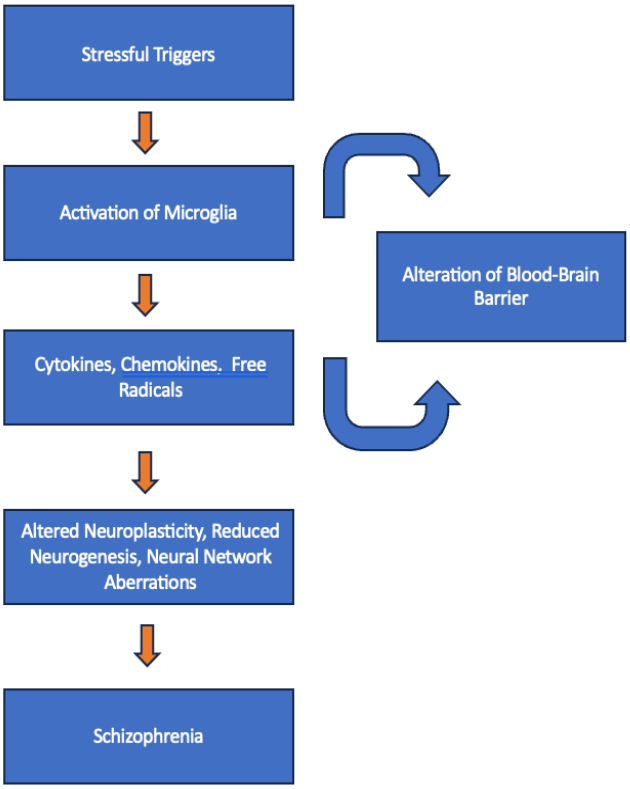
observations indicate that the control of immune function through dopaminergic mechanisms has significant importance. Investigating these pathways presents a potentially fruitful approach to addressing inflammation and illness [78]. Dopamine modulates the activation state, motility, and phagocytic activity of microglia, suggesting that it may play a recently identified immunomodulatory function in several diseases [68,79]. An expanding corpus of evidence indicates that the progression of schizophrenia may be influenced by inflammatory mechanisms occurring in the CNS, primarily through the action of proinflammatory cytokines, glial cells, and peripheral immune cells [80]. Altered levels of proinflammatory cytokines have been identified in the cerebrospinal fluid and serum of individuals diagnosed with schizophrenia. While the BBB restricts the ability of peripheral proinflammatory molecules to enter the CNS, there is evidence suggesting that a certain degree of permeability exists between the CNS and the periphery [64,81–83]

The investigation of neuroinflammation in schizophrenia has been rendered feasible in recent times through the implementation of PET and the creation of radiotracers that specifically target the 18 kDa translocator protein (TSPO). It has been hypothesized that the overexpression of TSPO in activated microglial cells and astrocytes is indicative of cerebral inflammation[81–83].

Neuroinflammation is mediated by pro-inflammatory molecules, such as cytokines and chemokines, released by activated microglia [85–88]. Furthermore, maternal infections that occur during pregnancy have the potential to elevate the risk of schizophrenia in the offspring, potentially by triggering neuroinflammation[84].

The utilization of human postmortem brain tissue has unveiled indications of immune activation, characterized by microglia activation and microgliosis, in specific regions of the brain, namely the dorsolateral prefrontal cortex and orbital frontal cortex, among a considerable subset of individuals diagnosed with schizophrenia, respect to individuals without schizophrenia [85–88]

**Figure 4 The Role of Activated Microglia in the Pathogenesis of the Schizophrenia**




*Various stress-inducing events, including prenatal stress, infection, emotional trauma, and autoimmune illnesses, have the potential to trigger the activation of microglia. This activation subsequently leads to the production of cytokines that disrupt the average balance of neurons and their surrounding environment. Furthermore, the activation of microglia leads to an augmentation in the permeability of the blood-brain barrier, exacerbating the impairment of neuronal function. This mechanism may potentially be attributed to the modifications seen in neural networks.*

Whether neuroinflammation is a cause or effect of the disorder remains unknown. Multiple factors, such as genetic predisposition, immune dysregulation, and environmental exposures, presumably interact reciprocally to contribute to the etiology of schizophrenia. In brief, although there exists evidence indicating a potential correlation between neuroinflammation and schizophrenia, further investigation is required to ascertain the precise characteristics of this association and its impact on the pathogenesis and advancement of the disorder. To better comprehend the mechanisms at play and the potential for therapeutic interventions that target neuroinflammatory processes in schizophrenia, additional research is required [50,89–91].

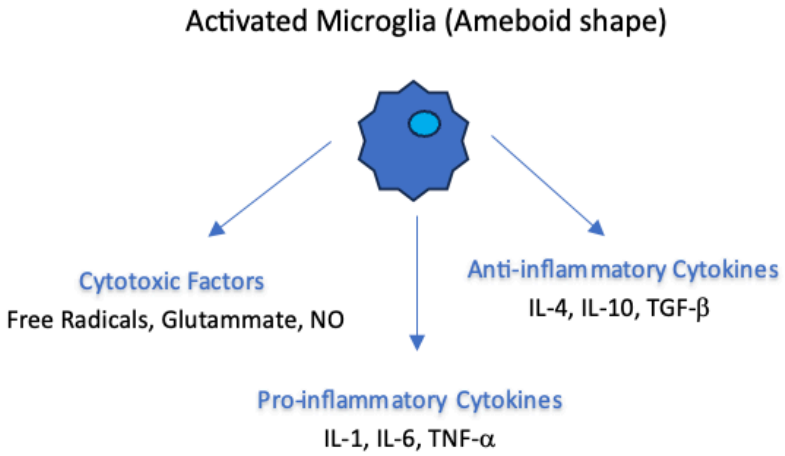
The neuropathology of schizophrenia is intricately linked to microglial activation, associated with secretion of cytokines/chemokines (Fig.4). These molecules not only induce direct neuronal damage but also hinder neurogenesis and induce white matter abnormalities in the brains of schizophrenia patients. Inhibiting microglial activation as a treatment modality could provide novel insights into the therapeutic approach toward schizophrenia [92].

### 2.3 Cytokines and Chemokines

Cytokines and chemokines are a class of secreted proteins that exhibit overlapping activities in growth, differentiation, and activation processes. The cytokine superfamily of proteins plays a crucial role in facilitating intercellular communication and is important for the development and regulation of the immune system. These proteins play a crucial role in the regulation and modulation of immune responses, as well as in the control of immune cell migration and the organization of immune response [94]. Cytokines are synthesized and secreted by a diverse array of cellular populations, including both immune cells, such as T cells, B cells, macrophages, and dendritic cells, and non-immune cells, including fibroblasts, endothelial cells, stromal cells, and microglia [93,94].

Inflammation is a biological response characterized by the release of cytokines, which can have pro-inflammatory or anti-inflammatory effects. Inflammatory cytokines, such as IL-1, IL-6, and TNF-α, play a pivotal role in the initiation and maintenance of inflammatory reactions, which are essential for the body's defense against infections. Interleukin-10 (IL-10), which falls under the category of anti-inflammatory cytokines, plays a crucial role in the resolution of inflammation and the prevention of exaggerated immune responses.

**Figure 5 The Activation of Microglia**




*Activated microglia release cytotoxic molecules anti-inflammatory and pro-inflammatory cytokines. The latter can trigger apoptosis over an extended period via the activation of caspases, glutamate excitotoxicity, and the occurrence of free radical damage. Additionally, it may lead to increased BBB permeability and the expression of CAMs, subsequently resulting in leukocyte diapedesis in the CNS. The events above underpin the neuroinflammation described in schizophrenia, and over an extended period, they are linked to the loss of neurons and neurodegenerative processes* [95].

*NO= Nitric Oxide, IL-4=Interleukin-4, IL-10=Interleukin-10, TGF-β= Transforming Growth Factor -beta, IL-1=Interleukin-1, IL-6=Interleukin-6, TNF-α= Tumor Necrosis Factor-alpha, BBB=Blood-Brain Barrier, CAMs=Cell Adhesion Molecules, CNS=Central Nervous System.*

Neuroinflammation can arise from a multitude of sources, such as infections, traumas, autoimmune illnesses, and neurodegenerative diseases. The initiation of the immune response occurs when the CNS is subjected to various stimuli such as pathogens, damage, or aberrant proteins found in neurodegenerative illnesses [72,95,96]. Within the CNS, immune cells like microglia and astrocytes are responsible for the production and release of pro-inflammatory cytokines (Fig.5). Cytokines, including IL-1β, IL-6, and TNF-α, play a crucial role in the initiation of the immune response, while some cytokines, as

Transforming Growth Factor- beta (TGF-β), negatively modulates neuroinflammation[95] (Fig.5). The disruption of the BBB and the expression of Cell Adhesion Molecules (CAMs) on endothelial surface, can be influenced by cytokines [95,97]. A compromised BBB can facilitate the enhanced infiltration of immune cells from the bloodstream into the brain, hence playing a role in the development of neuroinflammation. The process of inflammation amplification occurs when pro-inflammatory cytokines are generated and subsequently released, establishing a positive feedback loop that results in the escalation of the inflammatory response. The process of amplification might potentially result in detrimental effects on neurons and glial cells [93,94].

Chronic neuroinflammation arises when there is an imbalance in the synthesis of pro-inflammatory and anti-inflammatory cytokines, resulting in a sustained inflammatory response within the nervous system and the consequent risk of neuronal loss and neurodegeneration [93,94,98,99].

Another class of molecules involved in immune cells crosstalk are chemotactic cytokines, also known as chemokines. Chemokines are a class of cytokines that range in size from 7 to 12 kDa and are responsible for inducing directed chemotaxis in surrounding responder cells [100]. They communicate via heptahelical chemokine receptors coupled to G proteins on the cell surface. Their primary recognition stems from their capacity to induce cellular migration, precisely that of leukocytes, which are white blood cells. The process of leukocyte migration and trafficking, as well as inflammatory reactions, are all mediated by chemokines, which play an essential part in immune function [100]. As a result, chemokines are deeply implicated in the regulation and progression of the immune system, as well as in the initiation of all destructive or protective immune and inflammatory reactions. Classically viewed as inducers of directed chemotactic migration, it is now clear that chemokines can stimulate

a variety of other types of directed and undirected migratory behavior and induce cell arrest or adhesion [101,102].

### 2.4 Cytokines, Chemokines and Schizophrenia

Based on meta-analyses, cytokines are divided into the following four categories according to the alterations they undergo in schizophrenia patients [103–107]

*Increased cytokines*: IL-1, IL-6, TNF-α, TGF-β1, IL-12, IL-17, IL-18.
*Non-altered cytokines*: IL-2, IL-4, IL-17;
*Variable Levels of cytokines*: IL-8, interferon (IFN)-γ.

Notably, variations in cytokines may be different in different stages of schizophrenia, which are first-episode and drug-naive, stable chronic, and acute relapse. In addition, the duration of the condition, the severity of the symptoms, the domains of the symptoms, and cognitive functions are all correlated with levels of cytokines [57,105,108–112].

Investigating the levels of cytokines in patients with schizophrenia has some clinical utilities, some of which may include early diagnosis, the creation of novel therapeutic targets, the patient stratification necessary to select the most effective therapeutic protocol, and the prediction of both the prognosis and the treatment response [113]. Numerous studies have demonstrated that individuals diagnosed with schizophrenia exhibit potential dysregulation in the levels of cytokines and other inflammatory indicators within their blood and cerebral fluid [103,104,114]. Numerous studies have demonstrated that individuals diagnosed with schizophrenia exhibit potential dysregulation in the levels of cytokines and other inflammatory indicators within their blood and cerebral fluid [114,115].

For example, TGF-β1 showed to have the potential to serve as a diagnostic trait marker for patients suffering from psychosis (with a

sensitivity of 70.4% and a specificity of 80.6%) [116]. Furthermore, there is evidence from certain research that antipsychotic medication may be able to help in lowering the levels of pro-inflammatory cytokines that are present in persons who have schizophrenia[117–121].

Cytokines can disrupt neurotransmitter systems within the brain, including glutamate, serotonin, and dopamine, all of which have been implicated in the pathogenesis of schizophrenia [122–124]. Genetic and environmental factors may both play a role in the increased levels of cytokines observed in people diagnosed with schizophrenia. In addition to environmental factors such as infections, stress, and exposure to specific pollutants, genetic predisposition may influence an individual's immune response by inducing an immune response and contributing to cytokine elevation[122–124].

Chemokines are recognized as immunoregulatory proteins that play a multifaceted role, such as neuromodulation, neurogenesis, and neurotransmission [125].

The chemokines are classified into four families: CC, CX3C, CXC, and XC are the designations that correspond to the spacing of conserved cysteine motifs, with X denoting any amino acid [126]. The C-C motif: MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), Eotaxin-1 (CCL11), RANTES (CCL5); two ligands featuring the C-X-C motif*:* IL-8 (CXCL8), SDF-1α, GRO-alpha (CXCL1) and IP-10 (CXCL10).

The potential impact of peripheral inflammation on chemokine expression is a matter of concern, given that pro-inflammatory and anti-inflammatory cytokines modulate chemokine expression.

Chemokine levels in the CNS and blood are significantly increased in various neuroinflammatory disorders, including psychiatric conditions like schizophrenia [127–130]

In fact, meta-analyses confirmed the most reliable data, which indicated that patients with schizophrenia had elevated levels of CCL8/IL-8, CCL2/MCP-1, CCL4/MIP-1β, and CCL11/eotaxin-1 in

their blood [125]. Some researchers have shown that chemokines may have direct functions in the CNS, like neuroendocrine function, neurotransmission, and neurodegeneration[131] .

# CHAPTER 3
# RESEARCH PROJECT

## 3.1 Aim of the Research

In the present study authors aimed to examine the blood levels of cytokines/chemokines in patients with schizophrenia, specifically in relation to the intramuscular monthly administration of first-generation (Haloperidol Decanoate, HD) and second-generation antipsychotics (Paliperidone Palmitate- Long-Acting Injection, PAL-LAI). The long-acting formulation offers the clinical benefit of promoting greater adherence to treatment regimens and reducing fluctuations in blood concentrations caused by peak oral administration. This pharmacokinetic characteristic maintains stable drug concentrations in the bloodstream, thereby modifying the clinical course of the schizophrenia through its effects on dopaminergic and serotonergic neurotransmitter systems. Additionally, it has the potential to alter the neuroinflammatory biohumoral profile associated with schizophrenia. In this research project cytokines and chemokines plasma concentrations were measured in a sample of outpatients diagnosed with schizophrenia who were undergoing treatment with depot/long-acting antipsychotics. Cytokines evaluated included IL-1β, IL-18, TGF-β1. The panel we compiled comprised chemokines in innate and adaptive immunity. Some of these chemokines had dual functions, acting as homeostatic and inflammatory regulators (e.g., Eotaxin-1, IP-10). Additionally, it contained chemokines that were recognized for their functions in the central nervous system (e.g., MCP-1α, MIP-1β, Eotaxin-1, and IP-10) [132].

## 3.2 Material and Methods

Research was conducted on 18 patients with schizophrenia. The protocol underwent approval by the Ethics Committee at the University of Catania. The participants in the study were required to

give written informed consent. Participants were recruited from the Institute of Psychiatry of the University of Catania (Prof. Eugenio Aguglia, Prof. Maria Salvina Signorelli). The use of the Structured Clinical Interview for the DSM-5-TR established the diagnosis of schizophrenia [133]. The study collected data on sociodemographic variables, including age, education, gender, and smoking status, as well as illness-related variables such as the duration of illness (DUI), duration of untreated psychosis (DUP) and antipsychotic medication. Cytokines and chemokines assays were carried out by the BIOMETEC department, pharmacology section, and the Drug Science Department of the University of Catania (Prof. Renato Bernardini, Prof.Giuseppina Cantarella, Prof. Filippo Caraci)

The study's objective was to examine, in two stages at six-month intervals (T0 and T1) the levels of the following cytokines: IL-1β and TGF-β, IL-18 and of the following chemokines: MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), Eotaxin-1 (CCL11), RANTES (CCL5), IL-8 (CXCL8), SDF-1a, GRO-alpha (CXCL1) and IP-10 (CXCL10), in schizophrenia, and differentiating patients regarding recent antipsychotic HD and PAL-LAI treatment (< 2yr) and correlating with psychopathological domains. Individuals who had also received a diagnosis of substance addiction or cognitive impairments were ineligible to take part in the study. Moreover, patients who were older than 65 years, patients with a disease history for more than 10 years, and those who had internist comorbidities were not allowed to participate in the research. Those individuals who met the inclusion criteria and were deemed eligible to participate in the study were contacted by the research team and provided additional details regarding the investigation. The participant IDs were exclusively allocated for identification purposes and did not contain any specific information about the individuals to whom they were assigned.

### 3.2.1 Psychiatric Assessment

The assessment of negative and positive was conducted using interviewer-administered Positive and Negative Syndrome Scale (PANSS). For cognitive evaluation, the Montreal Cognitive Assessment (MoCA) was utilized.

**PANSS**

The Positive and Negative Syndrome Scale (PANSS) is widely recognized as one of the most rigorously tested tools for evaluating the presence of positive, negative, and general psychopathological symptoms in individuals diagnosed with schizophrenia. The PANSS is a validated and widely used clinical assessment tool that employs a structured interview format to evaluate the intensity and manifestation of positive and negative symptoms, along with general psychopathology, in individuals diagnosed with schizophrenia. Out of the total sample size of 30 items, seven things are classified as positive symptoms, seven items are classified as negative symptoms, and the other 16 items are categorized as general psychopathology symptoms. The intensity of symptoms for each item is assessed by determining which anchoring points on the 7-point scale (1 = absent; 7 = extreme) most accurately represent the manifestation of the symptom [134]

**MoCA**

The Montreal Cognitive Assessment (MoCA), developed by Nasreddine et al. [135], is a screening tool that is intended to identify cognitive impairments. Its administration duration is approximately 10 minutes. The MoCA evaluates the following cognitive domains: orientation, language, attention, memory, executive functions, abstraction, and calculation. Cognitive functioning is considered "normal" when the cumulative score is 26 or higher on a scale of 0 to 30. The MoCA total score incorporates years of education, with individuals who have completed 12 years or less having their total score adjusted by one point. MoCA is utilized in a clinical setting to screen patients with schizophrenia for cognitive impairment at the

bedside for a variety of reasons, including its short and straightforward administration, its availability for free use for clinical purposes, and, most significantly, its sufficient sensitivity in detecting cognitive impairment [136].

### 3.2.2 Cytokines and Chemokines Assays

Serum samples were collected and stored at -80°C until use. Serum samples were tested using multiplex immunobead assay technology (Th1/Th2 Cytokine & Chemokine 20-Plex Human ProcartaPlex™ Panel 1 Thermo Fisher Scientific, Vienna, Austria), according to the manufacturer's instructions. Cytokines and chemokines were detected with the Luminex MAGPIX instrument (Luminex Corp., Austin, TX) and data were analyzed with xPONENT® software (Luminex Corp., Austin, TX). Analytes with a concentration outside the linear range were excluded from the analysis.

### TGF-β1 measurement in plasma samples

To measure TGF-β1 levels in plasma samples obtained from patients at T0 and T1, we carried out the enzyme- linked immunosorbent assay (ELISA) in accordance with manufacturer's instructions (Bio-techne, R&D system, DB100C) as previously described (DOI 10.3389/fphar.2022.1075746). Briefly, after the activation procedure from latent TGF-β1 to the immunoreactive form, plasma samples were assayed at a 1: 10 dilutions. TGF-β1 standard curve and activated samples were assayed in duplicate. The optical density of each well was determined using a microplate reader Synergy HT (Agilent Bio Tek, Santa Clara, CA, United States) set to 450 nm, 540 nm, and 570 nm as suggested by the producer. Data were analyzed subtracting readings at 540 nm from the readings at 450 nm to correct the optical imperfections in the plate.

**The Acquiring of Data and Analyzing Statistical Information**

Following the completion of the PANSS, MoCA and the dosage of cytokines/chemokines, the data for each participant were gathered and recorded. After collecting them data were then entered into the 25th version of the SPSS statistical analysis program for the purpose of conducting additional research. Data were presented as the mean and standard deviation. The non-parametric U-Mann Whitney and Spearman test were used to compare the means of the groups and correlations, respect. In all the statistical studies, a level of significance was defined as a P-value of less than 0.05.

**3.3 Results**

This study comprised a total of 18 patients with schizophrenia; 11 patients were in treatment with PAL-LAI, and 7 patients were in treatment with HD. Both groups had been treated with PAL-LAI/HD antipsychotics for less than two years. In a nutshell, the comparison of the two groups of patients did not uncover any age, disease characteristics, or gender disparities that were statistically significant. Demographic data, the DUP, and DUI are reported in table 1. PANSS total score, positive syndrome scale, and negative syndrome did not change significantly between T0 and T1 in the two groups. The group treated with HD had a more significant presence of negative and cognitive symptomatology as well as greater degree of overall psychopathological impairment than the group treated with PAL-LAI for the same DUI, DUP, and demographic characteristics. The total score on the MoCA was higher in the group of PAL-LAI respect to HD. Visuospatial/executive, attention, and delayed recall were more deteriorated in the group treated with HD than PAL-LAI. There was no difference we observed in naming; attention was 5.01 (2.3), language was 2.09(1.01), abstraction was 1.21 (0.31), and delayed recall was 1.26 (0.24). Orientation was 5.43 (1.01).

Table 2 and 3 display the results of the measurements taken of TGF-β1, IL-1β and IL-18 cytokines levels and of chemokines RANTES

(CCL5) and Eotaxin (CCL11), that showed a significant variation in relation with treatment. Patients undergoing HD treatment exhibited a rise in pro-inflammatory cytokines from T0 to T1 (Graphic 2). In contrast, indices of neuroinflammation did not vary between T0 and T1 in patients treated with PAL-LAI, with the excpeption of Eotaxin. These differences were independent of DUI and DUP. There was no change in the concentrations MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), IL-8 (CXCL8), SDF-1α, GRO-alpha (CXCL1) and IP-10 (CXCL10) between two groups (PAL-LAI and HD patients).

| **Table 1 Demographic Variables** | **PAL-LAI (n=11)** | **HD (n=7)** |
|---|---|---|
| *Age* | 34.2 (5.1) | 42.2 (7.2) |
| *Sex* | 5 /6 (m/f) | 4/3 (m/f) |
| *Education* | 11.3 (5.2) | 9.3 (4.1) |
| *Marital Status* | Married 2; Unmarried 8; Divorced 1. | Married 1; Unmarried 6. |
| *Smokers* | 8 | 5 |
| *DUI* | 6.2 (3.2) yr | 7.3 (2.1) yr |
| *DUP* | 3.1 (1.4) yr | 4.2 (1.6) yr |

*Continuous variables are expressed as Mean (standard deviation); PAL-LAI- Paliperidone Long-acting; HD- Haloperidol Decanoate; DOI- Duration of illness; DUP- Duration of untreated psychosis.*

*No statistical difference was observed for demographic variables between the groups of patients, p >0.05.*

Pro-inflammatory cytokines, and specifically, IL-1β, IL-18, directly correlate with DUI. In the group of patients treated with HD, we found at T1 a significant reduction in the anti-inflammatory cytokine TGF-β1 and an increase in IL-1β (Graphic 1-2). In contrast, PAL- LAI did not affect the levels of cytokines TGF-β1 and IL-1β between T0 and T1 (Graphic 1). Specifically, in patients in treatment with PAL-LAI, TGF-β1 levels at T1 were significantly higher than those observed in the group of patients treated with HD (Graphic 1). Conversely, there

was a substantial elevation in the blood concentrations of IL-18 in both groups at T1 compared to T0 (Graphic 2).

Regarding chemokines, there was a statistically significant increase in RANTES levels exclusively within the HD group at T1 when compared to T0 (Graphic 2). In T1 RANTES levels were higher in HD group compared with PAL-LAI group (Graphic 2).

**Table 2 Cytokines concentration in T0 and T1 (pg/mL)**

**Graphic 1 Levels of TGF-β1 in PAL-LAI and HD patients**

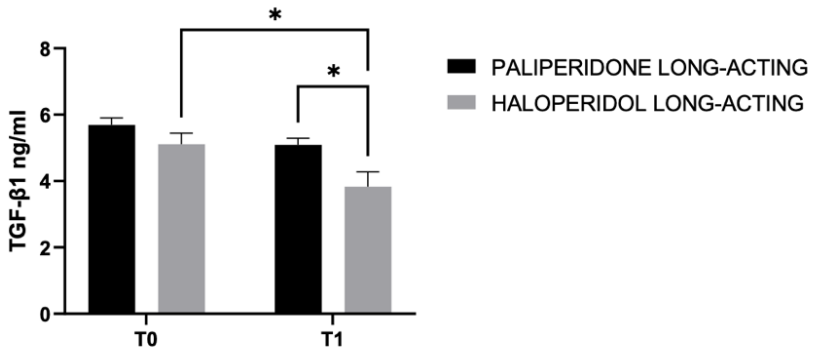
| | | | | |
|---|---|---|---|---|
| **TGF-β** | 5.6(0.7) | 5.0(0.6) | 5.1(0.9) | 3.8(1.2)* |
| **IL-1β** | 95.1(7.8) | 121.3(24.0) | 106.1(13.3) | 128.0(31.3)* |
| **IL-18** | 2.8(1.4) * | 6.3(3.2)* | 4.4(3.3)* | 5.5(3.0)* |

Data are expressed as mean (SD). Pal-LAI: Paliperidone Long-Acting; Hal-Depot: Haloperidol Decanoate. *p<0.05

**Table 3 Chemokines concentration in T0 and T1 (pg/mL)**

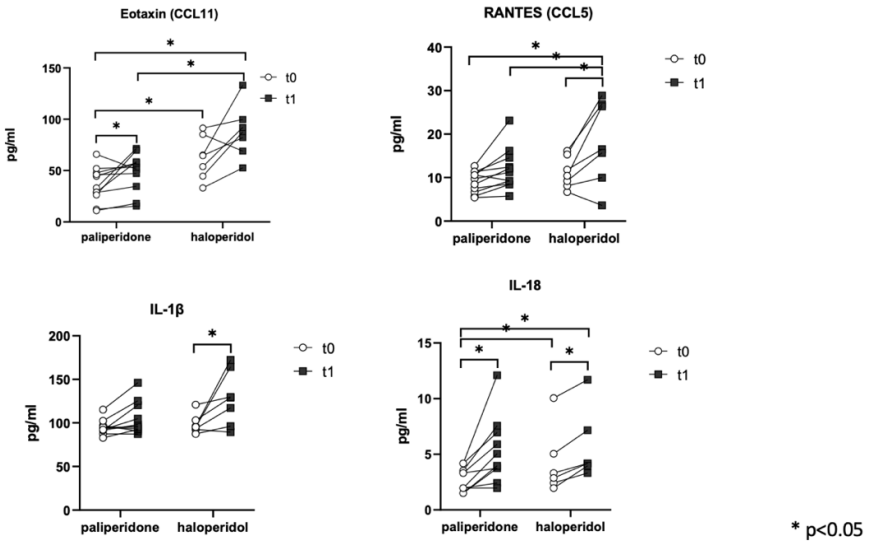
| | **Pal-LAI** | | **Hal-Depot** | |
|---|---|---|---|---|
| | **T0** | **T1** | **T0** | **T1** |
| **RANTES** | 10.5(2.1) | 11.5(4.8) | 10.7(3.6) | 27.5(2.2)* |
| **Eotaxin-1** | 29.7(14.4) | 55.4(13.3)* | 56.7(23.0) | 107.0(31.0) |

Data are expressed as mean (SD). Pal-LAI: Paliperidone Long-Acting; Hal-Depot: Haloperidol Decanoate. *p<0.05




*In comparison to patients treated with HD, TGF levels in the blood were greater in patients treated with PAL-LAI. Moreover, between T0 and T1, TGF levels were significantly decreased in HD-treated patients.*
*PAL-LAI- Paliperidone Long-Acting; HD-Haldol Decanoas.*

**Graphic 2 Levels of Chemokines and Citokynes in PAL-LAI and HD patients**




*The levels of pro-inflammatory cytokines IL1, Eotaxin, and RANTES were found to be significantly elevated in patients receiving HD treatment at T1 compared to T0. The pro-inflammatory cytokine levels in the cohort of patients taking PAL-LAI were stable between the initial time point (T0) and the subsequent time point (T1). The levels of IL-18 showed a statistically significant increase in both cohorts of patients.*
*PAL-LAI- Paliperidone Long-Acting; HD-Haldol Decanoas.*

**Table 4 PANSS and MOCA at T0 and T1**

| | Pal-LAI | | Hal-Depot | |
|---|---|---|---|---|
| | T0 | T1 | T0 | T1 |
| **$PANSS_{GEN}$** | 34 (6) | 30 (4) | 49 (6) | 52 (4) |
| **$PANSS_{POS}$** | 22 (3) | 19(2) | 25 (3) | 23(4) |
| **$PANSS_{NEG}$** | 16 (5) | 18(4) | 38 (4) | 41(6) |
| **MoCA** | 25 (4) | 26(3) | 22(4) | 23 (2) |

Data are expressed as mean (SD). Pal-LAI: Paliperidone Long-Acting;

Hal-Depot: Haloperidol Decanoate. PANSS: Positive and Negative Symptoms Scale;

$PANSS_{GEN}$: General Symptoms Subscale; $PANSS_{POS}$: Positive Symptoms Subscale;

$PANSS_{NEG}$: Negative Symptoms Subscale; MoCA: Montreal Cognitive Assessment.

Patients in the PAL-LAI group had lower average levels at both T0 and T1 of Eotaxin compared with HD-treated patients (Graphic 2). The correlation between blood levels of cytokines/chemokines and psychometric evaluations was investigated. The research demonstrated a direct correlation between TGF-β1 and MoCA scores. While an inverse correlation was observed between IL-1β , Eotaxin and MoCA scores (Tab. 5-7-9). General psychoapthology and positive symptoms correlate with IL-1β. IL-18 directly correlate with general psychopathology (Tab. 6). IL-6 values are missing, as unfortunately the IL-6 values did not fall within the standard of the assay curve.

**Table 5 Correlations between IL-1β and Psychopathological Domains**

| | IL-1β | |
|---|---|---|
| | **Spearman's Rho** | **p-Value** |
| $PANSS_{GEN}$ | 0.57 | 0.006 |
| $PANSS_{POS}$ | 0.64 | 0.006 |
| $PANSS_{NEG}$ | 0.41 | 0.07 |
| MoCA | -0.39 | 0.08 |

**Table 6 Correlations between IL-1β and Psychopathological Domains**

| | IL-18 | |
|---|---|---|
| | **Spearman's Rho** | **p-Value** |
| $PANSS_{GEN}$ | 0.51 | 0.05 |
| $PANSS_{POS}$ | 0.20 | 0.09 |
| $PANSS_{NEG}$ | 0.46 | 0.06 |
| MoCA | 0.49 | 0.06 |

**Table 7 Correlations between RANTES and Psychopathological Domains**

| | RANTES | |
|---|---|---|
| | **Spearman's Rho** | **p-Value** |
| $PANSS_{GEN}$ | 0.33 | 0.08 |
| $PANSS_{POS}$ | 0.22 | 0.09 |
| $PANSS_{NEG}$ | 0.40 | 0.07 |
| MoCA | - 0.38 | 0.08 |

**Table 8 Correlations between Eotaxin and Psychopathological Domains**

| | Eotaxin | |
|---|---|---|
| | **Spearman's Rho** | **p-Value** |
| $PANSS_{GEN}$ | 0.36 | 0.07 |
| $PANSS_{POS}$ | 0.48 | 0.06 |
| $PANSS_{NEG}$ | 0.41 | 0.06 |
| MoCA | -0.51 | 0.05 |

**Table 9 Correlation between TGF- β1 and Psychopathological Domains**

| | TGF-β1 | |
|---|---|---|
| | **Spearman's Rho** | **p-Value** |
| $PANSS_{GEN}$ | 0.44 | 0.07 |
| $PANSS_{POS}$ | 0.20 | 0.09 |
| $PANSS_{NEG}$ | - 0.46 | 0.06 |
| MoCA | 0.52 | 0.05 |

**Discussion**

The finding that individuals with schizophrenia exhibit dysregulated production of specific cytokines/chemokines in peripheral blood supports the concept that the peripheral cyto-chemokine network plays a role in the underlying pathogenetic mechanisms of schizophrenia.

To our knowledge, this is the first study to analyze cytokine/chemokine values in relation to long-acting/depot drug therapy. Recent research attempt to identify biomarkers that have a high predictive value for the diagnosis, severity of illness, or treatment resistance. Although schizophrenia is associated with activated immune-inflammatory pathways, such as higher levels of cytokines and chemokines, not much research has proven to determine the predictive qualities of such [137–142]

In patients treated with HD, we observed a significant rise in the levels of pro-inflammatory cytokines, such as IL-1β, IL-18, and of chemokines such as RANTES, and Eotaxin, between T0 and T1. Conversely, PAL-LAI was not associated with significant modifications of IL-1β and RANTES, between T0 and T1. On the

other hand, values significantly increased at T1 compared with T0 in both groups, although in the group treated with PAL-LAI, Eotaxin levels were higher than in the group treated with HD.

In our sample, patients treated with HD had higher levels of IL-1β. Multiple studies have provided data indicating a significant increase in IL-1β among individuals, with drug-resistant schizophrenia, receiving clozapine treatment compared to control groups [143].
IL-1β is a pro-inflammatory cytokine that plays a critical role in the initiation and regulation of host-defense mechanisms against infections and injuries [144]. Genetic polymorphism associated with IL-1β represents a vulnerability factor for schizophrenia [144]. Furthermore, IL-1β plays a role in both acute and chronic neurodegeneration, as well as in the embryonic development of the CNS [145]. During CNS neurodevelopment, IL-1β has a role in stimulating the proliferation and generation of cytokines and trophic factors, namely nerve growth factor (NGF), in astrocytes [146]. Additionally, IL-1β hinders the proper expression of brain-derived neurotrophic factor (BDNF) [145]. In this study, IL-1β was correlated with general psychopathology and positive symptoms prevalence. Patients with high levels of IL-1β showed more severe positive symptoms (delusions and hallucinations) and high levels of general psychopathology worsening.
Closely related to the IL-1β is the inflammosome, which according to some authors [146] plays a central role in the biochemical mechanisms leading to neuronal dysfunction and neuroinflammation. In Microglia, the NLRP3 (nod-like receptor pyrin domain-containing protein 3) (fig.6) inflammasome plays a crucial role in the innate immune system by facilitating the activation of caspase-1 and the release of proinflammatory cytokines IL-1β/IL-18 in response to microbial infection and cellular injury [146]. Nevertheless, the atypical activation of the NLRP3 inflammasome has related to several inflammatory conditions, such as Alzheimer's disease, diabetes, atherosclerosis, and schizophrenia [146–149]. A variety of stimuli

induces the activation of the NLRP3 inflammasome, such as mitochondrial malfunction, ROS, and lysosomal damage [147]. Recent research indicates that the involvement of the NLRP3 pathway may be implicated in the development of schizophrenia since systemic inflammation has been identified as a contributing factor. The NLRP3 inflammasome assumes a pivotal function within the innate immune system by facilitating the production of pro-inflammatory cytokines [146]. The NLRP3 inflammasome has been proposed as a potential biomarker for schizophrenia, and its pharmacological modulation has promise as a viable therapeutic strategy. Moreover, the authors suggest the hypothesis that the NLRP3 pathway may play a role in the etiopathogenesis of schizophrenia. Patients diagnosed with schizophrenia had elevated levels of NLRP3, IL-1β, and IL-18 mRNA in comparison to those without psychiatric disorder [148] .

IL-18 is a pleiotropic and pro-inflammatory cytokine that has been postulated to play a role in the development of schizophrenia [149–153]. Patients who had the chronic form of the disease exhibited considerably higher levels of IL-18 in their serum compared to both first-episode patients and controls [154].

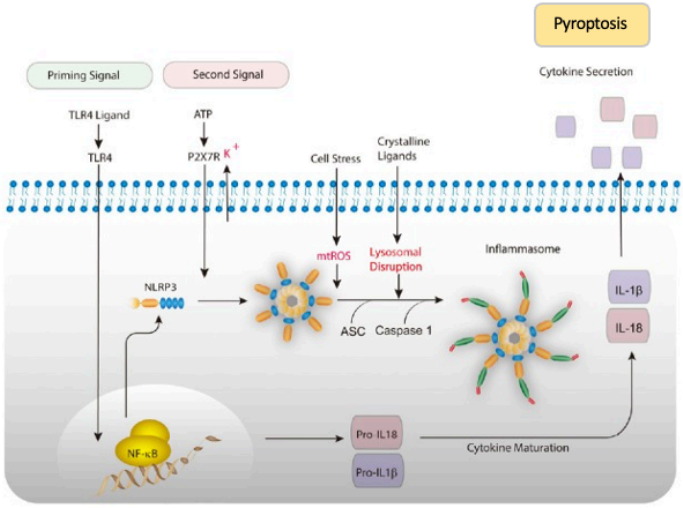
In our sample, IL-18 was found to be elevated in both groups, with an increase at T1 in both patients with schizophrenia taking HD and those treated with PAL-LAI. Despite, it was determined in a systematic review and meta-analysis that IL-18 does not contribute to the pathogenesis of the disorder [149], however, according to some authors, IL-18 might be helpful as a biomarker of schizophrenia and contribute to studies about the early detection and treatment of the disease [150].

Interestingly, in our group of patients treated with PAL-LAI, IL-18 was significantly higher than patients treated with HD. Moreover, the levels of IL-18 were increased at T1 in the PAL-LAI group.

Despite its pro-inflammatory activity, IL-18 is associate with cognitive function. Neurons across the brain contain components of the IL-18 receptor complex; furthermore, it is hypothesized that IL-

18 exerts a direct impact on neuronal function. This effect may explain a portion of the IL-18-induced effects on synaptic plasticity and functionality in the hippocampal system.

**Figure 6 The NLPR3 Inflammasome activation in Microglial Cell**




*The pathways associated with the NLRP3 inflammasome. Following a noxious agent, the process of NLRP3 inflammasome activation is initiated. In this phase, damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), and cytokines, including IL-1β engage with toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), or cytokine receptors. This connection elicits the activation of the NF-kB signaling pathways. Upon activation, the NF-kB undergoes translocation to the nucleus, subsequently initiating the production of many molecules including NLRP3, pro-IL-1β, pro-IL-18, and caspase-11 via the non-canonical route. During the process of activation, many inputs converge to initiate various cellular and molecular signaling processes. These events include mitochondrial malfunction, lysosomal damage, fluctuations in calcium and potassium ion levels, creation of reactive oxygen species (ROS), and release of adenosine triphosphate (ATP). The processes initiate the activation of the NLRP3 inflammasome, resulting in the enzymatic cleavage of procaspase-1 and subsequent release of the active caspase-1 form. The enzyme caspase-1 is responsible for the proteolytic cleavage of pro-IL-18 and pro-IL-1β, resulting in the generation of their active forms. The cellular lysis induced by cytokines is called Pyroptosis.*

Specifically, hippocampal synaptic plasticity would be enhanced by IL-18, which would not activate the nuclear factor-kB (NFkB) or inhibit BDNF [151–154] .

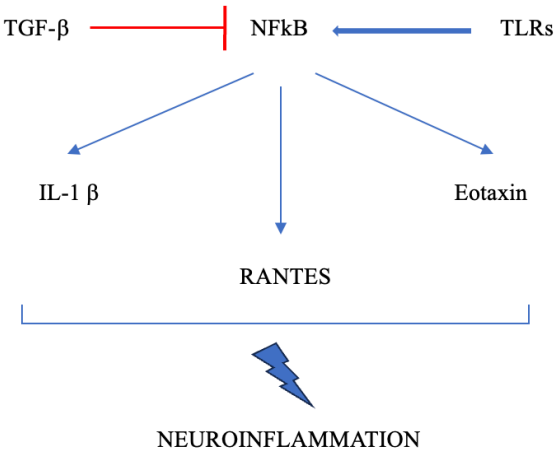
The action of the chemokine is effective leukocyte activator, which is a property that has the potential to be significant in a variety of inflammatory diseases. RANTES is an 8 kDa beta chemokine that plays a central function in the inflammatory immune response because of its capacity to attract and activate leukocytes [155]. RANTES levels were higher in the HD group than in the PAL-LAI patients at time T0. At T1, although the values of the various psychopathological domains were not substantially changed, RANTES levels increased in the same manner as already observed with IL-1β. The findings presented in this study indicate that modifications in the cytokines mentioned above may be associated with the disease itself. However, the use of antipsychotics can play a pivotal role in the production of cytokines and chemokines. Some in vitro studies have shown that haloperidol inducing macrophage activation results in stimulation of the production of pro-inflammatory cytokines, such as IL-1β[156]. The mechanism by which haloperidol affects the enhanced production of cytokines is elucidated through the activation of NFkB [157]. NFkB is a transcription factor that induces the expression of pro-inflammatory cytokines/chemokines (fig.7).

The administration of haloperidol has been seen to undergo a conversion process inside the brain, resulting in the formation of a free radical, with consequent oxidative neuron damage [158].

According to a literature review conducted by Nasrallah and Chen, it has been shown that haloperidol exhibits neurotoxic properties at various dosages in both in vitro and in vivo experiments. These neurotoxic effects are believed to be mediated by several molecular processes that ultimately lead to neuronal cell death[159]. The chronic administration of haloperidol has been linked to the occurrence of significant negative consequences, which may be attributed to the blocking of dopamine receptors and the subsequent neurotoxicity. These detrimental effects have been shown to be correlated with an elevation in the formation of ROS [160]. The metabolite known as haloperidol pyridinium exhibits a high level of toxicity and

contributes to the induction of oxidative stress, resulting in the occurrence of plasma membrane impairments[160,161].

**Figure 7 The Key Role of NFkB in Neuroinflammation**




*NFkB is a transcription factor that regulates innate immunity. It is expressed in* neurons and glia and activates transcription of cytokine genes involved in neuroinflammation. *The cascade activated by TLRs mediates the activation of NFkB and its subsequent translocation into the nucleus.*

*NFkB- Nuclear Factor-κB; TLRs- Toll Like Receptors*

Haloperidol has been found to induce oxidative stress through its modulation of cell metabolism. Specifically, it has been demonstrated that haloperidol treatment can increase mitochondrial activity, resulting in an elevated production of ROS [162,163]. In addition, the treatment of haloperidol has been shown to result in a reduction in the levels of BDNF, which is a neurotrophic factor that plays a role in neuronal survival and plasticity[164] .

Differently, SGAs have been shown to have neuroprotective properties via the upregulation of BDNF, which enhances cellular survival and promotes neurogenesis [165,166]

Multiple studies have shown the therapeutic efficacy of SGAs in modulating the activity of microglia, particularly in the decreasing of pro-inflammatory cytokines and ROS [165].

MacDowell et al. conducted an in vivo investigation wherein they demonstrated that the administration of paliperidone at a dosage of 1

mg/kg intraperitoneally could modulate the antioxidant and anti-inflammatory pathways in a rat model subjected to acute and chronic restraint stress [167]. The medicine exhibited the ability to increase the expression of nuclear factor erythroid-related factor 2 (Nrf2) and antioxidant response element-dependent antioxidant enzymes in response to acute stress. Additionally, it mitigated the downregulation of the body's natural antioxidant system caused by chronic stress. It is worth mentioning that paliperidone demonstrated the ability to enhance the levels of TGF-β1 and interleukin-10, as well as the population of M2-polarized microglial cells with an anti-inflammatory phenotype, in both acute and chronic stress [165,167]. Moreover, the potential antioxidant and anti-inflammatory effects of this SGAs may be attributed to its ability to block the production of nitric oxide (NO) and pro-inflammatory cytokines by activated microglia [168]. On the other hand, SGAs prevent the upregulation of interleukin IL-1β and TNF-α, as well as the activity of NO, cyclooxygenase, and NF-kB in the brain cortex. The distinctive characteristic of paliperidone lies in its capacity to regulate neuroinflammation through its interaction with Toll-like receptor 4 (TLR-4). In animals models the administration of paliperidone before the experiment effectively inhibited the activation of and mitigated neuroinflammation in the prefrontal cortex of rats subjected to stress. TLRs family are the fundamental components of innate immune response, which are responsible for detecting pathogen-associated molecular patterns and endogenous damage-associated molecular patterns (DAMPs)[169]. Lastly, an immunohistochemistry study revealed that the administration of paliperidone leads to a decrease in NFkB levels, resulting in a subsequent reduction in the expression of cytokines [170].

The blood levels of eotaxin were found to be significantly elevated in the group of patients receiving HD treatment compared to those receiving PAL-LAI treatment. In our sample patients with lower scores on MoCA had higher levels of Eotaxin. This data is consistent

with the fact that it implicates eotaxin-1/CCL11 in the age-related loss of hippocampus function, with cognitive decline, including memory and learning impairment[171–173]; it provides support for the idea of "accelerated aging" in schizophrenia [174–176].

Eotaxin levels are found to be elevated in patients with neurodegenerative illnesses and schizophrenia. Preclinical research suggests that eotaxin may be responsible for cognitive impairments[173]. The release of Eotaxin/CCL11 from active astrocytes causes oxidative stress to be triggered by the activation of microglia, which in turn increases glutamate-mediated neurotoxicity[172].

Expression of eotaxin, like IL-1β and RANTES, is stimulated by NFkB. This transcription factor can be inhibited by the anti-inflammatory cytokine TGF-β1, while it is activated by TLRs[177]. Recent data suggest that there is a link between the chemokine system in the brain and neurotransmitter systems. This interaction indicates that the endogenous chemokine system inside the brain works in combination with the neurotransmitter and neuropeptide systems to regulate the functioning of the brain. The chemokine system might be considered as the third prominent transmitter system within the brain [178].

In the HD-treated group, TGF-β1 levels were significantly reduced at 6 months. In contrast, TGF-β1 did not undergo a significant change in the PAL-LAI-treated group. Note, however, that at T1, TGF-β1 levels were significantly reduced more in the HD-treated group than in the PAL-LAI-treated group. In this study, the cognitive domain in patients with schizophrenia was directly correlated with levels of TGF-β1. Patients who received treatment with PAL-LAI had elevated levels of TGF-β1 and demonstrated superior cognitive function, as measured by the MoCA, compared to individuals who received treatment with HD.

In previous studies, the elevation of TGF-β1 was observed in individuals diagnosed with psychosis, as well as in individuals experiencing their first episode of psychosis and in those with relapsed schizophrenia. The analysis revealed that TGF-β1 exhibits potential as a significant biomarker for psychosis. The observed augmentation of anti-inflammatory/immunosuppressive activity in individuals with schizophrenia may represent a compensatory mechanism aimed at mitigating or constraining persistent pro-inflammatory mechanisms and modulating long-term inflammation [116]. TGF-β1 is involved in a variety of processes, some of which include anti-inflammatory, anti-apoptotic, protection against glutamate excitotoxicity, as well as neuroplasticity and neuroprotective action [186,187]. TGF-β1 activity is expressed through the induction of the SMAD (small mother against decapentaplegic) signaling, which facilitates neurogenesis and synaptogenesis [186]. Dopaminergic neurons use autocrine transmission of TGF-β1 to facilitate the development of axons and dendrites. Remarkably, the elimination of the TGF-β1 type II receptor in dopaminergic (DA) neurons results in a disturbance of both DA neurons and GABAergic receptors. Consequently, this disruption leads to an augmentation of inhibitory input, a reduction in excitatory synaptic input, and a modification of phasic firing patterns in dopaminergic neurons[179]. TGF-β1 signaling is crucial for neuronal function; it is possible that enhanced TGF-β1 signaling is necessary in the brain to preserve regular neuronal function amidst schizophrenia-related alterations. According to the so-called "GABAergic origin hypothesis" of schizophrenia, one possibility is that schizophrenia-like symptoms might be caused by NMDAR (N-methyl-D-aspartic acid-type glutamate receptors) hypofunction at GABAergic interneurons in particular [180].

Cortical interneurons are a type of inhibitory cells that are GABAergic in nature. They establish local connections within the neocortex and have a significant impact on the modulation of cortical network activity. It is postulated that the malfunction of these cells

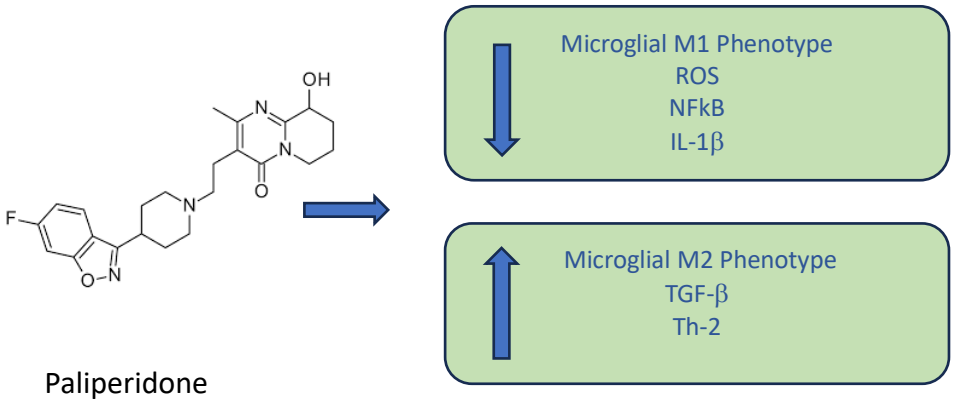
contributes to excessive excitation, which serves as the underlying mechanism for seizure-related disorders, including epilepsy, autism, and schizophrenia. The suppression of terminal differentiation of interneuron progenitors is observed with inhibition of TGF-β1 signaling, whereas the introduction of exogenous TGF-β1 expedites the transition of progenitors into postmitotic neurons [181].

In addition, the activity of NF-κB in normal cells is typically inhibited by TGF-β1 [182]. The fact that an increase in NF-κB was found in patients with treatment-resistant schizophrenia and in the initial stage of untreated schizophrenia patients suggests that NF-κB plays a role in the physiopathology of the condition from the very beginning [85,88,183,184].

Most of the cytokines and chemokines generated by microglial cells and astrocytes, both in their non-stimulated and activated states, are subject to direct regulation by the transcription factor NF-kB.

Numerous studies have demonstrated the capacity of antipsychotics to regulate the release of peripheral cytokines. This study aimed to assess the impact of PAL-LAI and HD, widely used as the second SGAs and first FGAs generation of antipsychotics, respectively, on the release of cytokines/chemokines. Nevertheless, the administration of haloperidol elicited a pro-inflammatory reaction, resulting in elevated levels of IL-1β, RANTES, and Eotaxin in the extracellular environment while concurrently reducing the presence of TGF-β. The administration of this conventional antipsychotic has the potential to elicit an inflammatory reaction through the activation of the p38 mitogen-activated protein kinase (p38 MAPK)/NFκB signaling pathways [185] The recent establishment of haloperidol's capacity to cause oxidative stress through a multi-modal mechanism, including enhanced dopamine metabolism, decreased glutathione content, stimulation of the NF-κB, and inhibition of complex I of the respiratory chain, has been documented [157,186].

**Figure 8 Neuroprotective Actions of Paliperidone**




Paliperidone

Emerging research indicates that oxidative stress plays a crucial role in the initial stages of schizophrenia pathogenesis. Moreover, a robust neurobiological association has been established between abnormalities in the dopaminergic system, excessive microglia activation, and oxidative stress. Various risk factors associated with schizophrenia contribute to the occurrence of oxidative stress, hence elevating the susceptibility to psychosis development. The development of extrapyramidal side effects is mainly contributed by oxidative stress generated by first-generation antipsychotics, such as haloperidol. Haloperidol additionally demonstrates neurotoxic properties through its ability to reduce levels of antioxidant enzymes, hence exacerbating pro-oxidant processes.

In contrast to haloperidol, SGAs, such as paliperidone, risperidone, clozapine, and olanzapine, demonstrate significant antioxidant effects in experimental models of schizophrenia. These effects are manifested through the restoration of the antioxidant system, leading to elevated levels of superoxide dismutase and glutathione (GSH) in the serum. SGAs have been found to enhance the antioxidant state and decrease lipid peroxidation in individuals diagnosed with schizophrenia. It is noteworthy that. SGAs, including risperidone, paliperidone, and notably clozapine, have the capacity to mitigate oxidative stress caused by excessive activation of microglia. This

reduction in oxidative stress is achieved by suppressing the generation of free radicals derived from microglia, ultimately safeguarding neurons against the detrimental effects of microglia-induced oxidative stress (fig.8) [165]. In contrast to neurotoxicity linked to the use of FGAs, several studies have indicated various neuroprotective effects connected with the SGAs [187].

Paliperidone exhibits inhibitory effects on some stress-induced inflammasome stimulations, with the aim of restoring the neuroinflammatory state that arises from stress. Given the growing recognition of inflammation's involvement in neuropsychiatric disorders, the exploration of novel pharmaceuticals that specifically target inflammasome pathways has significant potential as a viable strategy for future therapeutic interventions [188,189].

Neuroinflammation is mediated by innate immunity-related protein oligomers, the inflammasomes, as their primary motor. Various exogenous and endogenous noxious agents induce the formation of inflammasomes, the transmission of signals, and the production of proninflammatory cytokines (fig.6). The fundamental principle posits that prolonged activation of inflammasomes promotes neurodegeneration, whereas their transient activity reinstates tissue homeostasis [190].

In conclusion, our findings indicate that PAL-LAI and HD have distinct impacts on cytokines and chemokines levels, hence potentially exerting diverse influences on the neuroinflammatory processes linked to neuropsychiatric diseases.

**Conclusions**

Our findings, thereby providing further support for the hypothesis that schizophrenia is predicated on an immune system alteration.

Alterations in cytokine levels have been reported in the natural history of schizophrenia, specifically in patients with a duration of disease less than 10 years. Our study revealed a significant elevation in pro-inflammatory cytokines/chemokines, including IL-1β IL-18, RANTES, and Eotaxin, with a significant rise in the anti-inflammatory cytokine TGF-β1.

Both cytokines and chemokines correlate with the psychopathological dimensions of schizophrenia. IL-1β and IL-18 both correlate with the general symptomatologic scale, IL-1β in addition is directly correlated with positive symptomatology. Cognitive symptoms of schizophrenia correlate with TGF-β1 in a direct manner and with Eotaxin in an inverse manner. The correlations of the other cytokines/chemokines with the psychopathological dimensions of schizophrenia, did not reach the level of significance. Verisimilarly due to the small sample size.

Treatment with FGAs and SGAs affects cytokine/chemokine levels. HD tends to worsen pro-inflammatory cytokines, whereas Pal-LAI does not result in a worsening of neuroinflammation indices and on the contrary results in an increase in TGFβ1, which tends to increase to compensate for the increase in pro-inflammatory cytokines and has demonstrated neuroprotective activity.

Future studies on more extensive and heterogeneous samples, comparing patients at onset and patients with more years of disease history, are desirable.

It would also be desirable to compare oral treatments and treatments with long-acting antipsychotics, which allows to assess how and whether the pharmacokinetics of the molecules may impact the neuroinflammatory profile of schizophrenia.

**CONTENTS**

## References


[1] Dameshek: White blood cells in dementia praecox and... - Google Scholar n.d. https://scholar.google.com/scholar_lookup?title=White+blood+cells+in+dementia+praecox+and+dementia+paralytica&author=Dameshek+W&publication+year=1930&journal=Archives+of+Neurological+Psychiatry&volume=24 (accessed November 5, 2023).

[2] Lehmann-Facius H. Über die Liquordiagnose der Schizophrenien. Klin Wochenschr 1937; 16:1646–8. https://doi.org/10.1007/BF01776787/METRICS.

[3] SAUNDERS JC, MUCHMORE E. PHENOTHIAZINE EFFECT ON HUMAN ANTIBODY SYNTHESIS. Br J Psychiatry 1964; 110:84–9. https://doi.org/10.1192/BJP.110.464.84.

[4] Preble OT, Torrey EF. Serum interferon in patients with psychosis. Am J Psychiatry 1985; 142:1184–6. https://doi.org/10.1176/AJP.142.10.1184.

[5] Season of birth in schizophrenia: a review of evidence, methodology, and etiology - PubMed n.d. https://pubmed.ncbi.nlm.nih.gov/4080898/ (accessed November 5, 2023).

[6] Wright P, Takei N, Rifkin L, of RM-TA journal, 1995 undefined. Maternal influenza, obstetric complications, and schizophrenia. EuropepmcOrgP Wright, N Takei, L Rifkin, RM MurrayThe American Journal of Psychiatry, 1995•europepmcOrg n.d.

[7] Pollak TA, McCormack R, Peakman M, Nicholson TR, David AS. Prevalence of anti-N-methyl-d-aspartate (NMDA) receptor antibodies in patients with schizophrenia and related psychoses: a systematic review and meta-analysis‡. Psychol Med 2014; 44:2475–87. https://doi.org/10.1017/S003329171300295X.

[8] Upthegrove R, Barnes NM. The immune system and schizophrenia: an update for clinicians. Advances in Psychiatric Treatment 2014; 20:83–91. https://doi.org/10.1192/APT.BP.113.011452.

[9] Himmerich H, Sorge S, Kirkby KC, Steinberg H. [Schizophrenic disorders. The development of immunological concepts and therapy in psychiatry]. Nervenarzt 2012; 83:7–15. https://doi.org/10.1007/S00115-010-3205-3.

[10] Owen MJ, Sawa A, Mortensen PB. Schizophrenia. Lancet 2016; 388:86–97. https://doi.org/10.1016/S0140-6736(15)01121-6.

[11] Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR. Dementia praecox to schizophrenia: the first 100 years. Psychiatry Clin Neurosci 1999; 53:437–48. https://doi.org/10.1046/J.1440-1819.1999.00584.X.

[12] Shorter Edward. A history of psychiatry: from the era of the asylum to the age of Prozac 1997:436.

[13] E K, EJ E. Psychiatric observations on contemporary issues. Hist Psychiatry 1992; 3:253–6. https://doi.org/10.1177/0957154X9200301007.

[14] Moskowitz A, Heim G. Eugen Bleuler's Dementia Praecox, or the Group of Schizophrenias (1911): A Centenary Appreciation and Reconsideration. Schizophr Bull 2011; 37:471–9. https://doi.org/10.1093/SCHBUL/SBR016.

[15] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Diagnostic and Statistical Manual of Mental Disorders 2022. https://doi.org/10.1176/APPI.BOOKS.9780890425787.

[16] Tandon R. The Nosology of Schizophrenia. Toward DSM-5 and ICD-11. Psychiatric Clinics of North America 2012;35:557–69. https://doi.org/10.1016/j.psc.2012.06.001.
[17] Moskowitz A, Heim G. Eugen Bleuler's Dementia praecox or the group of schizophrenias (1911): a centenary appreciation and reconsideration. Schizophr Bull 2011;37:471–9. https://doi.org/10.1093/SCHBUL/SBR016.
[18] Kendler KS, Mishara A. The Prehistory of Schneider's First-Rank Symptoms: Texts From 1810 to 1932. Schizophr Bull 2019;45:971–90. https://doi.org/10.1093/SCHBUL/SBZ047.
[19] McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 2008;30:67–76. https://doi.org/10.1093/EPIREV/MXN001.
[20] Velligan DI, Rao S. The Epidemiology and Global Burden of Schizophrenia. J Clin Psychiatry 2023;84. https://doi.org/10.4088/JCP.MS21078COM5.
[21] McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia-An Overview. JAMA Psychiatry 2020;77:201–10. https://doi.org/10.1001/JAMAPSYCHIATRY.2019.3360.
[22] Luvsannyam E, Jain MS, Pormento MKL, Siddiqui H, Balagtas ARA, Emuze BO, et al. Neurobiology of Schizophrenia: A Comprehensive Review. Cureus 2022;14. https://doi.org/10.7759/CUREUS.23959.
[23] Cannon TD. The inheritance of intermediate phenotypes for schizophrenia. Curr Opin Psychiatry 2005;18:135–40. https://doi.org/10.1097/00001504-200503000-00005.
[24] Riley B, Kendler KS. Molecular genetic studies of schizophrenia. Eur J Hum Genet 2006;14:669–80. https://doi.org/10.1038/SJ.EJHG.5201571.
[25] Abi-Dargham A, Laruelle M, Aghajanian GK, Charney D, Krystal J. The role of serotonin in the pathophysiology and treatment of schizophrenia. J Neuropsychiatry Clin Neurosci 1997;9:1–17. https://doi.org/10.1176/JNP.9.1.1.
[26] McCutcheon RA, Krystal JH, Howes OD. Dopamine and glutamate in schizophrenia: biology, symptoms and treatment. World Psychiatry 2020;19:15. https://doi.org/10.1002/WPS.20693.
[27] de Jonge JC, Vinkers CH, Hulshoff Pol HE, Marsman A. GABAergic Mechanisms in Schizophrenia: Linking Postmortem and In Vivo Studies. Front Psychiatry 2017;8:118. https://doi.org/10.3389/FPSYT.2017.00118.
[28] Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G. A review on neural network models of schizophrenia and autism spectrum disorder. Neural Networks 2020;122:338–63. https://doi.org/10.1016/J.NEUNET.2019.10.014.
[29] Singh M, Badhwar R, Bagler G. Network biomarkers of schizophrenia by graph theoretical investigations of Brain Functional Networks n.d.
[30] Yang AC, Tsai SJ. New Targets for Schizophrenia Treatment beyond the Dopamine Hypothesis. Int J Mol Sci 2017;18. https://doi.org/10.3390/IJMS18081689.
[31] Sharkey RJ, Bacon C, Peterson Z, Rootes-Murdy K, Salvador R, Pomarol-Clotet E, et al. Neural Correlates of Positive and Negative Formal Thought Disorder in Individuals with Schizophrenia: An ENIGMA Schizophrenia Working Group Study. MedRxiv 2023. https://doi.org/10.1101/2023.06.06.23291034.
[32] Goghari VM, Sponheim SR, MacDonald AW. The Functional Neuroanatomy of Symptom Dimensions in Schizophrenia: A Qualitative and Quantitative Review of a Persistent Question. Neurosci Biobehav Rev 2010;34:468. https://doi.org/10.1016/J.NEUBIOREV.2009.09.004.
[33] Turner JA, Smyth P, Macciardi F, Fallon JH, Kennedy JL, Potkin SG. Imaging phenotypes and genotypes in schizophrenia. Neuroinformatics 2006 4:1 2006;4:21–49. https://doi.org/10.1385/NI:4:1:21.

[34] Birur B, Kraguljac NV, Shelton RC, Lahti AC. Brain structure, function, and neurochemistry in schizophrenia and bipolar disorder—a systematic review of the magnetic resonance neuroimaging literature. Npj Schizophrenia 2017 3:1 2017;3:1–15. https://doi.org/10.1038/s41537-017-0013-9.
[35] Karlsgodt KH, Sun D, Cannon TD. Structural and Functional Brain Abnormalities in Schizophrenia. Https://DoiOrg/101177/0963721410377601 2010;19:226–31. https://doi.org/10.1177/0963721410377601.
[36] Dietsche B, Kircher T, Falkenberg I. Structural brain changes in schizophrenia at different stages of the illness: A selective review of longitudinal magnetic resonance imaging studies. Http://DxDoiOrg/101177/0004867417699473 2017;51:500–8. https://doi.org/10.1177/0004867417699473.
[37] Davies C, Segre G, Estradé A, Radua J, De Micheli A, Provenzani U, et al. Prenatal and perinatal risk and protective factors for psychosis: a systematic review and meta-analysis. Lancet Psychiatry 2020;7:399–410. https://doi.org/10.1016/S2215-0366(20)30057-2.
[38] Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. The British Journal of Psychiatry 2011;198:173. https://doi.org/10.1192/BJP.BP.110.084384.
[39] Khokhar JY, Dwiel LL, Henricks AM, Doucette WT, Green AI. The Link Between Schizophrenia and Substance Use Disorder: A Unifying Hypothesis. Schizophr Res 2018;194:78. https://doi.org/10.1016/J.SCHRES.2017.04.016.
[40] González-Rodríguez A, Natividad M, Seeman M V., Paolini JP, Balagué A, Román E, et al. Schizophrenia: A Review of Social Risk Factors That Affect Women. Behavioral Sciences 2023;13. https://doi.org/10.3390/BS13070581.
[41] Jeppesen R, Benros ME. Autoimmune Diseases and Psychotic Disorders. Front Psychiatry 2019;10:131. https://doi.org/10.3389/FPSYT.2019.00131.
[42] Wiersma D, Nienhuis FJ, Slooff CJ, Giel R. Natural course of schizophrenic disorders: a 15-year followup of a Dutch incidence cohort. Schizophr Bull 1998;24:75–85. https://doi.org/10.1093/OXFORDJOURNALS.SCHBUL.A033315.
[43] Heilbronner U, Samara M, Leucht S, Falkai P, Schulze TG. The Longitudinal Course of Schizophrenia Across the Lifespan: Clinical, Cognitive, and Neurobiological Aspects. Harv Rev Psychiatry 2016;24:118. https://doi.org/10.1097/HRP.0000000000000092.
[44] Molstrom IM, Nordgaard J, Urfer-Parnas A, Handest R, Berge J, Henriksen MG. The prognosis of schizophrenia: A systematic review and meta-analysis with meta-regression of 20-year follow-up studies. Schizophr Res 2022;250:152–63. https://doi.org/10.1016/J.SCHRES.2022.11.010.
[45] Massachusetts General Hospital Handbook of General Hospital Psychiatry. Massachusetts General Hospital Handbook of General Hospital Psychiatry 2010. https://doi.org/10.1016/C2009-0-55410-4.
[46] DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: The Devil is in the Details. J Neurochem 2016;139:136. https://doi.org/10.1111/JNC.13607.
[47] Najjar S, Pearlman DM. Neuroinflammation and white matter pathology in schizophrenia: systematic review. Schizophr Res 2015;161:102–12. https://doi.org/10.1016/J.SCHRES.2014.04.041.
[48] Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. Acta Neuropathol 2001;101:249–55. https://doi.org/10.1007/S004010000284.

[49] Muzio L, Viotti A, Martino G. Microglia in Neuroinflammation and Neurodegeneration: From Understanding to Therapy. Front Neurosci 2021;15:742065. https://doi.org/10.3389/FNINS.2021.742065/BIBTEX.
[50] Comer AL, Carrier M, Tremblay MÈ, Cruz-Martín A. The Inflamed Brain in Schizophrenia: The Convergence of Genetic and Environmental Risk Factors That Lead to Uncontrolled Neuroinflammation. Front Cell Neurosci 2020;14:564901. https://doi.org/10.3389/FNCEL.2020.00274/BIBTEX.
[51] Madore C, Yin Z, Leibowitz J, Butovsky O. Microglia, Lifestyle Stress, and Neurodegeneration. Immunity 2020;52:222–40. https://doi.org/10.1016/J.IMMUNI.2019.12.003.
[52] Popa-Wagner A, Dumitrascu D, Capitanescu B, Petcu E, Surugiu R, Fang WH, et al. Dietary habits, lifestyle factors and neurodegenerative diseases. Neural Regen Res 2020;15:394–400. https://doi.org/10.4103/1673-5374.266045.
[53] Matzinger P, Kamala T. Tissue-based class control: the other side of tolerance. Nat Rev Immunol 2011;11:221–30. https://doi.org/10.1038/NRI2940.
[54] Midwood KS, Piccinini AM. DAMPening inflammation by modulating TLR signalling. Mediators Inflamm 2010;2010. https://doi.org/10.1155/2010/672395.
[55] O'Callaghan JP, Sriram K, Miller DB. Defining "neuroinflammation." Ann N Y Acad Sci 2008;1139:318–30. https://doi.org/10.1196/ANNALS.1432.032.
[56] Estes ML, McAllister AK. Alterations in immune cells and mediators in the brain: it's not always neuroinflammation! Brain Pathol 2014;24:623–30. https://doi.org/10.1111/BPA.12198.
[57] Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–55. https://doi.org/10.1016/S1471-4906(02)02302-5.
[58] Tremblay MÈ. Microglial functional alteration and increased diversity in the challenged brain: Insights into novel targets for intervention. Brain Behav Immun Health 2021;16:100301. https://doi.org/10.1016/J.BBIH.2021.100301.
[59] Christoffel DJ, Golden SA, Russo SJ. Structural and synaptic plasticity in stress-related disorders. Rev Neurosci 2011;22:535–49. https://doi.org/10.1515/RNS.2011.044/MACHINEREADABLECITATION/RIS.
[60] Bernardinelli Y, Nikonenko I, Muller D. Structural plasticity: Mechanisms and contribution to developmental psychiatric disorders. Front Neuroanat 2014;8:110995. https://doi.org/10.3389/FNANA.2014.00123/BIBTEX.
[61] Koizumi T, Kerkhofs D, Mizuno T, Steinbusch HWM, Foulquier S. Vessel-Associated Immune Cells in Cerebrovascular Diseases: From Perivascular Macrophages to Vessel-Associated Microglia. Front Neurosci 2019;13. https://doi.org/10.3389/FNINS.2019.01291.
[62] Dudvarski Stankovic N, Teodorczyk M, Ploen R, Zipp F, Schmidt MHH. Microglia–blood vessel interactions: a double-edged sword in brain pathologies. Acta Neuropathologica 2015 131:3 2015;131:347–63. https://doi.org/10.1007/S00401-015-1524-Y.
[63] Monji A, Kato TA, Mizoguchi Y, Horikawa H, Seki Y, Kasai M, et al. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog Neuropsychopharmacol Biol Psychiatry 2013;42:115–21. https://doi.org/10.1016/J.PNPBP.2011.12.002.
[64] Najjar S, Pahlajani S, De Sanctis V, Stern JNH, Najjar A, Chong D. Neurovascular Unit Dysfunction and Blood–Brain Barrier Hyperpermeability Contribute to Schizophrenia Neurobiology: A Theoretical Integration of Clinical and Experimental Evidence. Front Psychiatry 2017;8:1. https://doi.org/10.3389/FPSYT.2017.00083.

[65] Schöppe J, Ehrenmann J, Klenk C, Rucktooa P, Schütz M, Doré AS, et al. Crystal structures of the human neurokinin 1 receptor in complex with clinically used antagonists. Nat Commun 2019;10. https://doi.org/10.1038/S41467-018-07939-8.
[66] da Fonseca ACC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, et al. The impact of microglial activation on blood-brain barrier in brain diseases. Front Cell Neurosci 2014;8:1–13. https://doi.org/10.3389/FNCEL.2014.00362.
[67] Uranova NA, Vikhreva O V., Rakhmanova VI, Orlovskaya DD. Ultrastructural pathology of oligodendrocytes adjacent to microglia in prefrontal white matter in schizophrenia. NPJ Schizophr 2018;4. https://doi.org/10.1038/S41537-018-0068-2.
[68] Pike AF, Longhena F, Faustini G, van Eik JM, Gombert I, Herrebout MAC, et al. Dopamine signaling modulates microglial NLRP3 inflammasome activation: implications for Parkinson's disease. J Neuroinflammation 2022;19. https://doi.org/10.1186/S12974-022-02410-4.
[69] Zhang Y, Catts VS, Sheedy D, McCrossin T, Kril JJ, Shannon Weickert C. Cortical grey matter volume reduction in people with schizophrenia is associated with neuro-inflammation. Transl Psychiatry 2016;6. https://doi.org/10.1038/TP.2016.238.
[70] Zhang Y, Catts VS, Sheedy D, McCrossin T, Kril JJ, Shannon Weickert C. Cortical grey matter volume reduction in people with schizophrenia is associated with neuro-inflammation. Transl Psychiatry 2016;6. https://doi.org/10.1038/TP.2016.238.
[71] Lau JYF, Pine DS. Elucidating risk mechanisms of gene-environment interactions on pediatric anxiety: integrating findings from neuroscience. Eur Arch Psychiatry Clin Neurosci 2008;258:97–106. https://doi.org/10.1007/S00406-007-0788-1.
[72] Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. Journal of Neuroinflammation 2013 10:1 2013;10:1–24. https://doi.org/10.1186/1742-2094-10-43.
[73] Dickerson F, Stallings C, Origoni A, Schroeder J, Katsafanas E, Schweinfurth L, et al. Inflammatory Markers in Recent Onset Psychosis and Chronic Schizophrenia. Schizophr Bull 2016;42:134–41. https://doi.org/10.1093/SCHBUL/SBV108.
[74] Meyer JM, McEvoy JP, Davis VG, Goff DC, Nasrallah HA, Davis SM, et al. Inflammatory markers in schizophrenia: comparing antipsychotic effects in phase 1 of the clinical antipsychotic trials of intervention effectiveness study. Biol Psychiatry 2009;66:1013–22. https://doi.org/10.1016/J.BIOPSYCH.2009.06.005.
[75] Radewicz K, Garey LJ, Gentleman SM, Reynolds R. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. J Neuropathol Exp Neurol 2000;59:137–50. https://doi.org/10.1093/JNEN/59.2.137.
[76] Doorduin J, De Vries EFJ, Willemsen ATM, De Groot JC, Dierckx RA, Klein HC. Neuroinflammation in Schizophrenia-Related Psychosis: A PET Study. Journal of Nuclear Medicine 2009;50:1801–7. https://doi.org/10.2967/JNUMED.109.066647.
[77] Brisch R, Saniotis A, Wolf R, Bielau H, Bernstein HG, Steiner J, et al. The Role of Dopamine in Schizophrenia from a Neurobiological and Evolutionary Perspective: Old Fashioned, but Still in Vogue. Front Psychiatry 2014;5. https://doi.org/10.3389/FPSYT.2014.00047.
[78] Channer B, Matt SM, Nickoloff-Bybel EA, Pappa V, Agarwal Y, Wickman J, et al. Dopamine, Immunity, and Disease. Pharmacol Rev 2023;75:62–158. https://doi.org/10.1124/PHARMREV.122.000618.
[79] Vidal PM, Pacheco R. The Cross-Talk Between the Dopaminergic and the Immune System Involved in Schizophrenia. Front Pharmacol 2020;11:508364. https://doi.org/10.3389/FPHAR.2020.00394/BIBTEX.

[80] Momtazmanesh S, Zare-Shahabadi A, Rezaei N. Cytokine Alterations in Schizophrenia: An Updated Review. Front Psychiatry 2019;10:1. https://doi.org/10.3389/FPSYT.2019.00892.
[81] Meyer JH, Cervenka S, Kim MJ, Kreisl WC, Henter ID, Innis RB. Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. Lancet Psychiatry 2020;7:1064. https://doi.org/10.1016/S2215-0366(20)30255-8.
[82] Conen S, Gregory CJ, Hinz R, Smallman R, Corsi-Zuelli F, Deakin B, et al. Neuroinflammation as measured by positron emission tomography in patients with recent onset and established schizophrenia: implications for immune pathogenesis. Molecular Psychiatry 2020 26:9 2020;26:5398–406. https://doi.org/10.1038/s41380-020-0829-y.
[83] Marques TR, Ashok AH, Pillinger T, Veronese M, Turkheimer FE, Dazzan P, et al. Neuroinflammation in schizophrenia: meta-analysis of in vivo microglial imaging studies. Psychol Med 2019;49:2186. https://doi.org/10.1017/S0033291718003057.
[84] Choudhury Z, Lennox B. Maternal Immune Activation and Schizophrenia–Evidence for an Immune Priming Disorder. Front Psychiatry 2021;12:585742. https://doi.org/10.3389/FPSYT.2021.585742.
[85] Zhang Y, Catts VS, Sheedy D, McCrossin T, Kril JJ, Shannon Weickert C. Cortical grey matter volume reduction in people with schizophrenia is associated with neuro-inflammation. Transl Psychiatry 2016;6. https://doi.org/10.1038/TP.2016.238.
[86] Volk DW, Moroco AE, Roman KM, Edelson JR, Lewis DA. The Role of the Nuclear Factor-κB Transcriptional Complex in Cortical Immune Activation in Schizophrenia. Biol Psychiatry 2019;85:25–34. https://doi.org/10.1016/J.BIOPSYCH.2018.06.015.
[87] Fillman SG, Cloonan N, Catts VS, Miller LC, Wong J, Mccrossin T, et al. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. Mol Psychiatry 2013;18:206–14. https://doi.org/10.1038/MP.2012.110.
[88] Murphy CE, Walker AK, Weickert CS. Neuroinflammation in schizophrenia: the role of nuclear factor kappa B. Transl Psychiatry 2021;11. https://doi.org/10.1038/S41398-021-01607-0.
[89] Murphy CE, Walker AK, Weickert CS. Neuroinflammation in schizophrenia: the role of nuclear factor kappa B. Transl Psychiatry 2021;11. https://doi.org/10.1038/S41398-021-01607-0.
[90] Müller N. Inflammation in Schizophrenia: Pathogenetic Aspects and Therapeutic Considerations. Schizophr Bull 2018;44:973. https://doi.org/10.1093/SCHBUL/SBY024.
[91] Najjar S, Pearlman DM. Neuroinflammation and white matter pathology in schizophrenia: systematic review. Schizophr Res 2015;161:102–12. https://doi.org/10.1016/J.SCHRES.2014.04.041.
[92] Monji A, Kato TA, Mizoguchi Y, Horikawa H, Seki Y, Kasai M, et al. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog Neuropsychopharmacol Biol Psychiatry 2013;42:115–21. https://doi.org/10.1016/J.PNPBP.2011.12.002.
[93] Lewko WM, Oldham RK. Cytokines. Principles of Cancer Biotherapy: 5th Edition 2009:155–276. https://doi.org/10.1007/978-90-481-2289-9_8/COVER.
[94] Zhang JM, An J. Cytokines, Inflammation and Pain. Int Anesthesiol Clin 2007;45:27. https://doi.org/10.1097/AIA.0B013E318034194E.
[95] Smith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 2012;87:10. https://doi.org/10.1016/J.BRAINRESBULL.2011.10.004.
[96] Colton CA, Wilcock DM. Assessing activation states in microglia. CNS Neurol Disord Drug Targets 2010;9:174–91. https://doi.org/10.2174/187152710791012053.

[97] Boiko AS, Mednova IA, Kornetova EG, Semke A V., Bokhan NA, Ivanova SA. Cell Adhesion Molecules in Schizophrenia Patients with Metabolic Syndrome. Metabolites 2023;13. https://doi.org/10.3390/METABO13030376/S1.
[98] Falkai P, Schmitt A. Failed regeneration and inflammation in schizophrenia: two sides of the same coin? J Neural Transm 2022;129:611–5. https://doi.org/10.1007/S00702-022-02496-3/FIGURES/1.
[99] Aricioglu F, Ozkartal CS, Unal G, Dursun S, Cetin M, Müller N. Neuroinflammation in Schizophrenia: A Critical Review and The Future. Klinik Psikofarmakoloji Bülteni-Bulletin of Clinical Psychopharmacology 2016;26:429–37. https://doi.org/10.5455/BCP.20161123044657.
[100] Foxman EF, Campbell JJ, Butcher EC. Multistep navigation and the combinatorial control of leukocyte chemotaxis. J Cell Biol 1997;139:1349–60. https://doi.org/10.1083/JCB.139.5.1349.
[101] Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. FEBS J 2018;285:2944. https://doi.org/10.1111/FEBS.14466.
[102] Gebicke-Haerter PJ, Spleiss O, Ren LQ, Li H, Dichmann S, Norgauer J, et al. Microglial chemokines and chemokine receptors. Prog Brain Res 2001;132:525–32. https://doi.org/10.1016/S0079-6123(01)32100-3.
[103] Rodrigues-Amorim D, Rivera-Baltanás T, Spuch C, Caruncho HJ, González-Fernandez Á, Olivares JM, et al. Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. Schizophr Res 2018;197:19–33. https://doi.org/10.1016/J.SCHRES.2017.11.023.
[104] Reale M, Costantini E, Greig NH. Cytokine Imbalance in Schizophrenia. From Research to Clinic: Potential Implications for Treatment. Front Psychiatry 2021;12:536257. https://doi.org/10.3389/FPSYT.2021.536257/BIBTEX.
[105] Tremblay M-È, Schiavone S, Howells FM, Reale M, Costantini E, Greig NH. Cytokine Imbalance in Schizophrenia. From Research to Clinic: Potential Implications for Treatment. Frontiers in Psychiatry | WwwFrontiersinOrg 2021;1:536257. https://doi.org/10.3389/fpsyt.2021.536257.
[106] Amoli MM, Khatami F, Arzaghi SM, Enayati S, Nejatisafa AA. Over-expression of TGF-β1 gene in medication free Schizophrenia. Psychoneuroendocrinology 2019;99:265–70. https://doi.org/10.1016/J.PSYNEUEN.2018.10.009.
[107] Frydecka D, Misiak B, Pawlak-Adamska E, Karabon L, Tomkiewicz A, Sedlaczek P, et al. Sex differences in TGFB-β signaling with respect to age of onset and cognitive functioning in schizophrenia. Neuropsychiatr Dis Treat 2015;11:575. https://doi.org/10.2147/NDT.S74672.
[108] Marcinowicz P, Więdłocha M, Zborowska N, Dębowska W, Podwalski P, Misiak B, et al. A Meta-Analysis of the Influence of Antipsychotics on Cytokines Levels in First Episode Psychosis. Journal of Clinical Medicine 2021, Vol 10, Page 2488 2021;10:2488. https://doi.org/10.3390/JCM10112488.
[109] Patlola SR, Donohoe G, McKernan DP. The relationship between inflammatory biomarkers and cognitive dysfunction in patients with schizophrenia: A systematic review and meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry 2023;121:110668. https://doi.org/10.1016/J.PNPBP.2022.110668.
[110] Baek SH, Kim H, Kim JW, Ryu S, Lee JY, Kim JM, et al. Association between Peripheral Inflammatory Cytokines and Cognitive Function in Patients with First-Episode Schizophrenia. J Pers Med 2022;12. https://doi.org/10.3390/JPM12071137/S1.
[111] Momtazmanesh S, Zare-Shahabadi A, Rezaei N. Cytokine Alterations in Schizophrenia: An Updated Review. Front Psychiatry 2019;10:1. https://doi.org/10.3389/FPSYT.2019.00892.

[112] Tremblay M-È, Schiavone S, Howells FM, Reale M, Costantini E, Greig NH. Cytokine Imbalance in Schizophrenia. From Research to Clinic: Potential Implications for Treatment. Frontiers in Psychiatry | WwwFrontiersinOrg 2021;1:536257. https://doi.org/10.3389/fpsyt.2021.536257.
[113] Ermakov EA, Melamud MM, Buneva VN, Ivanova SA. Immune System Abnormalities in Schizophrenia: An Integrative View and Translational Perspectives. Front Psychiatry 2022;13. https://doi.org/10.3389/FPSYT.2022.880568.
[114] Dawidowski B, Górniak A, Podwalski P, Lebiecka Z, Misiak B, Samochowiec J. The Role of Cytokines in the Pathogenesis of Schizophrenia. J Clin Med 2021;10:10. https://doi.org/10.3390/JCM10173849.
[115] Momtazmanesh S, Zare-Shahabadi A, Rezaei N. Cytokine Alterations in Schizophrenia: An Updated Review. Front Psychiatry 2019;10:1. https://doi.org/10.3389/FPSYT.2019.00892.
[116] Borovcanin M, Dejanovic SD, Radosavljevic G, Jovanovic I, Stefanovic V, Arsenijevic N, et al. 2030 – Can TGF- β Be a Valuable Marker For Psychosis? European Psychiatry 2013;28:1–1. https://doi.org/10.1016/S0924-9338(13)76960-8.
[117] Patlola SR, Donohoe G, McKernan DP. Anti-inflammatory effects of 2nd generation antipsychotics in patients with schizophrenia: A systematic review and meta-analysis. J Psychiatr Res 2023;160:126–36. https://doi.org/10.1016/J.JPSYCHIRES.2023.01.042.
[118] De Witte L, Tomasik J, Schwarz E, Guest PC, Rahmoune H, Kahn RS, et al. Cytokine alterations in first-episode schizophrenia patients before and after antipsychotic treatment. Schizophr Res 2014;154:23–9. https://doi.org/10.1016/J.SCHRES.2014.02.005.
[119] Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: Clinical status and antipsychotic effects. Biol Psychiatry 2011;70:663–71. https://doi.org/10.1016/J.BIOPSYCH.2011.04.013.
[120] Noto MN, Maes M, Nunes SOV, Ota VK, Rossaneis AC, Verri WA, et al. Activation of the immune-inflammatory response system and the compensatory immune-regulatory system in antipsychotic naive first episode psychosis. European Neuropsychopharmacology 2019;29:416–31. https://doi.org/10.1016/J.EURONEURO.2018.12.008.
[121] Marcinowicz P, Więdłocha M, Zborowska N, Dębowska W, Podwalski P, Misiak B, et al. A Meta-Analysis of the Influence of Antipsychotics on Cytokines Levels in First Episode Psychosis. J Clin Med 2021;10. https://doi.org/10.3390/JCM10112488.
[122] Miller AH, Haroon E, Raison CL, Felger JC. Cytokine Targets in the Brain: Impact on Neurotransmitters and Neurocircuits. Depress Anxiety 2013;30:297. https://doi.org/10.1002/DA.22084.
[123] Hodo TW, de Aquino MTP, Shimamoto A, Shanker A. Critical Neurotransmitters in the Neuroimmune Network. Front Immunol 2020;11:523925. https://doi.org/10.3389/FIMMU.2020.01869/BIBTEX.
[124] Bourgognon J-M, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. Https://DoiOrg/101177/2398212820979802 2020;4:239821282097980. https://doi.org/10.1177/2398212820979802.
[125] Ermakov EA, Mednova IA, Boiko AS, Buneva VN, Ivanova SA. Chemokine Dysregulation and Neuroinflammation in Schizophrenia: A Systematic Review. Int J Mol Sci 2023;24. https://doi.org/10.3390/IJMS24032215.
[126] Wennerås C, Matsumoto K, Bochner BS, Cook-Mills JM, Asosingh K, Erzurum SC, et al. Eosinophil Trafficking. Eosinophils in Health and Disease 2013:121–66. https://doi.org/10.1016/B978-0-12-394385-9.00006-7.

[127] Stuart MJ, Baune BT. Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: a systematic review of biomarker studies. Neurosci Biobehav Rev 2014;42:93–115. https://doi.org/10.1016/J.NEUBIOREV.2014.02.001.
[128] Hong S, Lee EE, Martin AS, Soontornniyomkij B, Soontornniyomkij V, Achim CL, et al. Abnormalities in Chemokine Levels in Schizophrenia and Their Clinical Correlates. Schizophr Res 2017;181:63. https://doi.org/10.1016/J.SCHRES.2016.09.019.
[129] Stuart MJ, Singhal G, Baune BT. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. Front Cell Neurosci 2015;9:160259. https://doi.org/10.3389/FNCEL.2015.00357/BIBTEX.
[130] Beumer W, Drexhage RC, De Wit H, Versnel MA, Drexhage HA, Cohen D. Increased level of serum cytokines, chemokines and adipokines in patients with schizophrenia is associated with disease and metabolic syndrome. Psychoneuroendocrinology 2012;37:1901–11. https://doi.org/10.1016/J.PSYNEUEN.2012.04.001.
[131] Réaux-Le Goazigo A, Van Steenwinckel J, Rostène W, Mélik Parsadaniantz S. Current status of chemokines in the adult CNS. Prog Neurobiol 2013;104:67–92. https://doi.org/10.1016/J.PNEUROBIO.2013.02.001.
[132] Stuart MJ, Singhal G, Baune BT. Systematic Review of the Neurobiological Relevance of Chemokines to Psychiatric Disorders. Front Cell Neurosci 2015;9:1. https://doi.org/10.3389/FNCEL.2015.00357.
[133] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Diagnostic and Statistical Manual of Mental Disorders 2022. https://doi.org/10.1176/APPI.BOOKS.9780890425787.
[134] Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261–76. https://doi.org/10.1093/SCHBUL/13.2.261.
[135] Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. J Am Geriatr Soc 2005;53:695–9. https://doi.org/10.1111/J.1532-5415.2005.53221.X.
[136] Yang Z, Abdul Rashid NA, Quek YF, Lam M, See YM, Maniam Y, et al. Montreal Cognitive Assessment as a screening instrument for cognitive impairments in schizophrenia. Schizophr Res 2018;199:58–63. https://doi.org/10.1016/J.SCHRES.2018.03.008.
[137] Yan F, Meng X, Cheng X, Pei W, Chen Y, Chen L, et al. Potential role between inflammatory cytokines and Tie-2 receptor levels and clinical symptoms in patients with first-episode schizophrenia. BMC Psychiatry 2023;23:1–10. https://doi.org/10.1186/S12888-023-04913-7/FIGURES/1.
[138] Müller N. THEMED ISSUE Inflammation in Schizophrenia: Pathogenetic Aspects and Therapeutic Considerations. Schizophr Bull 2018;44:973–82. https://doi.org/10.1093/schbul/sby024.
[139] Liu JY, Chen HY, Lin JJ, Lu MK, Tan HP, Jang FL, et al. Alterations of plasma cytokine biomarkers for identifying age at onset of schizophrenia with neurological soft signs. Int J Med Sci 2020;17:255–62. https://doi.org/10.7150/IJMS.38891.
[140] Miller BJ, Goldsmith DR. Inflammatory biomarkers in schizophrenia: Implications for heterogeneity and neurobiology. Biomark Neuropsychiatry 2019;1:100006. https://doi.org/10.1016/J.BIONPS.2019.100006.
[141] Kim H, Baek SH, Kim JW, Ryu S, Lee JY, Kim JM, et al. Inflammatory markers of symptomatic remission at 6 months in patients with first-episode schizophrenia. Schizophrenia 2023 9:1 2023;9:1–7. https://doi.org/10.1038/s41537-023-00398-1.

[142] Mednova IA, Boiko AS, Kornetova EG, Semke A V., Bokhan NA, Ivanova SA. Cytokines as Potential Biomarkers of Clinical Characteristics of Schizophrenia. Life 2022;12. https://doi.org/10.3390/LIFE12121972/S1.
[143] Miller BJ, Goldsmith DR. Inflammatory biomarkers in schizophrenia: Implications for heterogeneity and neurobiology 2019. https://doi.org/10.1016/j.bionps.2019.100006.
[144] Papiol S, Rosa A, Gutiérrez B, Martín B, Salgado P, Catalán R, et al. Interleukin-1 cluster is associated with genetic risk for schizophrenia and bipolar disorder. J Med Genet 2004;41:219–23. https://doi.org/10.1136/JMG.2003.012914.
[145] Potter ED, Ling ZD, Carvey PM. Cytokine-induced conversion of mesencephalic-derived progenitor cells into dopamine neurons. Cell Tissue Res 1999;296:235–46. https://doi.org/10.1007/S004410051285.
[146] Moraes CA, Hottz ED, Dos Santos Ornellas D, Adesse D, de Azevedo CT, d'Avila JC, et al. Microglial NLRP3 Inflammasome Induces Excitatory Synaptic Loss Through IL-1β-Enriched Microvesicle Release: Implications for Sepsis-Associated Encephalopathy. Mol Neurobiol 2023;60:481–94. https://doi.org/10.1007/S12035-022-03067-Z.
[147] Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. Int J Mol Sci 2019;20. https://doi.org/10.3390/IJMS20133328.
[148] Liu R, Tang W, Wang W, Xu F, Fan W, Zhang Y, et al. NLRP3 Influences Cognitive Function in Schizophrenia in Han Chinese. Front Genet 2021;12. https://doi.org/10.3389/FGENE.2021.781625/FULL.
[149] Syed AAS, He L, Shi Y, Mahmood S. Elevated levels of IL-18 associated with schizophrenia and first episode psychosis: A systematic review and meta-analysis. Early Interv Psychiatry 2021;15:896–905. https://doi.org/10.1111/EIP.13031.
[150] Syed AAS, He L, Shi Y, Mahmood S. Elevated levels of IL-18 associated with schizophrenia and first episode psychosis: A systematic review and meta-analysis. Early Interv Psychiatry 2021;15:896–905. https://doi.org/10.1111/EIP.13031.
[151] Bossù P, Piras F, Palladino I, Iorio M, Salani F, Ciaramella A, et al. Hippocampal volume and depressive symptoms are linked to serum IL-18 in schizophrenia. Neurology - Neuroimmunology Neuroinflammation 2015;2. https://doi.org/10.1212/NXI.0000000000000111.
[152] Alboni S, Montanari C, Benatti C, Sanchez-Alavez M, Rigillo G, Blom JMC, et al. Interleukin 18 activates MAPKs and STAT3 but not NF-κB in hippocampal HT-22 cells. Brain Behav Immun 2014;40:85–94. https://doi.org/10.1016/J.BBI.2014.02.015.
[153] Prieto GA, Tong L, Smith ED, Cotman CW. TNFα and IL-1β but not IL-18 Suppresses Hippocampal Long-Term Potentiation Directly at the Synapse. Neurochem Res 2019;44:49–60. https://doi.org/10.1007/S11064-018-2517-8.
[154] Yamanishi K, Doe N, Mukai K, Ikubo K, Hashimoto T, Uwa N, et al. Interleukin-18-deficient mice develop hippocampal abnormalities related to possible depressive-like behaviors. Neuroscience 2019;408:147–60. https://doi.org/10.1016/J.NEUROSCIENCE.2019.04.003.
[155] Appay V, Rowland-Jones SL. RANTES: a versatile and controversial chemokine. Trends Immunol 2001;22:83–7. https://doi.org/10.1016/S1471-4906(00)01812-3.
[156] Da Cruz Jung IE, Machado AK, Da Cruz IBM, Barbisan F, Azzolin VF, Duarte T, et al. Haloperidol and Risperidone at high concentrations activate an in vitro inflammatory response of RAW 264.7 macrophage cells by induction of apoptosis and modification of cytokine levels. Psychopharmacology (Berl) 2016;233:1715–23. https://doi.org/10.1007/S00213-015-4079-7.

[157] Raudenska M, Gumulec J, Babula P, Stracina T, Sztalmachova M, Polanska H, et al. Haloperidol Cytotoxicity and Its Relation to Oxidative Stress. Mini-Reviews in Medicinal Chemistry 2013;13:1993–8. https://doi.org/10.2174/13895575113136660100.
[158] Subramanyam B, Rollema H, Woolf T, Castagnoli N. Identification of a potentially neurotoxic pyridinium metabolite of haloperidol in rats. Biochem Biophys Res Commun 1990;166:238–44. https://doi.org/10.1016/0006-291X(90)91936-M.
[159] Nasrallah H, - AC, 29 undefined, 3 undefined, 2017 undefined. Multiple neurotoxic effects of haloperidol resulting in neuronal death. IngentaconnectComHA Nasrallah, AT Chen, 29, 3, 2017•ingentaconnectCom n.d.
[160] Samad N, Haleem DJ. Antioxidant effects of rice bran oil mitigate repeated haloperidol-induced tardive dyskinesia in male rats. Metab Brain Dis 2017;32:1099–107. https://doi.org/10.1007/S11011-017-0002-8/METRICS.
[161] Murata T, Maruoka N, Omata N, Takashima Y, Igarashi K, Kasuya F, et al. Effects of haloperidol and its pyridinium metabolite on plasma membrane permeability and fluidity in the rat brain. Elsevier 2007;31:848–57. https://doi.org/10.1016/j.pnpbp.2007.01.023.
[162] Heiser P, Sommer O, Schmidt AJ, Clement HW, Hoinkes A, Hopt UT, et al. Effects of antipsychotics and vitamin C on the formation of reactive oxygen species. Http://DxDoiOrg/101177/0269881109102538 2009;24:1499–504. https://doi.org/10.1177/0269881109102538.
[163] Kropp S, Kern V, Lange K, Degner D, Hajak G, Kornhuber J, et al. Oxidative stress during treatment with first- and second-generation antipsychotics. Journal of Neuropsychiatry and Clinical Neurosciences 2005;17:227–31. https://doi.org/10.1176/JNP.17.2.227/ASSET/IMAGES/LARGE/RJ0012T2.JPEG.
[164] Pillai A, Veeranan-Karmegam R, Dhandapani KM, Mahadik SP. Cystamine prevents haloperidol-induced decrease of BDNF/TrkB signaling in mouse frontal cortex. J Neurochem 2008;107:941–51. https://doi.org/10.1111/J.1471-4159.2008.05665.X.
[165] Caruso G, Grasso M, Fidilio A, Tascedda F, Drago F, Caraci F. Antioxidant Properties of Second-Generation Antipsychotics: Focus on Microglia. Pharmaceuticals 2020, Vol 13, Page 457 2020;13:457. https://doi.org/10.3390/PH13120457.
[166] Kusumi I, Boku S, Takahashi Y. Psychopharmacology of atypical antipsychotic drugs: From the receptor binding profile to neuroprotection and neurogenesis. Psychiatry Clin Neurosci 2015;69:243–58. https://doi.org/10.1111/pcn.12242.
[167] MacDowell KS, Caso JR, Martín-Hernández D, Moreno BM, Madrigal JLM, Micó JA, et al. The Atypical Antipsychotic Paliperidone Regulates Endogenous Antioxidant/Anti-Inflammatory Pathways in Rat Models of Acute and Chronic Restraint Stress. Neurotherapeutics 2016;13:833–43. https://doi.org/10.1007/S13311-016-0438-2.
[168] Kato T, Monji A, Hashioka S, Kanba S. Risperidone significantly inhibits interferon-γ-induced microglial activation in vitro. Schizophr Res 2007;92:108–15. https://doi.org/10.1016/J.SCHRES.2007.01.019.
[169] MacDowell KS, Caso JR, Martín-Hernández D, Madrigal JL, Leza JC, García-Bueno B. Paliperidone Prevents Brain Toll-Like Receptor 4 Pathway Activation and Neuroinflammation in Rat Models of Acute and Chronic Restraint Stress. International Journal of Neuropsychopharmacology 2015;18:1–11. https://doi.org/10.1093/IJNP/PYU070.
[170] Rehman S, Nabi B, Javed A, Khan T, Iqubal A, Ansari MJ, et al. Unraveling enhanced brain delivery of paliperidone-loaded lipid nanoconstructs: pharmacokinetic, behavioral, biochemical, and histological aspects 2022. https://doi.org/10.1080/10717544.2022.2069880.

[171] Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 2011;477:90–6. https://doi.org/10.1038/NATURE10357.
[172] Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A. CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia 2015;63:2274–84. https://doi.org/10.1002/GLIA.22892.
[173] Sirivichayakul S, Kanchanatawan B, Thika S, Carvalho AF, Maes M. Eotaxin, an Endogenous Cognitive Deteriorating Chemokine (ECDC), Is a Major Contributor to Cognitive Decline in Normal People and to Executive, Memory, and Sustained Attention Deficits, Formal Thought Disorders, and Psychopathology in Schizophrenia Patients. Neurotox Res 2019;35:122–38. https://doi.org/10.1007/S12640-018-9937-8.
[174] Rizzo LB, Do Prado CH, Grassi-Oliveira R, Wieck A, Correa BL, Teixeira AL, et al. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord 2013;15:832–8. https://doi.org/10.1111/BDI.12121.
[175] Pedrini M, Massuda R, de Lucena D, Macêdo D, Paz AVC, Lobato MIR, et al. Differences in eotaxin serum levels patients with recent onset and in chronic stable schizophrenia: a clue for understanding accelerating aging profile. Schizophr Res 2014;152:528–9. https://doi.org/10.1016/J.SCHRES.2013.11.040.
[176] Teixeira AL, Gama CS, Rocha NP, Teixeira MM. Revisiting the Role of Eotaxin-1/CCL11 in Psychiatric Disorders. Front Psychiatry 2018;9:241. https://doi.org/10.3389/FPSYT.2018.00241.
[177] Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023. https://doi.org/10.1038/SIGTRANS.2017.23.
[178] Adler MW, Rogers TJ. Are chemokines the third major system in the brain? J Leukoc Biol 2005;78:1204–9. https://doi.org/10.1189/JLB.0405222.
[179] Luo SX, Timbang L, Kim JI, Shang Y, Sandoval K, Tang AA, et al. TGF-β Signaling in Dopaminergic Neurons Regulates Dendritic Growth, Excitatory-Inhibitory Synaptic Balance, and Reversal Learning. Cell Rep 2016;17:3233. https://doi.org/10.1016/J.CELREP.2016.11.068.
[180] Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, et al. GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology 2012;62:1574. https://doi.org/10.1016/J.NEUROPHARM.2011.01.022.
[181] Cruz MS, Li M. Identification of TGFβ signaling as a regulator of interneuron neurogenesis in a human pluripotent stem cell model. Neuronal Signal 2021;5. https://doi.org/10.1042/NS20210020.
[182] Wang H, Pan JQ, Luo L, Ning X jie X, Ye ZP, Yu Z, et al. NF-κB induces miR-148a to sustain TGF-β/Smad signaling activation in glioblastoma. Mol Cancer 2015;14:1–14. https://doi.org/10.1186/1476-4598-14-2/FIGURES/9.
[183] Long J, Tian L, Baranova A, Cao H, Yao Y, Rao S, et al. Convergent lines of evidence supporting involvement of NFKB1 in schizophrenia. Psychiatry Res 2022;312:114588. https://doi.org/10.1016/J.PSYCHRES.2022.114588.
[184] Roussos P, Katsel P, Davis KL, Giakoumaki SG, Siever LJ, Bitsios P, et al. Convergent Findings for Abnormalities of the NF-κB Signaling Pathway in Schizophrenia. Neuropsychopharmacology 2013;38:533. https://doi.org/10.1038/NPP.2012.215.
[185] Bobermin LD, da Silva A, Souza DO, Quincozes-Santos A. Differential effects of typical and atypical antipsychotics on astroglial cells in vitro. Int J Dev Neurosci 2018;69:1–9. https://doi.org/10.1016/J.IJDEVNEU.2018.06.001.

[186] Bošković M, Grabnar I, Terzič T, Kores Plesničar B, Vovk T. Oxidative stress in schizophrenia patients treated with long-acting haloperidol decanoate. Psychiatry Res 2013;210:761–8. https://doi.org/10.1016/J.PSYCHRES.2013.08.035.
[187] Chen AT, Nasrallah HA. Neuroprotective effects of the second generation antipsychotics. Schizophr Res 2019;208:1–7. https://doi.org/10.1016/J.SCHRES.2019.04.009.
[188] MacDowell KS, Munarriz-Cuezva E, Caso JR, Madrigal JLM, Zabala A, Meana JJ, et al. Paliperidone reverts Toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia. Neuropharmacology 2017;116:196–207. https://doi.org/10.1016/J.NEUROPHARM.2016.12.025.
[189] MacDowell KS, Martín-Hernández D, Ulecia-Morón C, Bris ÁG, Madrigal JLM, García-Bueno B, et al. Paliperidone attenuates chronic stress-induced changes in the expression of inflammasomes-related protein in the frontal cortex of male rats. Int Immunopharmacol 2021;90. https://doi.org/10.1016/J.INTIMP.2020.107217.
[190] Chiarini A, Armato U, Gui L, Dal Prà I. "Other Than NLRP3" Inflammasomes: Multiple Roles in Brain Disease. Https://DoiOrg/101177/10738584221106114 2022. https://doi.org/10.1177/10738584221106114.